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EXPERIMENTAL PHARMACOLOGY

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**Dovgan A.P.¹,
Urojevskaya J.S.,
Khavansky A.V.**

**POSSIBLE WAYS OF PHARMACOLOGICAL CORRECTION
OF ISCHEMIC DAMAGE TO THE LIVER WITH THE AGONIST
OF PERIPHERAL IMIDAZOLINE RECEPTORS C7070**

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Abstract

Introduction: We glad to introduce several variants of pharmacological correction of ischemic hepatic injury by imidazoline I₂ receptor agonist C7070.

Materials and methods: The experiment was carried out on 70 rats of both sexes, divided into 7 groups (n = 10): an intact group; Pseudo-operated animals (autopsy of the abdominal wall without ligation of the liver vessels); Ischemia / reperfusion group without drug correction; Animals undergoing ischemia / liver reperfusion + Metformin (50 mg / kg); Animals undergoing ischemia / liver reperfusion + Moxonidine (1 µg / kg); Animals undergoing ischemia / liver reperfusion + C7070 (1 mg / kg). For the evaluation, the coefficients calculated from the level of hepatic transaminases (ALT, AST), as well as morphometric ratios of the area of necrosis and deep ischemia of the liver, were used for the evaluation according to the histological examination.

Results and discussion: The indicated agonists of peripheral imidazoline I₂receptors (C7070) significantly reduces ischemically-reperfusion injury of the liver, in comparison with the preparations of moxonidine and metformine. Indirect sign of imidazoline activating mechanism of C7070 is decreasing of the hepatoprotective effect of C7070 by the preliminary administration of imidazoline receptor blocker BU-224. The coefficients for ALT / AST for C7070, moxonidine and metformin were 72.8 / 62.13, respectively; 44.99 / 34.20 and 36.88 / 21.02. The coefficients of the morphological hepatoprotective activity of the preparations were: C7070 – 82.61, moxonidine – 72.33, metformin – 38.96.

Conclusions: The imidazoline receptor agonists significantly and significantly reduce the functional and morphological manifestations of liver ischemia / reperfusion.

Keywords: Liver ischemia, liver reperfusion, diabetes mellitus, C7070, moxonidine, metformin, imidazoline receptor agonists.

Introduction

Ischemia may be considered as a trigger moment and part of pathway of numerous pathological conditions [1].

The development of new effective medicines for diabetes mellitus treatment is one of the most actual problems nowadays. As known, this

development is enable without studying of pharmacokinetic [2].

In case of diabetes mellitus and a metabolic syndrome the fatty dystrophy develops. It can rise to hepatic necrosis [3].

Modern drugs of metabolic syndrome and diabetes mellitus treatment do not protect liver effectively.

Remembering this, we are able to assert, that studying of additional pharmacological correction of standard therapy by biguanides is scientifically interests [4].

Materials and methods

The studying of hepatic anti-ischemic activity was conducted by D.A. Lopatin method [5].

In the experiment were included 50 both sex adult rats, divided in 5 groups: intact group, pseudo-operated group, control group (moderated pathology without therapy); and 3 groups with treatment by C7070 (1 mg/kg), moxonidine (1 µg/kg) and metformine (50 mg/kg).

The studying drugs were administrated before moderating a pathology.

The animals were narcotized by inroperitoneal administration of chloralhydrate in dose 300 mg/kg.

Narcotized animal was subjected to the median laparotomy through the lineaalba. Ligamentum hepatica major was stupid stood and pinched by atraumatic clamp for 15 minutes. Then, content of abdominal cavity was replaced back. A surgical wound was sawed in layers.

During 3 days studying drugs were administrated into the animals per os.

3 days after the end of experiment animals were euthanized by diethyl ether with subsequent taking blood from the heart. The blood was analyzed for ALT, AST.

There was none death during the experiment.

ALT and AST were chosen as biochemical markers of ischemic injury [6].

Histological sections were selected as morphological markers of ischemic injury [7].

All differences, detected by comparing of stated parameters, received in different groups were considered as statistically significant in case of $p<0.05$.

Results and discussion

Moderating of 15 minute ischemia with subsequent reperfusion caused to the increasing of ALT and AST level by 3rd day in 5 times (tab. 1). Pseudo-operated animals did not different from intact group. The same time, morphometric metering of ischemic injury area and necrosis area was 0.387 ± 0.014 and 0.207 ± 0.021 respectively (tab. 2).

Table 1

The influence of agonists of imidazoline receptors at the level of ALT and AST in modeling of ischemia/reperfusion of the liver ($M\pm m$, n=10)

Animal group	ALT (U/ml)	AST (U/ml)
Intact	102.89 ± 8.82	284.14 ± 19.36
Pseudo-operated	$110.27\pm21.96^*$	$289.80\pm16.29^*$
Ischemia/Reperfusion (I/R)	$526.90\pm17.97^{**}$	$1045.16\pm80.02^{**}$
I/R+C7070 (1 mg/kg)	143.27 ± 16.93^1	395.85 ± 33.31^1
I/R +Moxonidine (1 µ/kg)	289.86 ± 15.27^1	687.71 ± 28.37^1
I/R +Metformine (50 mg/kg)	332.56 ± 22.05^1	825.49 ± 22.46^1
I/R +C7070 (1 mg/kg)+BU224 (1 mg/kg)	300.45 ± 19.44^1	798.59 ± 21.34^1

Note: * – $p>0.05$ incomparing with intact group, ** – $p>0.05$ incomparing with pseudo-operated group, ¹ – $p>0.05$ incomparing with ischemia/reperfusion group.

Table 2

The influence of agonists of imidazoline receptors in the area of ischemic damage to the liver and area of the zone of necrosis of liver tissue by modeling of ischemia/reperfusion of the liver ($M\pm m$, n=10)

Animal group	Ischemia injury area, mm ²	Necrosis area, mm ²
Intact	n/a	n/a
Pseudo-operated	n/a	n/a
Ischemia/Reperfusion (I/R)	0.387 ± 0.014	0.207 ± 0.021
I/R+C7070 (1 mg/kg)	$0.058\pm0.029^*$	$0.046\pm0.013^*$
I/R +Moxonidine (1 µ/kg)	$0.090\pm0.025^*$	$0.075\pm0.015^*$
I/R +Metformine (50 mg/kg)	$0.238\pm0.052^*$	$0.125\pm0.020^*$
I/R +C7070 (1 mg/kg)+BU224 (1 mg/kg)	$0.159\pm0.031^*$	$0.104\pm0.008^*$

Note: * – $p>0.05$ incomparing with ischemia/reperfusion group.

Using of studying drugs statistically significant decrease the level of ALT and AST in experimental animals. Maximal activity had C7070 (1 mg/kg), the level of ALT and AST were 143.27 ± 16.931 and 395.85 ± 33.311 respectively (tab. 1).

The same time C7070 significantly decreased ischemia injury area and necrosis area till 0.058 ± 0.029 and 0.046 ± 0.013 respectively.

Metformine and moxonidine decreased biochemical and morphometrical markers of ischemia injury, but they were worse than C7070 (tab. 1-2).

For the ease evaluation we offer to compare the degree of AST and ALT level decreasing in different animal groups. It is possible with help of simple math formula 1.

$$K_{ALT} = 100 - \frac{ALT(exp)}{ALT(ctrl)} * 100\%, \quad (1)$$

Hepatoprotective activity C7070, moxonidine and Metformin in modeling of ischemia/reperfusion of the liver according biochemical research ($M \pm m$; $n=10$)

Hepatoprotective coefficient, U.	C7070 (1 mg/kg)	Moxonidine (1 μ /kg)	Metformine (50 mg/kg)
K_{ALT}	$72.81 \pm 1.71^*$	44.99 ± 1.23	36.88 ± 1.02
K_{AST}	$62.13 \pm 1.34^*$	34.20 ± 1.21	21.02 ± 1.49

Note: * – $p > 0.05$ in comparing with moxonidine and metformine groups.

Based on these data we are able to speak about maximal anti-ischemic activity of imidazoline I₂ agonist (C7070) through the all studied in frames of this studying drugs.

There are not enough data for the full assessment of anti-ischemic activity of studying drugs.

The coefficient of morphological hepatoprotection was chosen as additional data for full assessment of anti-ischemic activity for the studying drugs. This coefficient include data of ischemic injury area and necrosis area.

The coefficient was calculated by next formula (formula 3).

$$K = 100\% - \left(\frac{Mi(exp) + Mn(exp)}{Mi(ctrl) + Mn(ctrl)} * 100\% \right), \quad (3)$$

Mi (exp) – mean area of ischemic injury of liver in experimental animals;

Mn (exp) – mean necrosis area in liver of experimental animals;

Mi (ctrl) – mean area of ischemic injury of liver in control (I/R) animals;

ALT (exp) – ALT level in blood of experimental animals,

ALT (ctrl) – ALT level in blood of control (I/R) animals.

The same formula (formula 2) may be useful for AST studying.

$$K_{AST} = 100 - \frac{AST(exp)}{AST(ctrl)} * 100\%, \quad (2)$$

AST (exp) – AST level in blood of experimental animals,

AST (ctrl) – AST level in blood of control (I/R) animals.

With the help of previous formulas we can take next data of functional activity of studing drugs (tab.3).

Table 3

Hepatoprotective activity C7070, moxonidine and Metformin in modeling of ischemia/reperfusion of the liver according biochemical research ($M \pm m$; $n=10$)

Mn (ctrl) – mean necrosis area in liver of experimental animals.

Thus we receive next data of morphological anti-ischemic activity of studied drugs. (tab. 4)

Table 4

Hepatoprotective activity C7070, moxonidine and Metformin when modelirovani ischemia/reperfusion of the liver according moramerica research ($M \pm m$; $n=10$)

Animal group	Coefficient of hepatoprotective activity, U.
И/Р+C7070 (10 мг/кг)	$82.61 \pm 3.22^*$
И/Р+Moxonidine (1 мг/кг)	72.33 ± 1.04
И/Р+Metformine (50 мг/кг)	38.96 ± 5.69

Note: * – $p < 0.05$ in comparing with 2 и 3.

So, agonist of peripheral imidazoline receptor (I_2) C7070 has maximal anti-ischemic activity among all studied drugs.

Received data are able to be explained by different mechanism of action.

The base of metformine action is a decreasing of glucose production. It correlated with the decreasing of glycemia level. Metformine plays a role in increasing of peripheral insulin effects, decreasing gluconeogenesis and oxidation of free fat acids in liver, increasing of anaerobes way of glucose metabolism with the lactate formation, decreasing of lipolysis. In range of in vivo and in vitro studies was detected activating influence of metformine on the cell ferment AMP-kinase, playing a role in glucose transport by GLUT4 and oxidation of free fat acids. Probably, betterment of glycemic profile by metformine therapy may be related to the same aspects of its mechanism of action. Besides, dimethylbiguanide demonstrated an ability to decrease the rigidity of cell membranes, which often detected at patient with diabetes and can contribute the development of its complications [8].

Metformine activates AMPK – hepatic ferment, playing a role in insulin signalization, in total energy balance in organism and in glucose and lipid metabolism, including liver. Activation of AMPK is necessary for inhibition metformine effect to hepatic gluconeogenesis [9].

Resuming of previous, it can be argued that anti-ischemic action of metformine based on the accumulation of hepatocytes energy reserves and deceleration of spending generated current nutrients.

Moxonidine as an agonist of central (I_1) imidazoline receptor involved in redistribution of hepatic blood flow according to opening of collateral vessels, started from a. gastrica sinistra, free from the blocking. Also, additional activity can due to the central control by moxonidine on the opening of hepatic vessels in moment of reperfusion. It can't be excluded the influence of moxonidine to the peripheral imidazoline receptors [10].

The agonist of peripheral imidazoline (I_2) receptors C7070 releases its hepatoprotective action by mechanisms, like in case of skin flap ischemia. Obviously, its influence to the preservation of mitochondria using ATPase

canals, presented on the external and internal mitochondrial membranes. Slowing and blocking of avalanche ferric ion current decrease the oxidative stress with all its manifestations during the reperfusion of liver [11].

Activation of the imidazoline receptors results to the rise of arachnid acid synthesis and inhibition of Na^+/H^+ ion exchange canals. It seems, that imidazoline receptors belong to the neurocytokine receptors [12]. Activation of the central imidazoline (I_1) receptors leads to the decreasing of the blood pressure and slows a heartbeat. These all are the result of braking influence to the peripheral sympathetic nervous system.

During the time we know the new generation of the imidazoline receptors agotists there were many pre-clinical and clinical trials of effectiveness of these drugs.

So, Mukaddam-Daher in his trial shows, that intravenous administration of moxonidinerised rat diuresis and Na/K excretion. This effect was blocked by administration of imidazoline receptors antagonist epharoxan and decreased by effect of α -andrenoblocker – jobichinin [13].

Central imidazoline (I_1) receptors of hypothalamic area are involved into the glycemic level regulation, that was shown at the experiment with the selective I_1 receptor agonist agmatine, which causes the decreasing of glucose level in blood. The same action has moxonidine. Besides, it is assumed, that imidazoline receptors can be located in pancreas. Activation of these receptors is able to leads to increasing insulin secretion [14].

Using of moxonidine at Zucker line rats caused decreasing the level of hypothalamic neuropeptid Y, that may be one of the probably mechanism explaining the decrease in body mass during therapy with this drug [15].

It should be noted that not all of these effects can be explained by the activation of central I_1 –receptors. Apparently, some of them still mediated by α_2 –adrenergic receptors. In addition, a certain contribution is made by the peripheral action of drugs.

Representatives of the Kharkov pharmaceutical schools, by contrast, drew attention to the ability of agonists of peripheral imidazoline receptors influence glycemic control

[16]. According to their research, this group of drugs in its hypoglycemic action is not inferior to Metformin. But the unwanted side effects they have much less. Moreover, unlike Metformin, agonists peripheral imidazoline receptors do not cause the development of hypoproteinemia and hyperlactacidemia.

In addition to its other localizations, imidazoline receptors located on the membranes of adipocytes — fat cells. Stimulation of these receptors leads to increased lipid metabolism.

In the treatment of moxonidine (0.4 mg/day) for 8 weeks in 20 patients with hypertension there was a significant decrease in blood pressure, but the levels of total lipids, oxidized low-density lipoprotein and the ratio of different subtypes of lipoproteidov low density did not change significantly [17]. In an 8-week study, involving 51 patients with hypertension and hypercholesterolemia did not affect the level of blood lipids and rilmenidine [18].

The history of biguanide derived from guanidine – thing of the past. In the Middle ages for the treatment of patients with diabetes used an extract from the root of the French lilac. The drug Galega officinalis contained a substance "guanidine" which was aimed at reducing the clinical symptoms of diabetes.

Since, at this time established a significant correlation of insulin resistance with the activity of the sympathetic nervous system, it is logical that you need a "universal" tool that would control and glycemia, and insulin resistance and SNS activity.

Besides, do not forget that the role of endothelial cells in the development of cardiovascular disease was an important discovery for understanding pathogenesis including diabetic vascular lesions [19].

Modern hypoglycemic medicines may have positive pleiotropic effects on the pancreatic tissue. However, their activity is insufficient to prevent the development spotswoode diabetes complications [20].

In view of the foregoing, it becomes apparent the advantage of agonists of peripheral imidazoline receptors (IR2) as drugs that affect the early survival of patients with diabetes mellitus type II.

Conclusion

1. Theimidazoline I₂ receptors agonist C7070 at a dose of 1 mg/kg at 4.5 times prevents the increase of ALT and AST and at 2.5 times reduces areaof ischemic injury and necrosis in the modereating of a 15-minute ischemia of the liver. Hepatoprotective effect of C7070 50% decreased by antagonist of peripheral imidazolinereceptors BU224 (1 mg/kg)

2. Moxonidin andmetformine also has a hepatoprotective effect and their coefficients are 36.88% to 44.99% for moxonidine (ALT and AST) and 34.20/21.02 for Metformin (ALT/AST). Factors histological hepatoprotective activity amounted to 72.33 and 38.96 for moxonidine and metformine respectively.

3. On hepatoprotective activity of the drug was inferior C7070 (72.81/62.13/82.61 ALT/AST/histology, respectively).

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Conflicts of interest

The authors have no conflict of interest to declare.

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**Krut U.A.¹,
Shaposhnikov A.A.,
Korokin M.V.****RESEARCH OF WOUND HEALING EFFECT
OF PHYTOMINERALSORBENT ON THE BASIS
OF MONTMORILLONITE**

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Corresponding author, ¹e-mail: krut@bsu.edu.ru**Abstract**

Introduction: The results of preclinical investigation of the wound healing effect of phytomineralsorbent (PMS) based on montmorillonite are presented.

Objectives: Study of the possibilities of pharmacological correction of purulent wound process with the help of various medicinal forms of phytomineral sorbent based on montmorillonite.

Methods: Purulent wounds were modeled in white rats of the Wistar line in the interblade area 3 cm². Wound dressing was performed daily for 21 days by PMS in the form of powder and gel, and also by the comparison preparation Dexpanthenol (Russia, Pharmstandard). The wound healing effect was carried out according to morphological parameters of histological sections, clinical and biochemical parameters of blood and exudate of the animals under study.

Results and discussion: The wound defect of rats, the ligation of which was carried out by phytomineral sorbents, was significantly less than the control 2 times, the depth of necrosis in the acute phase of the wound process was up to 0.85 microns deep, which is 1.7 times less than in the control group. The use of Dexpanthenol in the acute stage was characterized by a suppuration reaction and the presence of microbes. When studying the dynamics of the most important rat blood parameters during wound healing, it was established that the content of immature neutrophils in the regeneration phase in animals using PMS in the form of powder was lower by a factor of 2 compared to the control group and group of animals using the drug Dexpanthenol; the concentration of the total protein in the blood of animals in experimental groups with the use of the preparation Dexpanthenol and PMS based on montmorillonite in the forms of powder and gel were significantly lower than the control one by 18-25% on the third day of the experiment. Exudation of purulent wounds of animals, wound dressings which were performed by PMS in the form of powder, ceased on the second day, the remaining animals lasted up to 5 days.

Conclusion: PMS in the form of powder has best effect of wound healing on inflammatory stage of injury.

Keywords: phytomineralsorbent, montmorillonite, wound healing, injury.

Introduction

Wound and consequent purulent processes are among the most common pathologies in surgery. They have a different etiology, are widespread and are accompanied by various complications [1]. The wound process can not be considered as a purely local phenomenon, since many body systems are affected to a greater or lesser extent [2]. Local and general infection leads

to a variety of disorders of the body's systems and functions. Violated metabolism and hematopoiesis, microcirculation is changing, there is a suppression of liver functions [3].

There is a wide spectrum of various methods and means of treatment of purulent wounds. For today in the arsenal of physicians [2, 4]. In this case, the method of local treatment of wounds remains the most common due to its availability,

low cost, and most importantly effectiveness [6]. Over time, many funds lose their effectiveness due to the development of resistance of pathogenic microflora to this drug [7, 8].

Studies in the field of wound healing agents have been carried out for many decades, including the influence of sorbents on the wound process [7-12]. Sorbents are able to remove toxic substances, purulent exudate, products of cellular decay, blood elements from the wound, as well as pathogenic microflora, aggravating the severity of the disease [13-15].

Previously, it was proved that sorbents based on montmorillonite with high sorption ability, successfully cope with undesirable microflora, optimize the process of wound healing [16-18].

Among the basic requirements for modern wound coverings, in addition to protecting the wound from infection, care should be taken to ensure vapor permeability and sorption capacity sufficient to maintain the wet state of the wound, as well as the ability to maintain adhesion and cell growth [19].

In this regard, it becomes relevant to study ecologically safe, biologically effective sorption compositions for the treatment and prevention of purulent consequences of wound injuries on the basis of mineral and plant raw materials.

Objective: To study the possibilities of pharmacological correction of purulent wound process with the help of various dosage forms of phytomineral sorbent based on montmorillonite.

Materials and methods

Investigation of wound healing properties of phytomineral sorbents (PMS) in the form of powder and gel, as well as Dexpanthenol spray, was performed on white linear Wistar rats (80 pieces), which were selected as a biological model. FMS is a complex sorption composite based on the inorganic minerals of the montmorillonite group and the extract of the medicinal plant *Thymus serpylum*. For the study, animals weighing 180-200 g were taken without external signs of the disease, who had passed the quarantine regime. Operations and other manipulations in rats were carried out under conditions of general anesthesia by intraperitoneal administration of an aqueous solution of chloral hydrate at a dose of 300 mg / kg. The animals were divided into groups by stratified randomisation with stratification by

body weight, diet and nutrition conditions, and by operations and manipulations. All experiments were approved by the Ethics Committee of the "Belgorod State National Research University". Vivisection was carried out in accordance with the ethical principles of the treatment of laboratory animals "The European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No. 123".

Wounds were applied in the interblade area with an average diameter of 2.83 cm², which was 1% of the total surface of the skin of the animals. In the area of injury, using a sutured suture, a special port was sewed (for collection of exudate and local examination of the wound), a certain agent corresponding to the experimental groups was inserted into it [20]. Further, a strain of *Escherichia coli* was applied to the wound region at a fixed infectious dose of $2 \cdot 10^8$ microbial bodies.

All the animals were divided into five experimental groups, 20 each in each group: group I – control (the wound was washed with NaCl 0.9%); Group II – wound dressing Dexpanthenol; Group III – dressing wounds PMS in the form of powder; IV group – dressing wounds PMS in the form of gel. Bandaging with PMS (powder, gel) was carried out by applying 0.1 g of the drug to the damaged area daily during the entire period of the experiment once a day. Dexpanthenol was applied daily to the affected area of the skin at a distance of 10-20 cm once a day so that the entire affected area was coated with the drug (before use, the balloon was shaken). This dressing technique was chosen based on the instructions for using the drug.

The experiment was carried out for 21 days, which corresponds to the generally accepted wound healing time. The entire period of wound healing was divided into three stages: inflammation, regeneration, scar formation.

Morphological examination was applied to the cutaneous muscle flap of the wound from rats of various groups taken on the third, ninth and twentieth day of the course of the wound process. The cutaneous muscle flap was removed surgically, then it was fixed for 24 hours in a 10% formalin solution and degreased in growing spirits, filled into paraffin and histological

preparations with a cut thickness of 3-5 μm , stained with hematoxylin-eosin.

The study of blood in laboratory animals was carried out every third day. Blood sampling for general and biochemical analysis was carried out from the tail vein into tubes with EDTA anticoagulant, under the action of the 1st stage of anesthesia, using analgesics.

Blood tests were performed on the hematological analyzer "Sysmex XP-300" (Germany), and the biochemical "Cobas® 4000" (Germany) c with sets of standard reagents.

A general clinical blood test was examined in terms of: Hb; Ht; the number of erythrocytes, platelets, leukocytes; leukocyte formula: neutrophils, basophils, eosinophils lymphocytes). Biochemical analysis: (total protein, albumin, ALT, AST, urea, creatinine, bilirubin, glucose, electrolytes: K⁺, Na⁺, iron, Cl⁻, pH).

Investigation of exudate purulent wounds was carried out in the acute phase of the wound process, according to the following parameters: pH, concentration of total protein and glucose. Sampling is carried out from the surface of the wound (by sampling an average sample) into special tubes with a lid.

The pH was determined by a potentiometric method using the pH meter of Metter-Toledo AG (Switzerland). In addition, the pH of the isotonic solution and the extract of thyme were determined.

The total protein concentration was determined by a biuret reaction using a

spectrophotometric method using the Specord 210 Plus device (Germany). Glucose concentration was determined by titrimetric method.

Results and discussion

All laboratory animals had swelling of the edges, presence of purulent exudate, white-green color, viscous consistency with an unpleasant odor a day after the application of wounds. Since 3 days the animals have died. The most intensive death was in the control group of animals. By the third day in this group, 40% of the animals died. By 20 days 7 animals out of 20 survived. The death of animals was the result of the developed sepsis of diffuse peritonitis. At the autopsy, a part of the dead animals found a reactive adhesive process in the abdominal cavity and intestinal obstruction.

A planimetric study of the wound defect showed that on the 18th day in the group where the wound treatment was performed with the gel form of FMS, the area of the wound surface was significantly less than the original value by 81%. The area of the wound surface in animals where Panthenol Pharmstandard was used was the lowest (65% less on average for the rest of the groups). However, the use of FMS in powder form stimulated a more early reduction in the area of the wound defect than in other animals (Fig. 1).

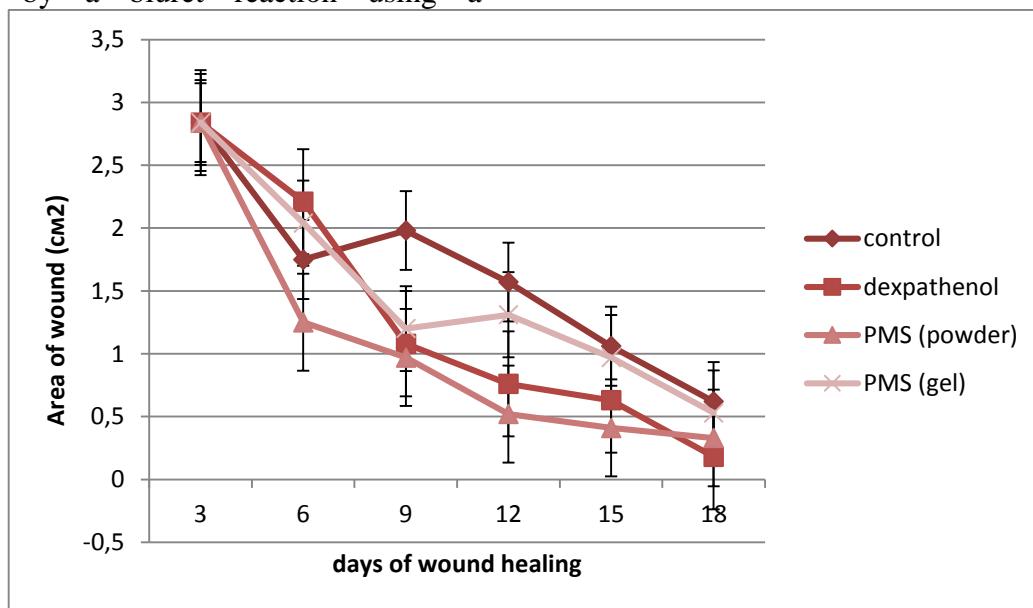


Fig. 1. Change in the area of the wound defect in moulding a purulent wound in rats in the experiment

The morphological pattern of the purulent wound in the control group is the same in all cases: in the acute phase, a large layer of necrotic tissue up to 1.5 μm is present on the wound surface, indicating an active inflammatory process, mast cells and macrophages, (Fig. 2 A). In animals treated with Dexpanthenol, the scab had a thickness of 0.15 to 0.6 μm , consisting of fragments of necrotic tissue with fragments of leukocytes, a thickness of inflammatory

infiltration up to 1 μm (Figure 2 B). In rats fed PMS in the form of powder on the acute phase of the wound process, necrotic tissue with dense infiltration of neutrophilic leukocytes and blood clots was up to 0.85 microns deep (Fig. 2C). The area of the wound defect in animals treated with FMS as a gel is covered in the acute phase with a necrotic tissue with neutrophils, the depth of necrosis is up to 1 μm (Fig. 2 D).

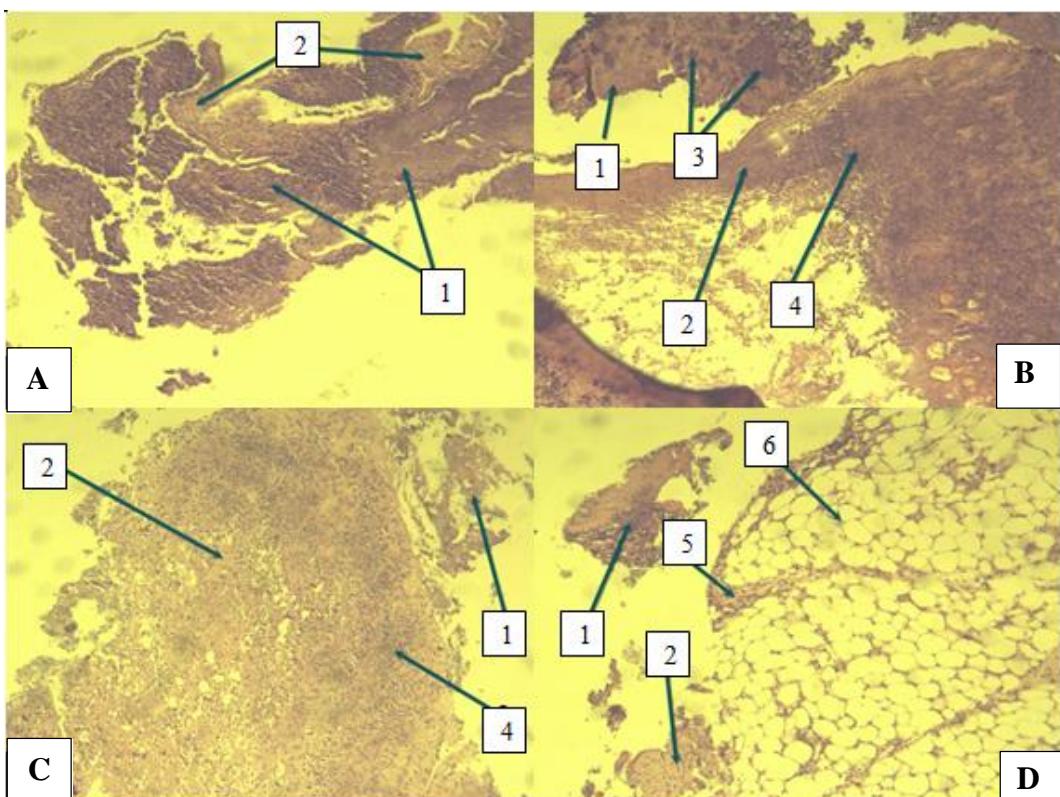


Fig. 2. Section of the skin of a rat after modeling a purulent wound in the interblade area on day 9 under different experimental conditions (regeneration phase). Note: A – control; B – wound dressing Dexpanthenol;

B – wound dressing PMS in the form of powder; D – dressing wound PMS in the form of gel.

1 – necrotic tissue; 2 – connective tissue; 3 – colony of microbes; 4 – neutrophilic leukocytes with their fragments; 5 – lymphocytic infiltration; 6 – adipose tissue

Note: staining with hematoxylin-eosin. Magnification x 200.

As a result of the conducted microscopic studies of the wound defect, it was found that when using PMS in the early stages of the wound process, lymphonodiocyte infiltration and rapid restoration of the connective tissue are facilitated. Secondary reinfection did not occur, due to the high sorption ability of the drugs. The use of Dexpanthenol in the application of purulent wounds caused the decomposition of tissue in the area of scab formation, the scab was rejected before the wound was healed, a secondary

infection with a copious purulent discharge was attached. It should be noted that at the final stage of the wound process, neutrophilic leukocytes were present in the histological preparations of only the control group, which indicates the effectiveness of using both PMS and Dexpanthenol in the treatment of purulent wounds.

Throughout the entire experiment, we studied the morphological parameters of peripheral blood in animals of experimental and control groups.

The clinical picture of the blood of rats in the acute phase of the wound process and shows that in all experimental animals acute blood loss and severe inhibition of the functions of the formation of new cells were observed.

A study of the red blood system of injured animals showed that on the 3rd day hemoglobin in all groups of authentically initial indicator was on average 11% (Table 1). During the

regeneration phase (9th and 12th days) in animals where the FMS in the form of powdered Hb was used for dressing the wounds, there were 142.50 ± 1.575 and 143.15 ± 1.602 , which may indicate the restoration of hematopoiesis processes that were activated earlier than in the animals of the control group and the application of Dexpanthenol.

Table 1

Concentration of Hb in the blood (g / l), ($M \pm m$; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			136.25 ± 2.61	
3 day	128.00 ± 1.67*	123.33 ± 2.56*	121.50 ± 0.84*	123.50 ± 1.58*
6 day	152.00 ± 3.37*	116.13 ± 1.69*°	115.00 ± 1.69*°	134.67 ± 4.24°
9 day	149.00 ± 1.67	135.50 ± 0.84°	142.50 ± 1.56*°	140.13 ± 2.58°
12 day	135.43 ± 2.06	131.30 ± 2.60	143.15 ± 1.60*°	138.00 ± 2.74
15 day	132.02 ± 1.91	141.36 ± 2.52°	137.65 ± 2.40	140.36 ± 2.03°
18 day	150.96 ± 1.36*	148.21 ± 1.95*	148.54 ± 2.65*	151.87 ± 2.79*

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats (p < 0.05)

Adhesion of platelets to the endothelium and subendothelial matrix is the initial stage of hemostasis and thrombosis. The number of platelets was significantly increased on the 6th day after the application of the model wound, which indicates a high thrombus formation (Table 2). The level of platelets at this stage in animals treated with PMS in the forms of powder and gel

was 611.33 ± 19.313 and 826.00 ± 16.859 , respectively, which is lower than in the control group of rats by 35 and 11%. During the reorganization phase of the rumen, the platelet count gradually decreases, thus the integrity of the endothelium of the blood stream is restored.

Table 2

Platelet concentration in the blood ($\times 10^9 / l$), ($M \pm m$; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			483.00 ± 43.40	
3 day	624.33 ± 8.65*	595.67 ± 7.01*°	612.00 ± 3.37*	555.67 ± 8.60°
6 day	935.33 ± 25.31*	950.00 ± 50.58*	602.67 ± 53.98*°	826.00 ± 16.86*°
9 day	560.45 ± 18.65	513.67 ± 28.60	611.33 ± 19.31*	620.28 ± 19.29*
12 day	439.25 ± 32.23	451.15 ± 41.38	402.46 ± 35.62	398.25 ± 26.65
15 day	419.02 ± 45.21	387.33 ± 43.222	451.21 ± 42.37	400.00 ± 44.44
18 day	520.23 ± 43.37	501.04 ± 45.37	511.36 ± 45.60	487.69 ± 48.46

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats (p < 0.05)

Reduction of the containing of leukocytes in the peripheral blood during the first three days can

be explained by the general reaction of the organism to the local infectious process (Table 3).

Table 3

Concentration of leukocytes in the blood (* 10⁹ / l), (M ± m; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			18,67 ± 1,404	
3 day	16.52 ± 0.73	15.13 ± 0.26*	16.83 ± 0.35	15.18 ± 0.30*
6 day	18.20 ± 0.34	13.20 ± 0.67*°	11.00 ± 1.01*°	14.60 ± 0.84*°
9 day	16.80± 0.73	1410± 1.26*	14.30± 0.96*	16.40± 1.66
12 day	14.30± 1.69	13.93± 0.88*	13.32± 1.22*	14.44± 1.56*
15 day	14.30± 0.99	14.04± 1.86	13.76± 1.73*	14.18± 1.92
18 day	11.36± 0.95*	11.54± 1.36*	11.60± 1.65*	10.67± 1.49*

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats (p <0.05)

Analyzing the indicators of the leukogram, it should be noted that during the first three days after the application of wounds, neutrophilia was registered, the content of stab neutrophils was increased 2-fold (Table 4). On the 6th day in the

groups where treatment of wounds of PMS occurred, a regenerative shift of neutrophils to the left was observed, with normalization of these indices to the twelfth-fifteenth day.

Table 4

Indicator of the content of stab neutrophils (%) in the blood (M ± m; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			1.60 ± 0.52	
3 day	2.33 ± 0.97	3.16 ± 0.49*	2.17 ± 0.49	2.35 ± 0.87
6 day	3.00 ± 0.18*	2.17 ± 0.49	2.04 ± 0.12°	2.28 ± 0.429
9 day	2.00 ± 0.97	2.37± 0.92	1.13± 0.42	2.70± 0.36
12 day	2.00± 0.31	2.32± 0.33	1.02± 0.152	2.20± 0.25
15 day	1.80± 0.45	2.00± 0.22	2.02± 0.31	1.20± 0.50
18 day	2.00± 0.65	2.02± 0.75	1.00± 0.12	2.36± 0.16

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats (p <0.05)

Studies of blood biochemical analysis of rats with model purulent wounds showed that the concentration of total protein on the 3rd and 6th day was decreased, which indicates catabolic processes in organs and tissues (Table 5). When using the drug Dexpanthenol and PMS based montmorillonite in the forms of powder and gel,

the concentration of total protein in the blood of animals was significantly lower than the control protein by 18-25% on the third day of the experiment. By the 9th day there is an increase in the total protein content in the blood plasma, which indicates anabolic processes.

Table 5

Concentrations of total protein in blood serum (g / l), (M ± m; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			74.78 ± 7.23	
3 day	86.75 ± 1.41	70.87 ± 4.24°	65.00 ± 3.37°	70.20 ± 3.372°
6 day	53.00 ± 5.06*	65.83 ± 5.15	66.87 ± 7.60	59.33 ± 7.60
9 day	56.70 ± 6.74	63.43 ± 5.15	59.71 ± 7.60	53.53 ± 7.602
12 day	60.12 ± 7.23	62.45 ± 6.29	59.56 ± 6.74	58.53 ± 7.15
15 day	68.50 ± 6.95	69.53 ± 7.26	54.05 ± 7.46	45.98 ± 6.23*
18 day	76.00 ± 6.98	69.34 ± 7.46	72.36 ± 7.26	69.58 ± 7.75

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats ($p < 0.05$)

The activity of aminotransferases throughout the experiment was increased, with a decrease towards the end of the experiment. It should be noted that according to the data of studies, the activity of ALT and AST (Tables 6 and 7) was

increased by 2.5 times using Dexpanthenol, while when using PMS, the activity of these enzymes was only 2 times at the acute stage of the wound healing process.

Table 6

Activity of ALT (ME / L) in serum (M ± m; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			38.40 ± 6.39	
3 day	48.00 ± 0.84	50.70 ± 3.37	64.20 ± 1.69°*	46.70 ± 1.69
6 day	49.93 ± 7.60	59.70 ± 5.06*	38.00 ± 6.74	27.30 ± 6.74
9 day	50.65 ± 6.39	51.41 ± 5.06	49.82 ± 6.74	41.93 ± 6.74
12 day	41.33 ± 6.390	41.06 ± 6.74	37.36 ± 7.60	39.96 ± 6.80
15 day	55.00 ± 7.95	53.81 ± 6.96	55.82 ± 6.852	46.73 ± 6.36
18 day	31.00 ± 7.12	41.92 ± 6.16	41.13 ± 5.65	38.94 ± 6.26

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats ($p < 0.05$)

Table 7

ACT activity (ME/ L) in blood serum (M ± m; n = 6)

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline			97.90 ± 8.62	
3 day	265.67 ± 6.81*	67.30 ± 5.06°*	371.40 ± 5.06°*	285.20 ± 8.43°*
6 day	202.40 ± 8.921*	102.90 ± 8.56°	129.20 ± 5.09.*°	158.63 ± 7.50.*°
9 day	135.90 ± 7.50*	121.74 ± 8.56*	120.43 ± 9.39*	154.12 ± 8.92*
12 day	115.10 ± 8.56	119.70 ± 0.51	121.90 ± 8.92	144.30 ± 9.39*
15 day	337.64 ± 28.65*	146.67 ± 7.99**°	130.65 ± 8.16°*	136.62 ± 7.24°*
18 day	118.90 ± 9.12	109.15 ± 8.85	117.97 ± 8.74	134.79 ± 8.37*

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats ($p < 0.05$)

The concentration of iron in the blood plasma in the acute phase of the wound process was increased 2.5-fold in animals whose wound dressings were performed by Dexpanthenol,

compared with the initial index and 1.5-fold compared with the control group of animals (Table 8). Such an increase in iron ions may indicate a strong hemolysis of red blood cells.

Table 8

Concentration of iron ions (mmol / L) in blood serum ($M \pm m$), n = 5

Group of animals	Control	Dexpanthenol	PMS (powder)	PMS (gel)
Baseline		19,86 ± 0,460		
3 day	33.74 ± 1.69*	51.75 ± 2.58**	22.12 ± 1.69°	22.48 ± 0.81**
6 day	25.30 ± 0.56*	26.90 ± 0.36*	19.60 ± 0.47°	20.90 ± 0.51°
9 day	23.63 ± 0.56*	25.51 ± 0.02**	26.40 ± 0.02**	21.81 ± 0.51°
12 day	25.00 ± 0.02*	21.41 ± 0.02°	20.90 ± 0.56°	21.70 ± 0.47°
15 day	19.10 ± 0.02	19.70 ± 0.36	21.40 ± 0.56°	20.10 ± 0.47
18 day	19.80 ± 0.51	20.10 ± 0.47	20.50 ± 0.07	19.2 ± 0.19

Note: * significant differences from the corresponding values in animals with a baseline; ° – significant differences in indices compared with the control group of rats ($p < 0.05$)

In animals, the application of wounds, which were performed by PMS in the forms of powder and gel, the concentration of iron was slightly higher than the initial value throughout the experiment.

Biochemical changes in exudate – a fluid released from tissues and blood vessels during inflammation play an important role in diagnosis and selection of a method for treating purulent wounds.

pH values of effervescent liquids are used in clinical practice to diagnose the transition of serous exudate to serous-purulent. The common boundary is the pH of 7.20.

The data obtained for the 1st day of purulent process indicate that in rats in groups II and III of the experimental groups with characteristic signs of suppuration, they have a slightly acidic or neutral character: the pH value was in the range from 5.88 to 7.09, which is below the boundary criterion pH is equal to 7.20. This aspect indicates the violation of acid-base balance of cells and tissues and, as a consequence, about acidosis. However, in rats in the experimental group, where the wound dressing was performed with a dry form of PMS, exudation stopped already on the 3rd day of the experiment, and the most uniform dynamics of pH change was observed (Table 9).

Table 9

Parameters of the pH value of suppurative exudate of model I wounds in white rats ($M \pm m$; n = 6)

Group of animals	pH		
	1st day	2nd day	3rd day
(I) Control	7.26 ± 0.04	5.76 ± 0.03	7.46 ± 0.04.
(II) Dexpanthenol	7.09 ± 0.04*	7.25 ± 0.04*	7.05 ± 0.04*
(III) PMS (powder)	7.07 ± 0.04*	7.04 ± 0.04*	-
(IV) PMS (gel)	7.25 ± 0.04	6.77 ± 0.04*	7.24 ± 0.04*

Note: * significant differences compared to Group I animals, $p \leq 0.05$

Determining the concentration of total protein in the effusions is the main point in the study of purulent wounds. With mild vascular damage, not only low-molecular albumin seeps into the focus of inflammation, in more severe lesions, large-molecule globulins appear in the exudate, and, finally, the largest molecules of fibrinogen, which turn into tissues into fibrin. A common value that

distinguishes exudate from a transudate is that the protein content in the serum of a biological material should be more than 25 g / ml [21].

The data on the protein content in wound exudate are presented in Table 10. The calculation was carried out by the equation: $y = 0.0032x + 0.0061$; $R = 0.9944$, at the analytical wavelength: $\lambda = 547$ nm.

The results of the study show that in I, II and IV experimental groups of animals, an increase in the concentration of total protein in the wound exudate was observed. On the third day of the experiment in these groups, the protein content in the sweat was greater than 25 g / ml, which indicates that the effusion from the wound is characterized as

exudate. In the III experimental group, the total protein concentration had a negative dynamics, effusions ceased on day 3 of the experiment. On the first day of the experiment, the protein concentration in the exudate was 25 g / ml, and on the 2nd day – 10 g / ml, which is less than the usual value for exudate.

**Concentration of total protein (g / l) in exudate purulent wounds in rats
in the experiment ($M \pm m$; $n = 6$)**

Group of animals	1st day	2nd day	3rd day
	$C_p, \text{mg/cm}^3$		
(I) Control	34.00 ± 0.19	21.60 ± 0.12	90.00 ± 0.50
(II) Dexamphenol	$29.40 \pm 0.16^*$	21.20 ± 0.12	$77.30 \pm 0.43^*$
(III) PMS (powder)	$25.00 \pm 0.14^*$	$10.20 \pm 0.06^*$	-
(IV) PMS (gel)	$54.51 \pm 0.31^*$	$35.10 \pm 0.20^*$	$108.80 \pm 0.61^*$

Note: *significant differences compared to Group I animals, $p \leq 0.05$

With an increase in the permeability of the walls of the vessels, glucose also passes into the exudate of the wound. The presence of glucose in the effervescent fluids creates a favorable environment for the development of pathogenic microflora.

The glucose concentration indices in the wound exudate are presented in Table 11. Based

on the results of the study, an uneven change in the glucose concentration is observed in the experimental groups. In rats I, III and IV of the experimental groups, the glucose concentration changes in wave form: it rises on the 2nd day of the experiment and decreases by the third. In groups II and III the glucose content decreases on the second day and rises to the third.

The concentration of glucose (mmole / l) in exudate purulent wound ($M \pm m$; $n = 6$)

Group of animals	1st day	2nd day	3rd day
	$C_g, \text{mmole/l}$		
(I) Control	0.77 ± 0.01	2.27 ± 0.02	0.72 ± 0.01
(II) Dexamphenol	$0.22 \pm 0.01^*$	$1.39 \pm 0.01^*$	$1.51 \pm 0.02^*$
(III) PMS (powder)	$0.33 \pm 0.01^*$	$1.11 \pm 0.01^*$	-
(IV) PMS (gel)	$0.49 \pm 0.01^*$	2.22 ± 0.02	$1.72 \pm 0.02^*$

Note: * significant differences compared to Group I animals, $p \leq 0.05$

Thus, it can be concluded that the use of FMS in the exudative phase of the wound healing process helps to reduce the concentration of total protein and glucose, which favors the formation of regeneration processes.

Conclusions

Comparative study of purulent wounds in the control group and groups with the use of the studied drugs showed that phytomineral sorbent

based on montmorillonite in the form of powder contains the most pronounced effect on the healing of purulent wounds. This is expressed in the absence of a suppuration reaction in the wound bottom, a significant decrease in the severity of morphological signs of inflammation, a smaller thickness of the scab, lack of microbe, accelerated epithelialization of the lesion, and a good development of the granulation tissue. The

survival rate of animals with phytomineral sorbents was more than 65%, while in the control one it was less than 35%.

When studying the dynamics of the most important rat blood parameters during wound healing, it was established that the content of immature neutrophils in the regeneration phase in animals using PMS in the form of powder was lower by a factor of 2 compared to the control group and group of animals using the drug Dexpanthenol; the concentration of total protein in the blood of animals in experimental groups with the use of the preparation Dexpanthenol and PMS based on montmorillonite in the forms of powder and gel were significantly lower than the control one by 18-25% on the third day of the experiment; transamination enzyme activity in animals where treatment was performed with the investigational drug was significantly lower than the control group by 25% (for ALT) and by 31% (for AST); the concentration of iron ions in blood plasma in animals where wounds were applied with dry and gel forms of PMS was significantly lower by a factor of 1.5 compared with the corresponding values in the control group.

The results of investigation of the local inflammatory, purulent and regenerative processes in the wound exudate showed that the concentration of the total protein in the group of rats that treated PMS on the basis of montmorillonite in the form of powder was significantly lower than the control – by 26% in the first day after the start of treatment, by 52 % – in the second, on the third day, exudation in animals of this group stopped. In the remaining experimental groups, exudation lasted up to 5 days.

Conflicts of interest

The authors have no conflict of interest to declare.

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**TADALAFIL AS AN AGENT OF PHARMACOLOGICAL
PRECONDITIONING IN ISCHEMIC – REPERFUSION BRAIN
INJURY**

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Corresponding author, e-mail: m.olga91@mail.ru**Abstract**

Introduction: Ischemic stroke or cerebral infarction is the main pathology among severe forms of vascular lesions of the brain. One of the more effective non-medicamental methods of treatment is pharmacological preconditioning. Pharmacological neuroprotection is one of the treatment areas to reduce the damage in ischemic stroke and other modifications of brain ischemia. Therefore, the development and introduction of new pharmacological agents that can reduce the degree of ischemic-reperfusion injury of the brain, remains one of the major challenges of modern medicine. The most promising to explore, from our point of view, is a PDE-5 inhibitor.

Goal: Improving the efficiency of pharmacological cerebroprotective with pharmacological preconditioning of the PDE-5 inhibitor (tadalafil) in comparison with recombinant erythropoietin («Epocrin») and a neuroprotectant "Gliatilin".

Materials and methods: In the pilot study used an integrated approach to the study of neuroprotective effects of pharmacological preconditioning in animals with ischemia-reperfusion brain damage in four-vascular total ischemia of the brain. In the complex of methods included evaluation of neurological deficit, behavioral status, level of markers of brain damage S100b and NSE, morphometry. To compare the efficacy of tadalafil (1 mg/kg) in the experiment used recombinant erythropoietin «Epocrin» (50 IU/kg) and the neuroprotectant "Gliatilin" (85.7 mg/kg).

Results and discussion: Prophylactic intraperitoneal administration of PDE-5 inhibitor, tadalafil (1 mg/kg) exerted cerebroprotective effect in modeling of ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8 ± 0.21 points), compared with the control group (of 2.05 ± 0.49 points); increase in the number of stands at 1.7 times and 2.2 hanging times; not a big increase in overall activity, patterns of movement, maximum speed, total distance increased 1.5 times, decrease rest time by 1.2 times; the reduction in the concentration of damage markers S100b 3.5 times and the NSE in 2 times. A number of distinctive characteristics the morphometric study, as well as a set of symptoms, manifestations of behavioral reactions confirm the fact of cerebroprotective properties of tadalafil in comparison with the control group animals.

Conclusions: the conducted research showed cerebroprotective property of an inhibitor of phosphodiesterase type 5, tadalafil. The results of the study clearly indicate the prospects of its use in vascular pathology of the brain.

Key words: pharmacological preconditioning, ischemia of the brain, tadalafil.

Introduction

Ischemic stroke or cerebral infarction is the main pathology among severe forms of vascular lesions of the brain [1, 2]. In Russia, this pathology ranks first as the cause of disability, and in the

structure of total mortality is second [3]. The problem of timely pathogenetic treatment of this pathology is crucial due to the widespread prevalence, high mortality, disability and social exclusion suffered its patients [4]. To date, there are

two approaches to solve this problem: the use of medication (pharmacological) medications and non-pharmacological methods affecting the mobilization of domestic genetically-determined protective mechanisms, evolutionary acquired. With therapy achieves the restoration of blood flow in the ischemic region. Such therapies include: the destruction of blood clots, anticoagulant and antiplatelet therapy and hemodilution (using low molecular weight dextrans). To date, there is a need for search and development of new effective cerebroprotectors that could improve the course of disease, prevent the development of neurodegenerative processes in the brain, it is more useful to provide emergency assistance, reduce mortality, reduce the duration of the acute period, to reduce the disabling effects of the disease. One of the more effective non-medicamental methods is pharmacological preconditioning [5, 6]. Pharmacological neuroprotection is one of the treatment areas to reduce the damage in ischemic stroke and other modifications of brain ischemia. Therefore, the development and introduction of new pharmacological agents that can reduce the degree of ischemic-reperfusion injury of the brain, remains one of the major challenges of modern medicine. The most promising to explore, from our point of view, is a PDE-5 inhibitor.

Currently in the neuro emergency care requires a constant introduction of new methods for the early detection and prevention of secondary damaging factors, including and stroke. The latest guidelines of American Heart Association / American Stroke Association and the Institute of cerebrovascular pathology and stroke are not the proposed methods of predicting the course of AI. According to a leading international clinical recommendations on the treatment and prevention of the consequences of stroke and to data obtained by the majority of large randomized trials to study the efficacy of cerebroprotective drugs, there was no neuroprotective regimen of drugs, which showed significant improvement of outcome of stroke. Now known for a large number of mechanisms that forms the basis preconditioning: open launcher agents, substances – mediators, a number of final targets [5, 6, 7]. One of the many branches of the study of agents that have a pharmacological preconditioning is the impact on the path of NO – cGMP – protein kinase G (PK –

G) [8]. Recently, a new potential therapeutic strategy for inhibitor of phosphodiesterase type 5 that it has a protective effect on the brain neurogenic, neurodegenerative diseases and memory loss [9, 10, 11]. Currently, no drugs based on iPDE – 5 approved for clinical use in stroke, therefore, it seems appropriate to study the presence of cerebroprotective properties of iPDE – 5 and their effectiveness.

Goal: Improving the efficiency of pharmacological cerebroprotective with pharmacological preconditioning of the PDE-5 inhibitor (tadalafil) in comparison with recombinant erythropoietin («Epocrin») and a neuroprotectant "Gliatilin".

Materials and methods

The study was performed on 410 weinbrenner adult male rats line "Wistar" 5-6 months of age weighing 220-250 g. the animals comply with all the rules of good laboratory practice in preclinical studies in Russia. Animals were kept under standard conditions, corresponding to sanitary regulations (No. 1045-73), approved by the USSR health Ministry 06.04.73 on the device, equipment and maintenance of experimental biological clinics (vivariums) and GOST R 53434-2009. Vivisection was carried out according to ethical principles for the treatment of laboratory animals "of the European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes. CETS No.123".

Methods of research in vivo. All the experiments were performed in accordance with methodological recommendations on preclinical study of drugs for treatment of disorders of cerebral circulation and migraine [1, 4]. In this study we used several methods reproduce the experimental models of cerebral ischemia: the "two-vascular" and "four-vascular". Ischemic stroke ischemic was simulated by a temporary occlusion of two common carotid arteries with subsequent reperfusion or by coagulation of two vertebral carotid artery with temporary occlusion of two common carotid arteries with subsequent reperfusion. Depending on the purpose of the study the duration of episodes of ischemia-reperfusion can vary [12]. Evaluation of adequacy of implementation of occlusion of the arteries supplying the brain, was carried out using the recording of the electrical activity of the brain of the animal and the level of microcirculation in the

sclera of the eye using laser Doppler flowmetry on the device Biopac Systems Inc. MP150 EEG100C and LDF 100C. The methods were implemented using the software AcqKnowledge 4.2. The criterion for correct execution of the techniques was the reduction of EEG amplitude and a decrease in the level of the microcirculation [13, 14, 15]. To assess the neurological status of rats used several methods:

1. *Point scale of evaluation of the McGraw stroke in the modification of I. V. Gannushkina* [13, 16]. Within the group of rats with signs of neurological deficit were divided into animals with mild, moderate and severe symptoms of neurological deficit. If the animal has several signs of neurological deficit, the scores are summed.

2. "Elevated cross maze" behavioral test to examine the activity, emotional state and level of anxiety in laboratory animals during the experiment [17]. In the experiment, was used to install the labyrinth of the firm Panlab Harvard Apparatus LE 846.

3. "Infrared activity monitor" – IR Actimeter allows you to test arbitrary locomotor activity, numbers and duration of episodes of getting up on his hind legs, stereotypical movements and exploratory behavior in the model of "perforated field" in terms of day and night illumination [17]. Is used to assess exploratory behavior. Were used in the experiment installation firm Panlab Harvard Apparatus LE 8825.

Methods in vitro. For more accurate results and correct interpretation of animal plasma is examined for two markers of brain damage – S100b and NSE. Determined by their concentration in the serum [18]. Morphometric study of histological [19, 20, 21] of brain slices was performed under a microscope MIKMED-6 (LOMO, Russia). The Protocol of the study. Justification of doses. At the beginning of the experiment, all animals were randomized according to the degree of resistance to hypoxia. The experiment involved animals with average resistance. Under the objectives of the study, we have developed a model of pathology. For this simulated local two-vascular IGM group of rats ($n = 10$) and total IGM four-vascular group of rats ($n = 10$). As the comparison drugs used recombinant erythropoietin «Epocrin» and neuroprotectant "Gliatilin". Tadalafil (Eli Lilly Vostok S. A., Switzerland) were administered once

intragastrically at a dose of 1 mg/kg for 60 min prior to the ischemia simulation. The selected dose corresponds to an average therapeutic dose for humans calculated by the formula interspecies transfer [8]. «Epocrin» – recombinant erythropoietin (FGUP GosNII OCHB, Saint-Petersburg, Russia) was administered once at a dose of 50 IU/kg intraperitoneally 30 minutes before the modeling of the IGM. The dose chosen in order to avoid stimulation of erythropoiesis and the presence of the protective effect is proven in many organs and tissues [22, 23, 24, 25, 26, 27]. "Gliatilin" (ITALFARMACO, S. p.A. Italy) was injected once intragastrically at a dose of 85.7 mg/kg in the first case, for 30 min prior to simulation of ischemia, the second after 30 min after. The selected dose corresponds to the dose calculated by the formula interspecies transfer of doses. Glibenclamide is a blocker of ATP-sensitive potassium channels ("Maninil", Berlin-Chemie AG), was injected once intragastrically at a dose of 5 mg/kg over 60 min. before simulation of the IGM [29].

The study Protocol included the following stages: modeling cerebral ischemia; evaluation of the level of the electroencephalogram of an animal and microcirculation by LDF; assessment of behavioral status (2 d) and neurological deficits at 1, 3, 7 and 14 days after modelling pathology; determining the presence of specific markers of brain damage S100b and NSE; removal of animals from the experiment and the taking of material for morphometric studies, analysis of blood for the presence of markers of brain damage S100b NSE. In the Protocol of study of protective action of iPDE -5, EPO and "Gliatilin" included the following groups of animals (each group 10 animals):

1. The group of intact animals.
2. The group of false-operated animals.
3. Group two-vascular model 4-min.
4. Group four-vascular model 3-min.
5. Group four-vascular model 4-min.
6. Group four-vascular model for 4,5-min.
7. Group four-vascular model 4-min + iFDE-5.
8. Group four-vascular model 4-min + EPO.
9. Group four-vascular model 4-min + "Gliatilin" (CI).
10. Group four-vascular model 4-min + "Gliatilin" (after CI).
11. Group four-vascular model 4-min + iFDE-5+ "Gliatilin".

12. Group glib + iFDE-5 + four-vascular model 4-min.

13. Group glib+ EPO + four-vascular model 4-min.

For all of the data was used descriptive statistics. The received data is checked for normality of distribution. Using the Shapiro-Wilk test have chosen the type of distribution. In the case of the normal distribution was calculated the average value M and standard error of the mean m. In cases of abnormal distribution were calculated median and quartile Me the scope of QR. Intergroup differences were analyzed by parametric (student's t-test) or nonparametric (Mann-Whitney test) methods, depending on the distribution type. Differences were determined at 0,05 level of significance. Statistical analysis was performed using software Statistica 10.0 [30, 32, 33].

Results and discussion

In the beginning of the experiment was developed a set of methods for quantitative evaluation of disorders in ischemic-reperfusion brain injury, as described earlier. The true criterion of the performed techniques was to reduce the amplitude of the EEG and the level of microcirculation in the sclera of the eye by LDF. Also was selected as the optimal model of pathology – four-vascular and experimentally matched the duration of the ischemic period of 4 min. This model of pathology was characterized by the development of neurological deficits (of

2.05 ± 0.49 points); reduction of the number of racks in 2 times and hanging 2.7 times roll "Elevated cross maze"; violation of behavioral status in the test of actimetry, which was manifested in the decline in overall activity in 2 times, reducing the number of patterns of movement in 2 times, reduce the maximum speed by 1.5 times, decrease of passing the total distance of 2.3 times the increase in leisure time in 1.7 times; the increase in the concentration of damage markers S100b 2.5 times and the NSE in 2 times; morphological changes: the presence of 90% hyperchromic neurons in the frontal lobes and the hippocampus. Depending on the research method for the control data received intact or false-operated animals.

Cerebroprotective effects of PDE-5 inhibitor, tadalafil. Neurological deficit in rats on the background correction pharmacological preconditioning tadalafil (1 mg/kg) was more mild symptoms compared to the group of animals with CI without drug administration. 3, 7, 14 days was still polutes right eye. In behavioral test, ECM group of rats with CI and correction tadalafil manifested itself more actively in comparison with the control group. When comparing the locomotor activity of animals in the control group with the group in the correction tadalafil in the test of actimetry for infrared activity monitor IR Actimeter the activity of rats with pre-administration of the drug is higher (figure 1).

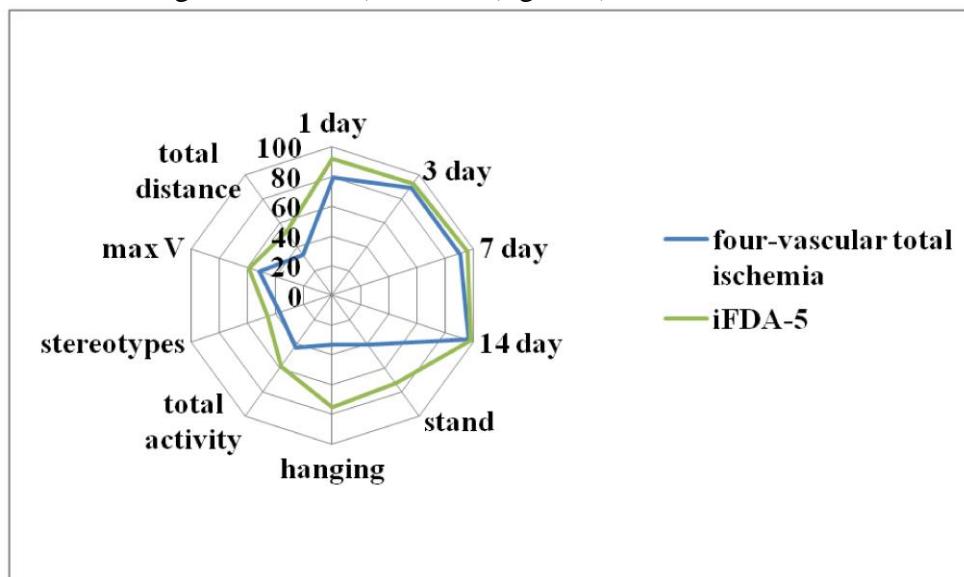


Fig. 1. Effect of tadalafil on indicators of activity of the animals in the modeling of cerebral ischemia (iFDE-5)

When analyzing the level of S100b and NSE in animals of the group with CI, with a

preliminary correction tadalafil observed a decrease in the levels of markers of damage even below the level of the control group (figure 2).

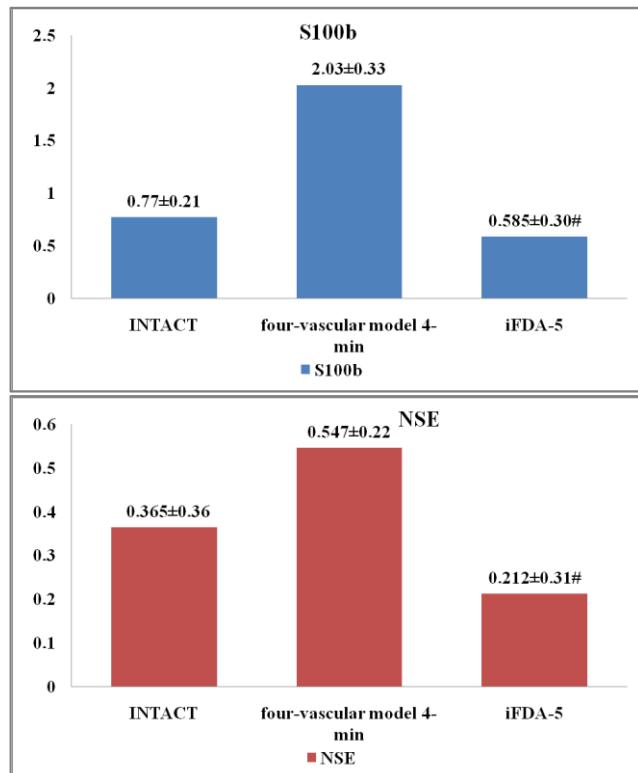


Fig. 2. Effect of tadalafil on the concentration of markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) ($M \pm m$; $n = 10$). Note – * $p < 0.05$, # – $p < 0.05$ in relation to intact rats

In brain slices of rats with a preliminary introduction tadalafil observed: hyperchromia neurons in the frontal lobes 43.4% and in the area of the hippocampus 90%; hypochromia neurons in the frontal lobes of 43.4% (in the area of the hypochromic hippocampal neurons were absent); neurons with two ravines in the frontal lobes of 13.2% and in the area of the hippocampus 10%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 3).

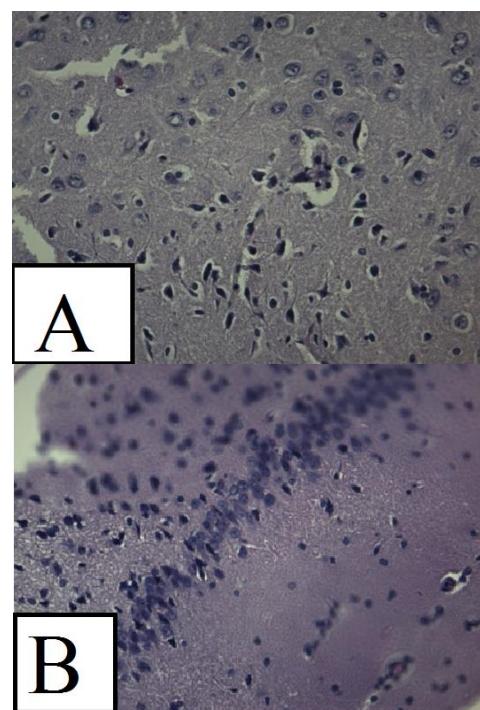


Fig. 3. Brain Slices rat CI and prior to the introduction of tadalafil

A- the frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400 Deposit.
hematoxylin+eosin

Cerebroprotective effects of recombinant erythropoietin «Epocrin». Neurological deficit in rats with pre-introduction "Epocrin" (50 IU/kg) were with mild symptoms, compared to control group animals. 3, 7, 14 day remained the floor ptosis right eye. In behavioral test, ECM group of rats with correction "Epocrin" proved to be active. When comparing the locomotor activity of animals in the control group with the group in the correction of "Apocrine" in the test of actimetry for infrared activity monitor IR Actimeter the activity of the rats with pre-infusion is much higher relative to the control group (figure 4).

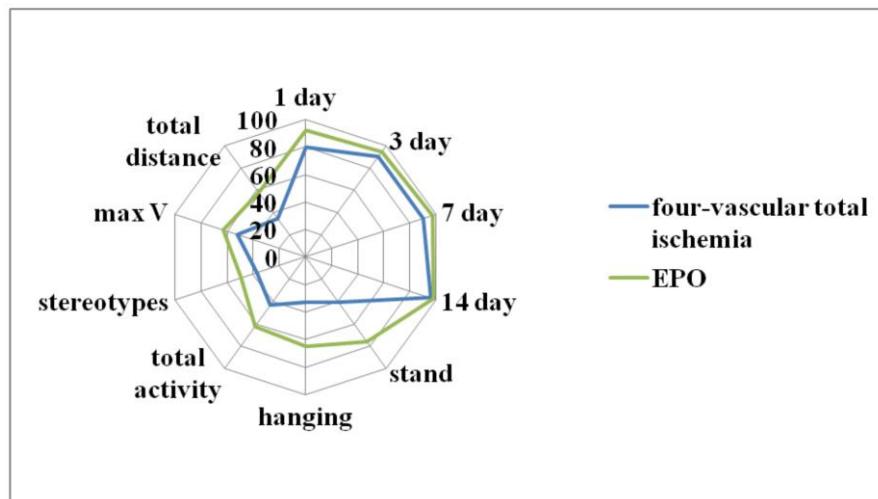


Fig. 4. The Effect of recombinant erythropoietin («Epocrin») on indicators of activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in animals of the group with CI, with a preliminary introduction of "Epocrin" observed the decrease in the concentration of markers of damage below the level of intact animals ($p > 0.05$) (figure 5).

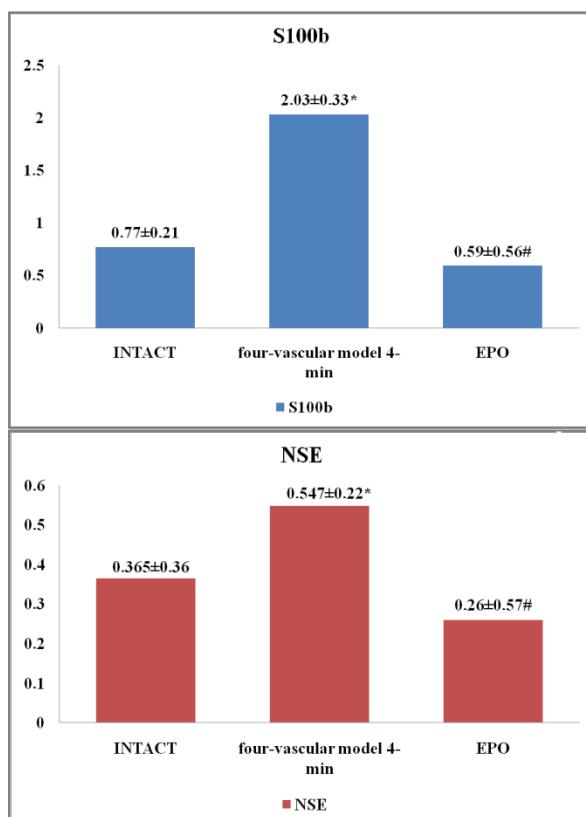


Fig. 5. Effect of recombinant erythropoietin («Epocrin») on markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) ($M \pm m$; $n = 10$). Note – * $p < 0.05$, # – $p < 0.05$ in relation to intact rats

In brain slices of rats with a pre-introduction "Epocrine" was also observed by hyperchromia neurons is 43.3% in the frontal lobes and 69.7% in the area of the hippocampus; neurons hypochromia – 43.4% in the frontal lobes and 20.3% in the area of the hippocampus; neurons with two ravines neurons and 13.3% in the frontal lobes and 10% in the area of the hippocampus. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 6).

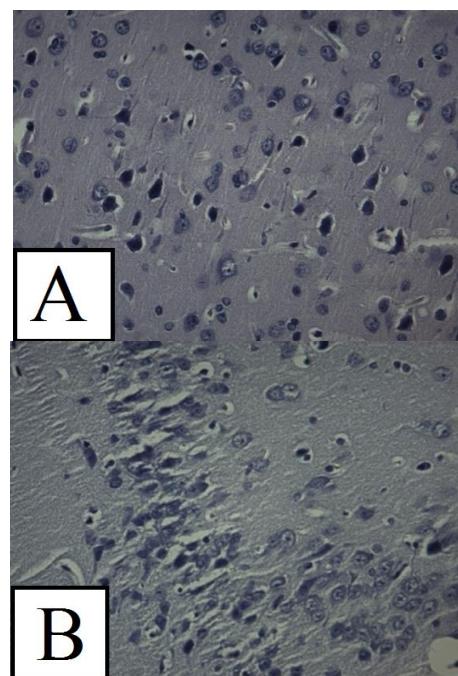


Fig. 6. Brain Slices rat IGM and prior to the introduction of "Epocrin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400 Deposit. hematoxylin+eosin

Cerebroprotective effects of the neuroprotectant "Gliatilin". Neurological deficit of the groups of rats as a prophylactic and therapeutic introduction "Gliatilin" (85.7 mg/kg) was with a medium degree of gravity. 3, 7, 14 days was preserved paralysis of hind left limb and remained the floor ptosis, ptosis of the right eye.

The group of rats with the introduction of the drug for medicinal purposes in the modeling of the pathology observed the same symptoms of neurological deficit except Manege movements. Visually the activity of rats in these experimental groups did not differ from the control group as in the test ECM and test altimetry (figure 7).

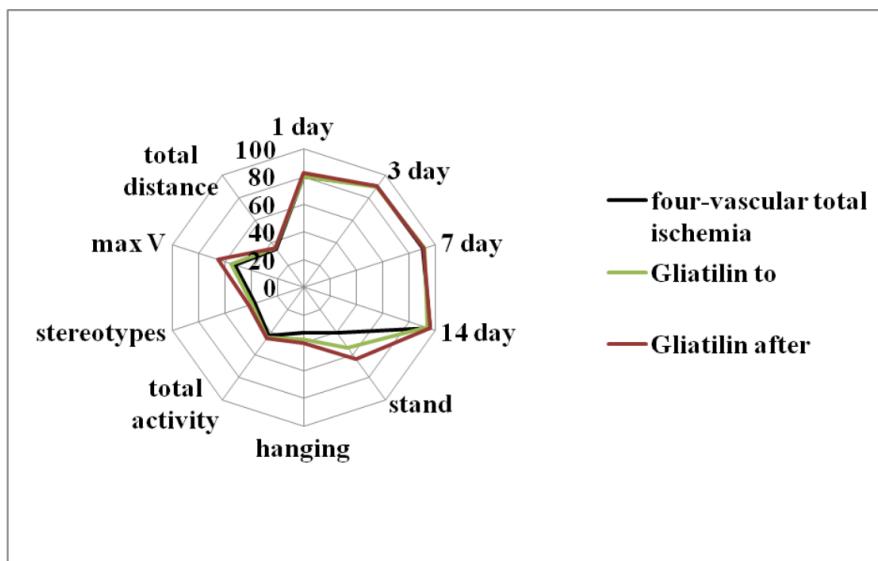


Fig. 7. Influence of neuroprotectant "Gliatilin" on indicators of activity of animals in modeling cerebral ischemia

The level of S100b and NSE in the animals of group a prophylactic introduction of "Gliatilin" above the level of the control group. The level of

markers of damage a group of rats with a therapeutic drug is lower than in the control group (figure 8).

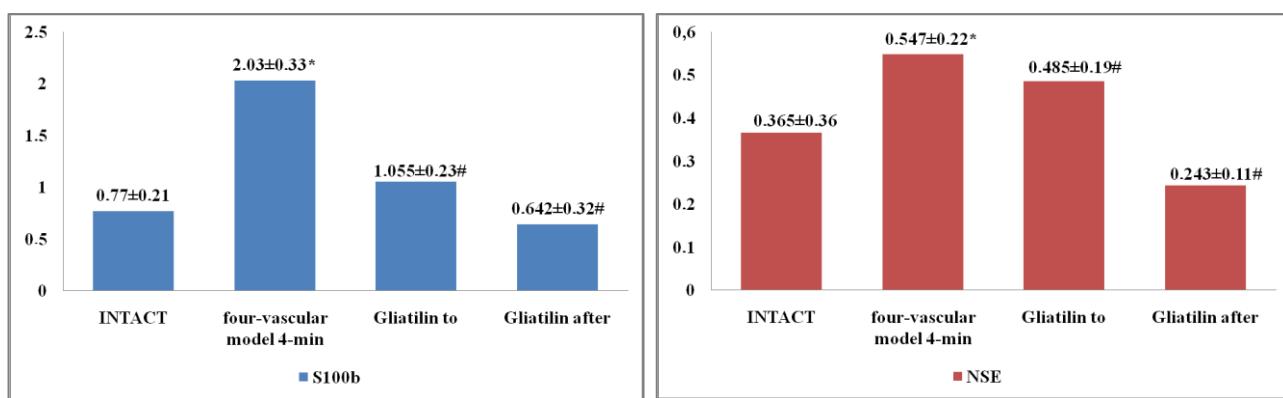


Fig. 8. Influence of neuroprotectant "Gliatilin" markers of brain damage in the plasma of animals in 3 days (S100b- μ g/l; NSE – ng/ml) ($M \pm m$; n = 10).

Note – * – p < 0.05, # – p < 0.05 in relation to intact rats

In brain slices of rats in the correction of "Gliatilin" both preventive and therapeutic purposes observed: hyperchromia neurons were 92% and 88% in the frontal lobes, 89% and 84% in the area of the hippocampus; neurons hypochromia – 8% and 12% in the frontal lobes, in the area of the hypochromic hippocampal

neurons were absent; neurons with two ravines neurons in the frontal lobes absent, in the area of the hippocampus, their number corresponded to 11% and 16%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figures 9, 10).

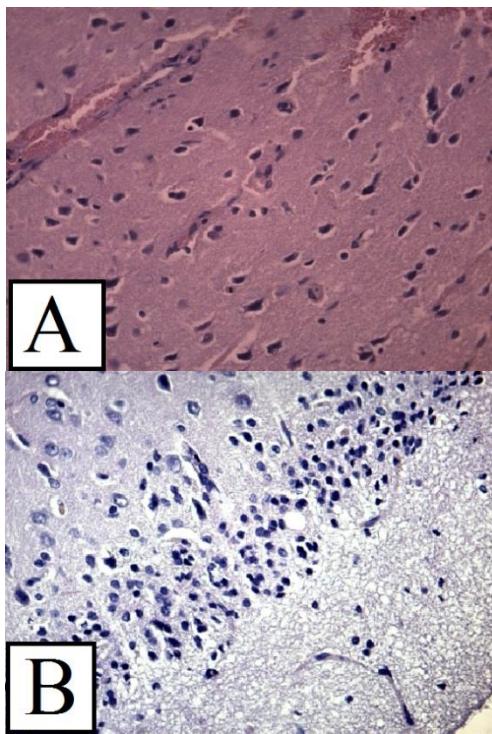


Fig. 9. Brain Slices of a rat a prophylactic introduction of "Gliatilin":

A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus X 400, hematoxylin+eosin

Additive effect of combined use of PDE-5 inhibitor, tadalafil and neuroprotectant "Gliatilin". Neurological deficit in rats, "iFDE-5+CI+Gliatilin" (1 mg/kg; 85,7 mg/kg) were with mild symptoms. 3, 7, 14 days was preserved the floor ptosis right eye. In behavioral test, ECM group of rats", iFDE-5+CI+Gliatilin" were active, compared with the group CI. Compared with

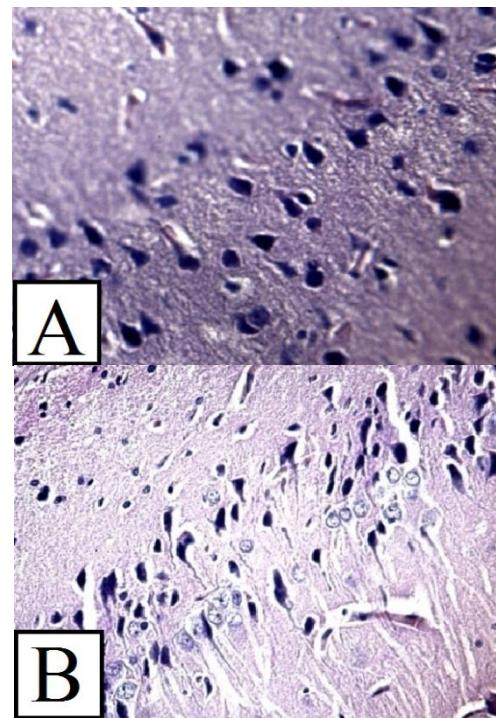


Fig. 10. Brain Slices of the rat with a medical introduction of the "Gliatilin":

A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

groups of rats in monotherapy, this group has a significantly greater activity. Locomotor activity of rats ", iFDE-5+CI+Gliatilin" in the test of actimetry for infrared activity monitor IR Actimeter higher than in rats with BL a group of rats in monotherapy (figure 11).

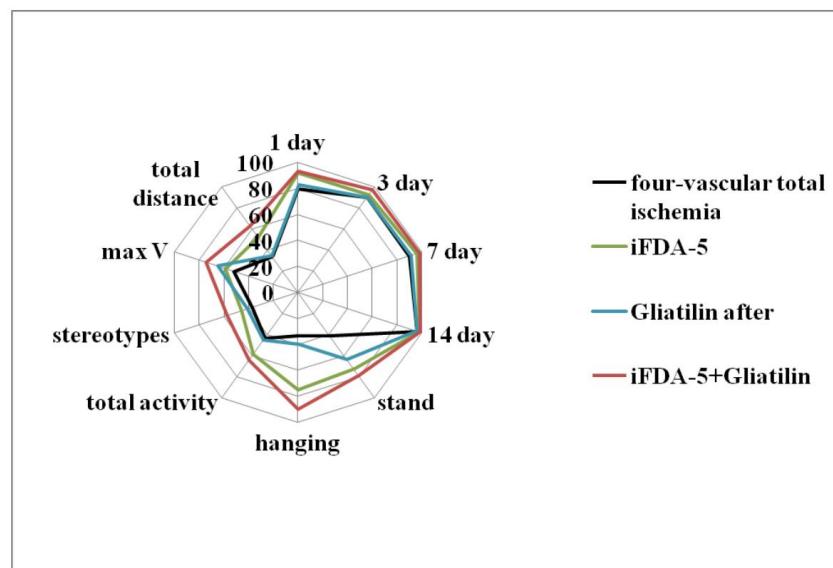


Fig. 11. The Effect of combination of tadalafil (iPDE -5)and neuroprotectant "Gliatilin" on indicators of activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in the animals of group " iPDE -5+IGM+Gliatilin" observed the decrease in the concentration of markers of damage below the level of intact animals ($p > 0.05$) (figure 12).

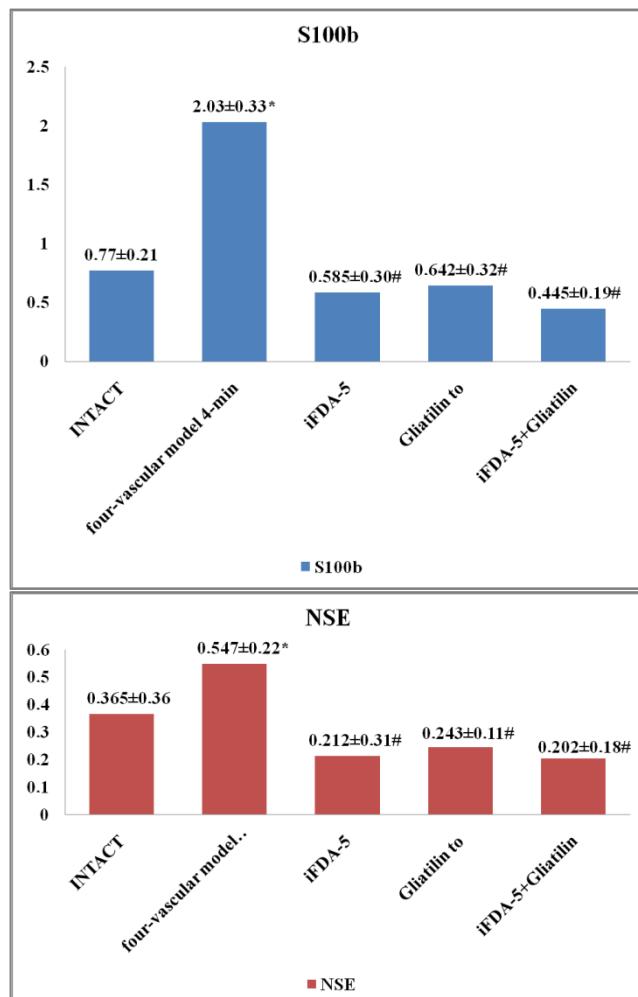


Fig. 12. Effect of combination of tadalafil (iPDE-5) and neuroprotectant "Gliatilin" markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) (M ± m; n = 10).

Note – * $p < 0.05$, # – $p < 0.05$ in relation to intact rats

In brain slices of rats ", iFDE-5+CI+Gliatilin" observed: hyperchromia neurons and 32% in the frontal lobes and 70% in the area of the hippocampus; hypochromia neurons is 54.8% in the frontal lobes and 15% in the area of the hippocampus; neurons with two ravines

neurons of 13.2% in the frontal lobes and 15% in the area of the hippocampus. Also recorded pericellular and perivascular edema, hyperemia of the capillaries (figure 13).

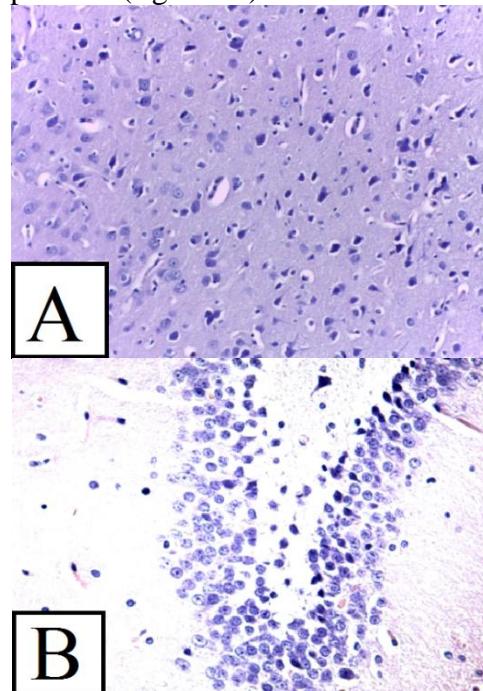


Fig. 13. The brain Slices of the rat group "iPDE-5+IGM+Gliatilin":
A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

The contribution of K⁺ATP-channels to the implementation of the cerebroprotective effects of pharmacological preconditioning. Neurological deficit in animal groups "Glib + iFDE-5 + CI" and "Glib + EPO + CI" (5 mg/kg) were medium severity, which was not statistically different from the group with only IGM. 3, 7, 14 days was preserved paralysis of left rear leg and the floor ptosis /ptosis of the right eye. In the behavioral test ECM behavior of groups of rats "Glib + iFDE-5 + CI", "Glib + EPO + CI" was characterized by decreased horizontal activity, a significant reduction in vertical activity. The activity of rats between these experimental groups are similar, which confirms the cancellation preconditioning properties of drugs due to prior administration of blocker of K⁺ATP channels (figure 14).

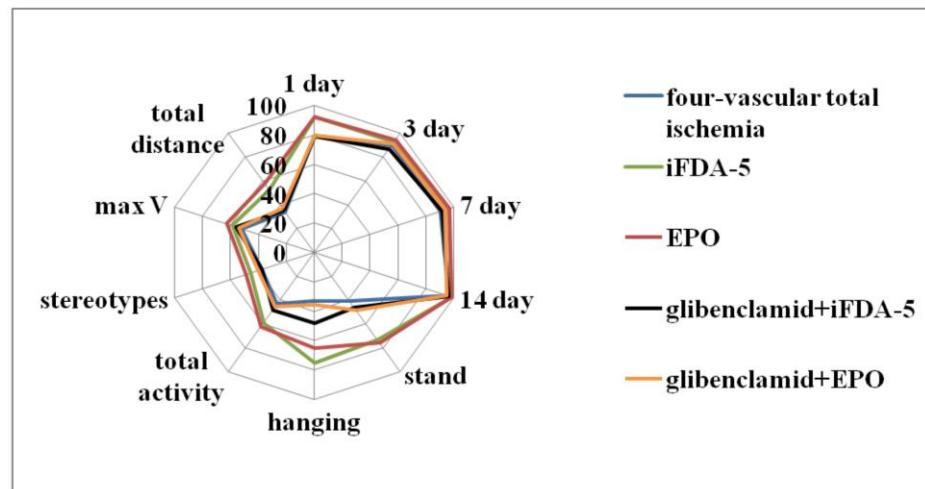


Fig. 14. Effect of tadalafil (iPDE-5), "Epocrin" with the prior introduction of glibenclamid on the activity of animals in modeling cerebral ischemia

When analyzing the level of S100b and NSE in the animals of group "Glib + iFDE-5 + CI" and "Glib + EPO + CI" was observed by increasing the concentration of markers of damage. Their

concentration is statistically significantly different from concentration in intact rats ($p < 0.05$) (figure 15).

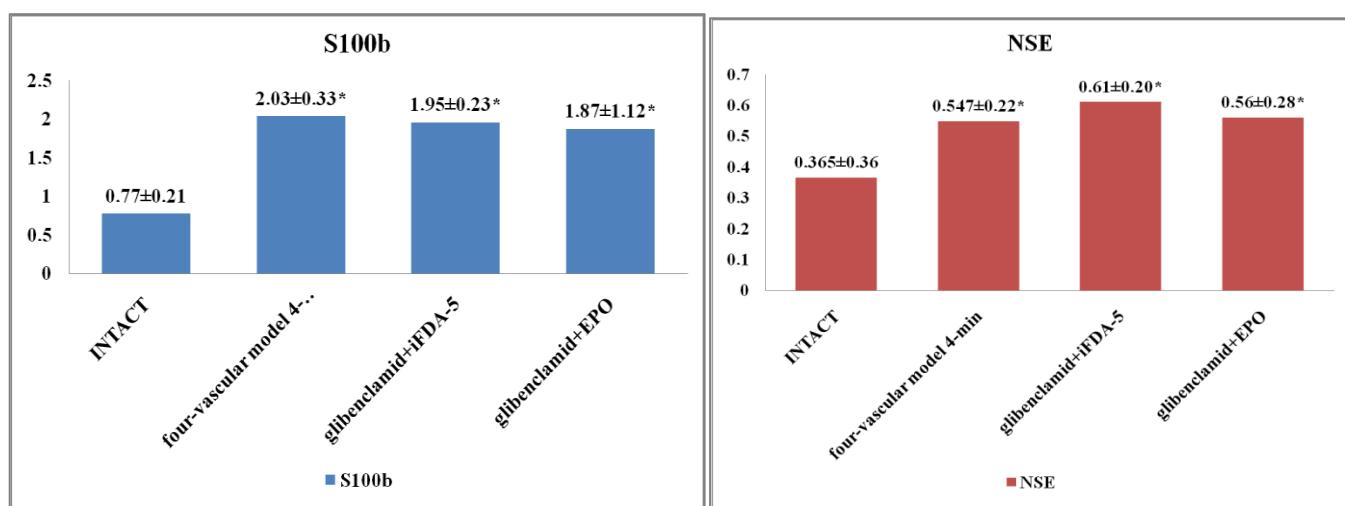


Fig. 15. Effect of tadalafil (iPDE-5), "Epocrin" with the prior introduction of glibenclamid on the concentration of markers of brain damage in the plasma of animals in 3 days (S100b-g/l; NSE – ng/ml) ($M \pm m$; $n=10$).

Note – * $p < 0.05$, # – $p < 0.05$ in relation to intact rats

Histological preparations of the brain of rats of the group "Glib + EPO + CI" similar to the preparations of the group "Glib + iFDE-5 + CI". These groups were observed scattered neurons, the neurons hyperchromia to 83.6% and 70% in the frontal lobes, 63.4% and 80% in the area of the hippocampus; hypochromia neurons to 10% and 20% in the frontal lobes, 26.6% and 10% in the area of the hippocampus; neurons with two ravines neurons in the frontal lobes absent, in the

area of the hippocampus, their number corresponded to 10% and 10%. Also recorded pericellular and perivascular edema, hyperemia of the capillaries.

Based on the measurements obtained after morphometric studies and examination of brain slices, it is clear that glibenclamid preconditioners cancels the action of tadalafil and recombinant erythropoietin (figures 16, 17).

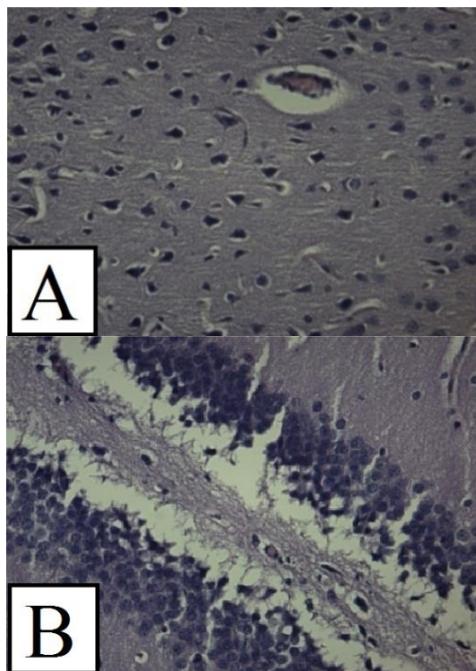


Fig. 16. Brain Slices of the rat group "Glib +iFDE-5+ CI":

A – frontal lobe, X 400, env. hematoxylin+eosin;
B – hippocampus, X 400, env. hematoxylin+eosin

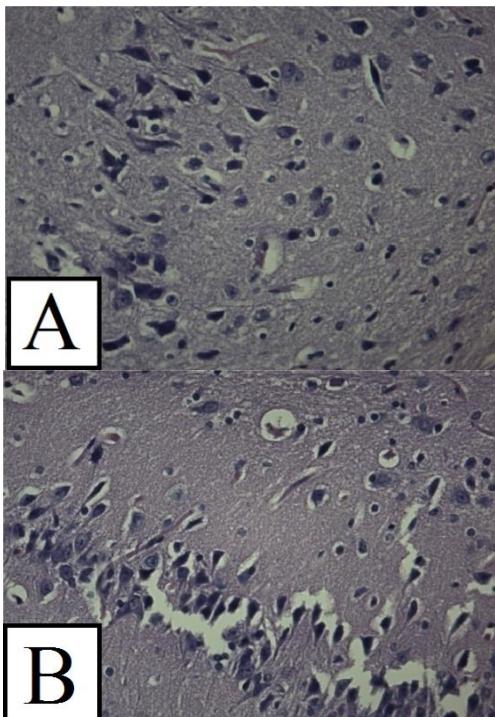


Fig. 17. Brain Slices of the rat group "Glib +EPO+ CI":

A – frontal lobe, X 400, hematoxylin+eosin;
B – hippocampus, X 400, hematoxylin+eosin

In the result of the study was chosen as the optimal model to study four-vascular model 4-minute ischemia of the brain with the justification of a temporary simulation mode.

A single administration of tadalafil (1 mg/kg) and "Epocrin" (50 IU/kg) led to rapid recovery of EEG amplitude after the ischemic period, preserving the electrophysiological activity of the retina, improve behavioral status, reducing the level of neurological deficit, markers of brain damage S100b and NSE, to increase the number of hypochromic and presence of two nuclei in neuron in histological sections of brain.

Prophylactic administration of a neuroprotectant "Gliatilin" (85.7 mg/kg) did not produce positive results. The performance of rats in this group did not differ from indicators of control CI ($p > 0.05$).

Concomitant use of prophylactic administration of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) has an additive cerebroprotective effect, which is manifested in the improvement of all criteria for the integrated assessment of this pathology.

Prior administration of glibenclamid (5 mg/kg) neutralized the positive effects tadalafil (1 mg/kg) and "Epocrin" (50 IU/kg) ischemic preconditioning, confirming the implementation of cerebroprotective by preconditioning, with the participation of ATP-sensitive potassium channels.

For visualization of the obtained data was constructed charts on indicators of behavioral status and neurological deficits of the animals (as a percentage) calculated area of each shape, and dynamics of changes in markers of brain damage (figure 18-23). The smaller the area of the figure of the group, the harder the degree of ischemia of the rat brain (figure 23).

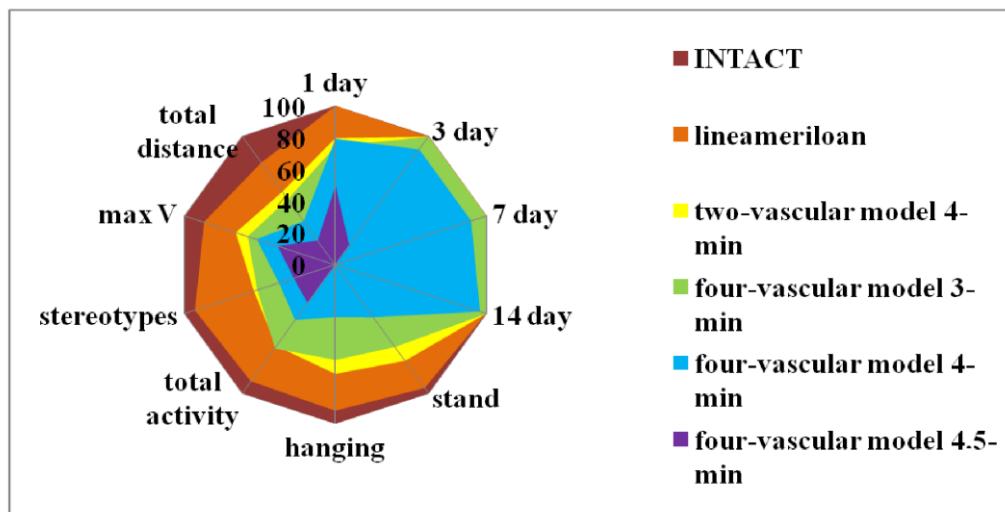


Fig. 18. Impact of duration and severity of the ischemic episode on the indicators of activity of animals in the experiment

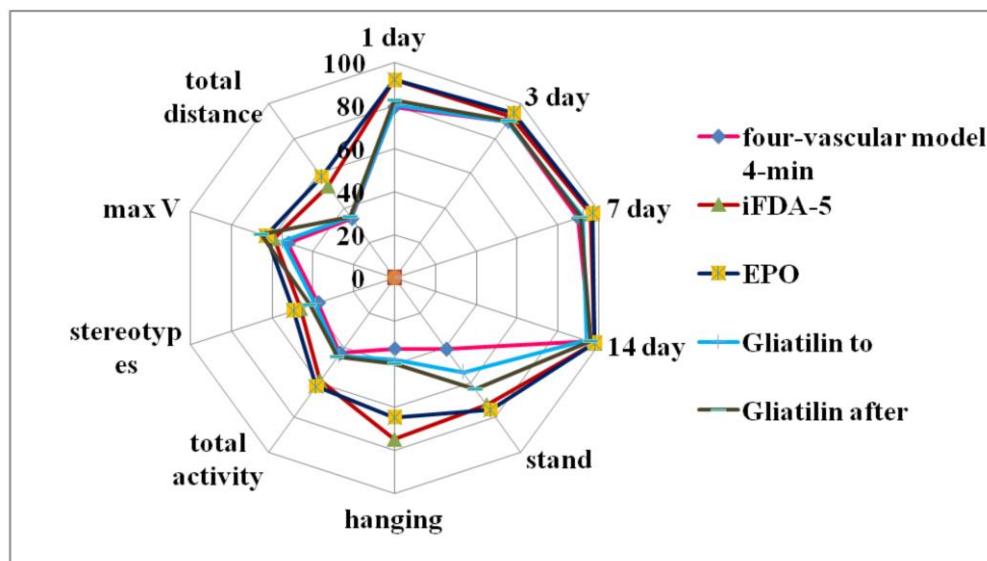


Fig. 19. Influence of used drugs on the activity rate of animals with cerebral ischemia

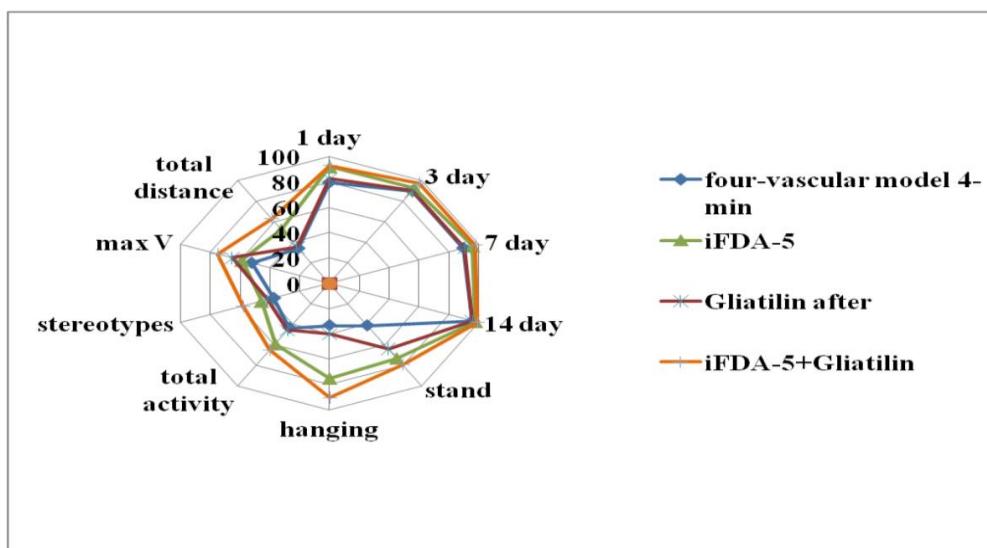


Fig. 20. Influence of used drugs on the activity rate of animals with cerebral ischemia

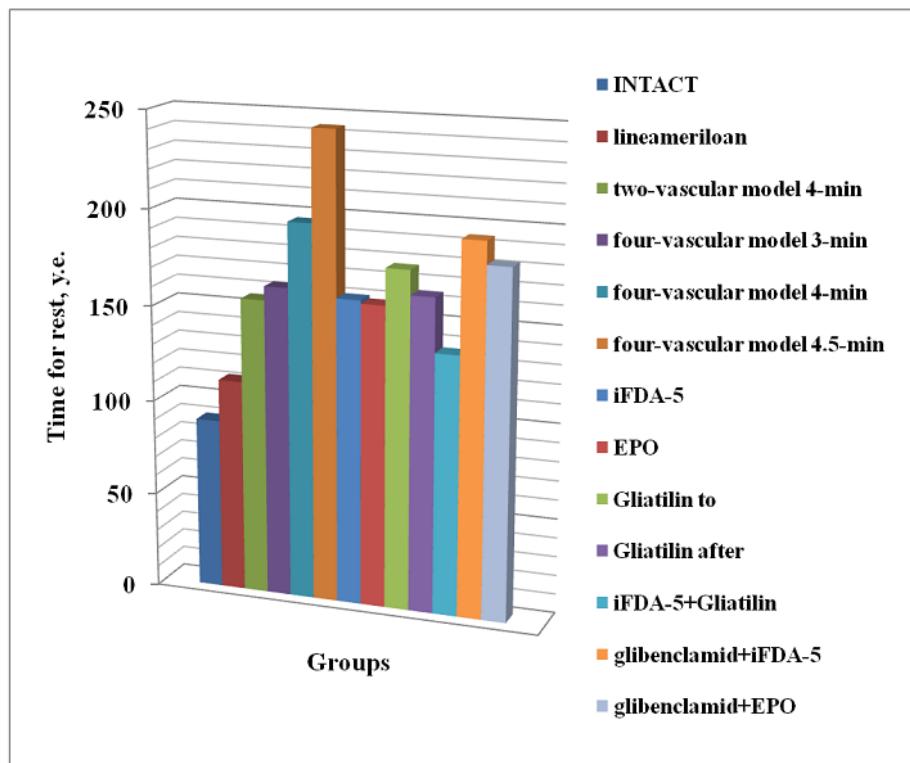


Fig. 21. Influence of used drugs on the duration of stay of animals in the test of actimetry

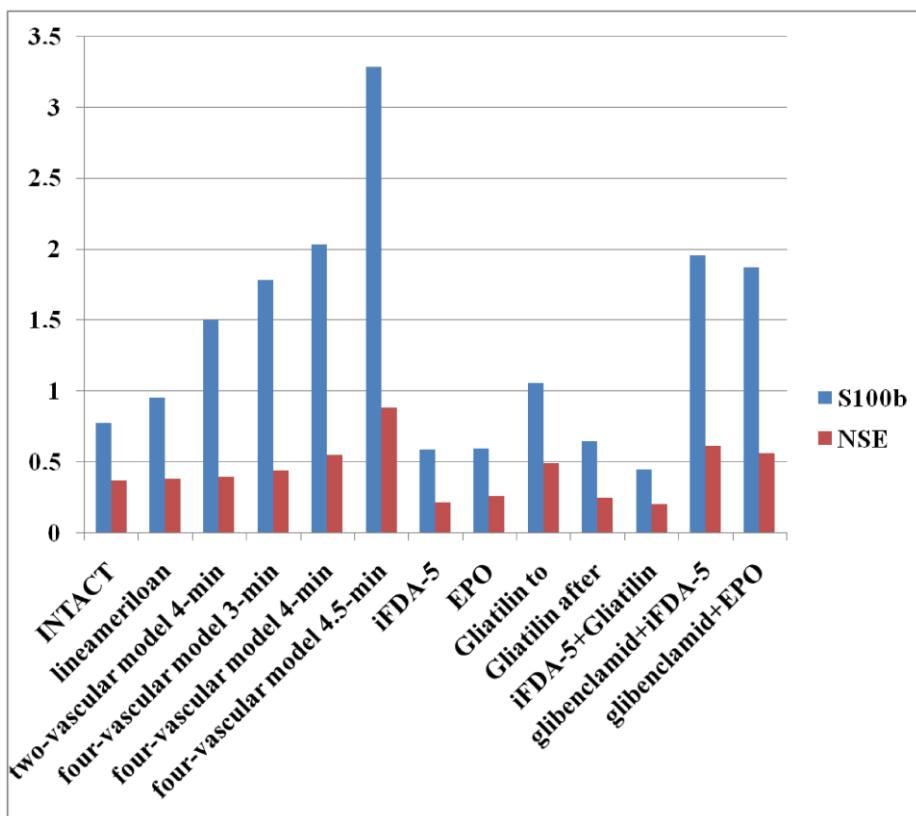


Fig. 22. Influence of used drugs on the level of specific markers of damage of the rat brain S100b and NSE

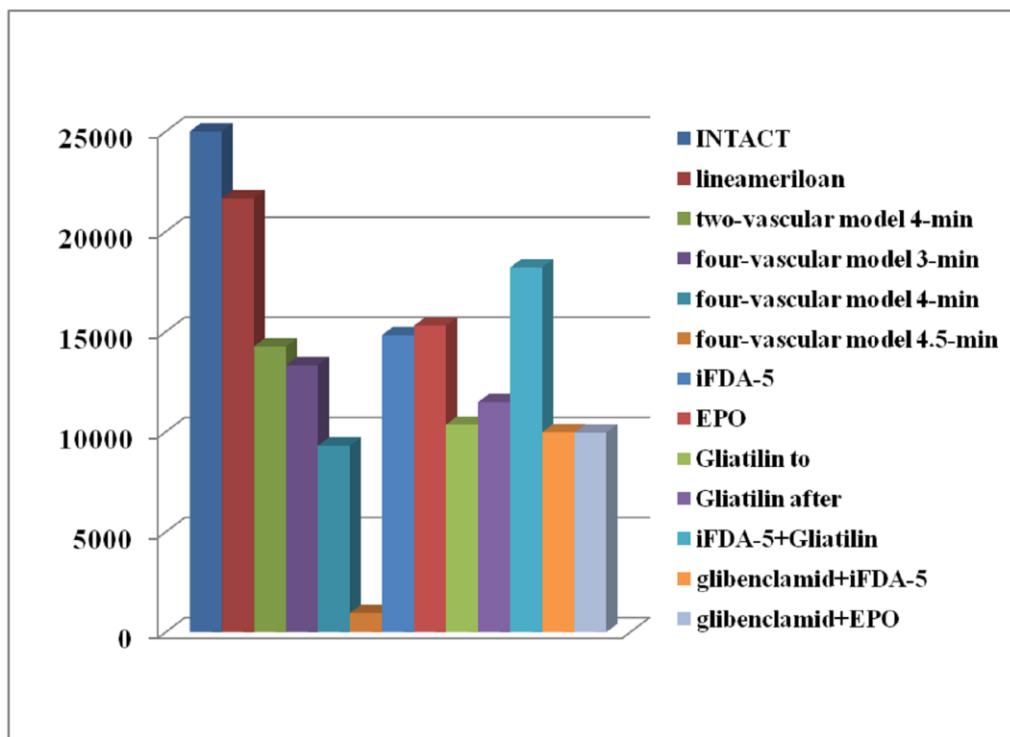


Fig. 23. Area values radar chart of the experimental groups

The received results convincingly testify to the long term development of pharmacological methods and approaches of correction of ischemic brain injury based on triggering mechanism of pharmacological preconditioning. To do this, but recombinant erythropoietin can be used inhibitor of phosphodiesterase type 5.

Conclusions

1. Four-vascular model the 4-minute model of brain pathology in rats was characterized by the development of neurological deficits (of 2.05 ± 0.49 points); a decrease in the number of racks in 2 times and hanging 2.7 times roll "Elevated cross maze"; violation of behavioral status in the test of actimetry, which was manifested in the decline in overall activity in 2 times, reducing the number of patterns of movement in 2 times, reduce the maximum speed by 1.5 times, decrease of passing the total distance of 2.3 times the increase in leisure time is 1.7 times; the increase in the concentration of damage markers S100b 2.5 times and the NSE in 2 times; morphological changes: the presence of 90% hyperchromic neurons in the frontal lobes and the hippocampus.

2. Prophylactic intraperitoneal administration (60 min) of PDE-5 inhibitor, tadalafil (1 mg/kg) exerted cerebroprotective effect in modeling of

ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8 ± 0.21 points), compared with the control group (of 2.05 ± 0.49 points); increase in the number of stands at 1.7 times and 2.2 hanging times; not a big increase in overall activity, patterns of movement, maximum speed, total distance increased 1.5 times, decrease rest time by 1.2 times; the reduction in the concentration of damage markers S100b 3.5 times and the NSE in 2 times. A number of distinctive characteristics the morphometric study, as well as a set of symptoms, manifestations of behavioral reactions confirm the fact of cerebroprotective properties of tadalafil in comparison with the control group animals.

3. Prophylactic intraperitoneal administration of recombinant erythropoietin «Epocrin» (50 IU/kg) exerted cerebroprotective effect in modeling of ischemia-reperfusion, expressed in reducing the severity of neurological deficit (0.8 ± 0.21 points) compared with the control group (of 2.05 ± 0.49 points); increase in the number of racks and hanging 2 times; not a big increase in overall activity, patterns of movement, maximum velocity, an increase in the total distance of 1.7 times, reduction of time of stay 1.2 times; the reduction in the concentration of damage markers S100b in 3.3 times and the NSE in 2 times. Morphometry

confirmed the neuroprotection "Epocrin" the brain of the rats.

4. The use of intraperitoneal prophylactic neuroprotectant "Gliatilin" (85.7 mg/kg) had a weak cerebroprotective action, which is expressed in the acceleration of recovery of neurological deficit (2.0 ± 0.46 points); no significant increase in the number of racks and hanging, overall activity, patterns of movement, maximum speed, total distance and decrease rest time; not a significant prevention of the increase of the values of neuron specific enolase and protein S100b in the blood serum. Therapeutic use "Gliatilin" (85.7 mg/kg) exerted a more pronounced cerebroprotective action compared to the prophylactic administration of the drug, which is manifested in the acceleration of recovery of neurological deficit (1.75 ± 0.13 points) and locomotor activity compared with the effects of prophylactic purpose. Morphometry in the application of "Gliatilin" as in prophylactic and therapeutic purposes show a lower neuroprotective response in comparison with tadalafil.

5. Concomitant use of prophylactic administration of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) has an additive cerebroprotective action, which is expressed in the presence of mild neurological deficit (0.55 ± 0.07 points); increase in the number of racks in 2 times and hanging 2.6 times; an increase in overall activity and patterns of movement by 1.5 times, the maximum speed of 1.4 times, an increase in the total distance in 2 times, decrease rest time to 1.5 times; approximation of values of neuron specific enolase and protein S100b in the blood serum to indicators of intact animals. Morphometry of combined use of tadalafil (1 mg/kg) and neuroprotectant "Gliatilin" (85.7 mg/kg) significantly increase the resistance of rat brain to ischemia-reperfusion injury compared with the monotherapy.

6. Blockade of K⁺ATP- channels glibenclamid (5 mg/kg) removes the effects of pharmacological preconditioning of the PDE-5 inhibitor and tadalafil does not affect neuroprotection mediated "Gliatilin".

Conflicts of interest

The authors have no conflict of interest to declare.

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PRECLINICAL STUDY OF PHARMACOLOGICAL ACTIVITY OF ENTEROSORBENTE ON THE BASIS OF MONTMORILLONITE

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Abstract

Introduction: At present, enterosorbents based on mineral raw materials are in high demand among the population. However, there are no enterosorbents on the Russian pharmaceutical market on the basis of domestic mineral raw materials.

Objectives: to study the pharmacological activity of enterosorbent based on montmorillonite of Russian origin under experimental conditions.

Methods: The methodological approach was based on the implementation of a complex of theoretical, pharmacological, toxicological, histological, biochemical, statistical methods. Models of experimental diarrhea, acute and toxic liver damage, acute experimental pancreatitis were selected.

Results and discussion: Enterosorbent based on montmorillonite Crim_04 has a dose-dependent antidiarrhoeal effect, which is manifested in an increase in the time of onset of diarrhea from 50.4% to 82.6% with various models of diarrhea, a reduction in the number of defecations from 50.4% to 64.4% liquid in them. Enterosorbent on the basis of montmorillonite has a high sorption activity to *E.coli* enterotoxin, inhibiting the outflow of fluid into the luminal cavity by 95.1%. In addition, the use of enterosorbent Crim_04 significantly improves biochemical indices in the blood serum of rats when modeling acute and chronic liver damage and acute pancreatitis.

Conclusion: The enterosorbent under the Crim_04 cipher has a dose-dependent anti-diarrhea, detoxification activity, high sorption activity for *E.coli* enterotoxin, high therapeutic efficacy in experimental pancreatitis, most pronounced at a dose of 3320 mg / kg. It can be recommended for further complex toxicological studies and clinical trials.

Keywords: enterosorbents, montmorillonite, preclinical studies, diarrhea, intoxication.

Introduction

The pathological conditions associated with the syndrome of endogenous intoxication, as well as exogenous intoxications with salts of heavy metals, toxins of fungi and bacteria are extremely common today. This is due to the growth of

atmospheric and aqueous pollutants, contamination of food and drinking water by bacterial and endotoxic agents [1, 2]. In Russia, about 90 thousand poisonings are annually registered [3]. One of the most common diseases, having water or food origin, is diarrhea.

According to WHO, diarrhea is one of the leading causes of death in the world, ranking 8th in the frequency of causes of death. In 2015, 1.39 million people died of diarrheal diseases, most of them children. In 2013, diarrhea was the second most frequent cause of death of children. Annually more than 1.7 billion cases of diarrhea are registered in the world. These statistics relate mainly to countries with low and medium-low incomes [4, 5]. The syndrome of endogenous intoxication is also accompanied by exogenous intoxications and diseases of internal organs, for example hepatitis, acute pancreatitis [6, 7, 8].

One of the most common, effective and accessible methods of detoxification of the body is the method of enterosorption [9, 10, 11, 12]. Of particular interest in this regard is mineral raw materials, in particular montmorillonite. Montmorillonite, due to its properties [13, 14, 15], has an antacid [16], antidiarrhoeal [17, 18, 19], cytotoxicoprotective [20], anti-inflammatory and anti-cytokine [21], high sorption activity against bacterial toxins [22, 23, 24, 25], salts of heavy metals [26], aflatoxin [24, 27], herbicides [28] for oral administration. Enriched with silver nanoparticles, montmorillonite has a bactericidal effect [22, 23, 24]. The structure, physical and chemical properties, mechanisms of sorption of toxins and xenobiotics on the surface of montmorillonite have been studied quite thoroughly [13, 29, 30].

Enterosorbent preparations based on mineral raw materials, mainly smectite dioctahedral, are in high demand among the population of Russia, far outstripping other enterosorbents [30, 31]. At the moment on the Russian pharmaceutical market there are 4 medicines with enterosorption activity on the basis of smectite minerals. However, they are all made from foreign substances. The development of medicines based on domestic substances is an urgent task for the public health and pharmaceutical industry and is consistent with the "Pharma Strategy-2020" [32].

Degree of elaboration of the research topic. The sorption properties of clay minerals have attracted the attention of scientists for the last half-century [33, 34]. Over the past decade, many works on structure, physicochemical properties, experimental and clinical studies of the effectiveness of smectites, including montmorillonite, have been published [35, 36]. To date, there are several drugs based on smectite

clay minerals. These are mainly preparations based on smectite dioctahedral. However, the clinical efficacy of the montmorillonite calcium preparation in the treatment of aflatoxicosis (NovaSil, the manufacturer of Engelhard Chemical Corporation, USA) has been thoroughly investigated. However, there is no enterosorbent based on layered aluminosilicates produced on the basis of domestic raw materials on the Russian pharmacological market. To solve this problem, it is necessary to study montmorillonite of Russian origin with high sorption activity at the preclinical level.

Objectives: to study the pharmacological activity of enterosorbent on the basis of montmorillonite under experimental conditions.

Materials and methods of research

The work was performed on the basis of Belgorod State University in the preclinical research laboratory of the Center for Preclinical and Clinical Studies on 430 white mature rats, Wistar males (weight 200 ± 20 g) and 460 laboratory mice of both sexes (weight 25 ± 2 g). All stages of the research were carried out in accordance with the requirements of GOST ISO/IEC 17025-2009, GOST R ISO 5725-2002 and the "Rules of Laboratory Practice" approved by Order No. 708n of the Ministry of Healthcare and Social Development of the Russian Federation of August 23, 2010, in compliance with the "European Convention for the Protection of Vertebrates, used for experiments or for other scientific purposes "[Directive 2010/63 / EU]. Vivisection was carried out in accordance with the principles of the "European Convention for the Protection of Vertebral Animals Used for Experimental and Other Scientific Purposes." CETS No. 123".

Object of study

The object of the study was a prototype of enterosorbent based on montmorillonite of the Crimean deposit under the laboratory code Crim_04, provided by the company "Krympharmmamed". The experimental sample of enterosorbent Crim_04 is a powder from a yellowish- or grayish-white color to a grayish or brownish-yellow color with the smell of vanillin. The mass fraction of montmorillonite is 62.4%, silver – 0.15%. The size of the most common particles in the suspension is $7.08\text{ }\mu\text{m}$.

Comparison preparations: Enterosorbent "Smecta" (Beaufuor Ipsen Industrie, France) and loperamide ("Janssen-Cilag", France).

Methods of research

A study of the acute toxicity of montmorillonite-based enterosorbent under the laboratory code Crim_04 was carried out on laboratory mice of both sexes weighing 25 ± 2 g in accordance with the "Guidelines for preclinical drug research" [37, 38].

Calculation of doses was carried out individually for each animal in mg / kg. The calculation of the optimal therapeutic dose was carried out using tables of dose recalculation, taking into account the average therapeutic dose of the drug "Smecta" for humans, the average weight of a person is 70 kg, the average weight of the rat is 200 g, the average mouse weight is 25 g [38]. As a result, the optimal therapeutic dose for the rat was 770 mg/kg, for the mouse – 1660 mg/kg.

Laboratory mice were used to model acute diarrhea. Acute diarrhea was modeled by intraperitoneal single administration of serotonin hydrochloride (5-hydroxytryptamine hydrochloride, 5-HT) at a dose of 0.32 mg / kg (n=20 animals) [39]. In the modeling of diarrhea induced by castor oil, castor oil was injected intragastrically at a dose of 0.5 ml per individual (n=20 animals) [40], magnesium sulfate ($MgSO_4$) was used intragastrically at a dose of 2 g/kg to model $MgSO_4$ -induced diarrhea [40] (n=20 animals) [41]. Serotonin hydrochloride was used 30 minutes after intragastric administration of enterosorbents and loperamide. Castor oil and magnesium sulfate were used 30 minutes prior to administration of the investigational pharmacological agents. In the group of intact animals 0.9% sodium chloride solution 10 ml/kg (n=20 animals) was used.

In the experimental groups, the aqueous suspensions of enterosorbents Crim_04 were injected single times in animals at doses of 880 mg/kg, 1660 mg/kg, 3320 mg/kg and Smecta in a dose of 1660 mg/kg, loperamide at an effective dose of 10 μ g/kg, taking into account the recalculation of doses from the average therapeutic daily dose for humans. In the control group, the animals received an equivalent volume of 0.9% sodium chloride solution.

After modeling the pathology, the time of onset of diarrhea, the number of defecations, and

the weight of stool for 4 hours were taken into account. To calculate the severity of inhibition (SI) diarrhea used formula (1).

$$SI(\%) = \left[\frac{(Dk - Di)}{Dk} \right] \times 100\% \quad (1),$$

where Dk – the number of wet and liquid defecations in the control group, Di – the number of wet and liquid defecations in the study groups.

Calculation of the coefficient of severity of diarrhea (CSD) was carried out with the help of a scale scale for assessing the consistency of stool mass: 1 point – normal excrement, 2 – semi-liquid, wet defecation, 3 points – liquid defecation. The indicator was calculated using formula (2).

$$CSD = \frac{(N \times 1 + S \times 2 + L \times 3)}{\Delta D} \quad (2),$$

where N is the amount of normal excrement, S is the number of semi-liquid excrement, L is the amount of liquid excrement, and ΔD is the total number of defecations during observation. Stressed defecations at the beginning of the experiment were not taken into account when calculating the total number of fecal outcrops during the experiment.

After 4 hours of observation, the animals were anesthetized from the experiment. For pathomorphological examination, the small intestine sites were taken from animals [42].

Wistar male rats were used to model isolated gut loop. Before the experiment, the animals starved for 1 day with free access to water. Chloral hydrate 300 mg/kg was used for anesthesia intraperitoneally. After epilation of the hair on the abdomen, a laparotomy of 2.5 cm was carried out. At a distance of 5 cm from the stomach, the first ligation was applied to the intestine with a constant irrigation of 0.9% sodium chloride solution. Then, in steps of 2.5 cm, ligatures were applied to the intestine to create isolated loops, avoiding ligation of vascular feeding beams. A 0.9% sodium chloride solution was injected into the lumen of the first two loops in a volume of 0.2 ml (internal control, intact loops), the rest – thermolabile cholera-like *E.coli* enterotoxin (TCET, Sigma-Aldrich, USA) at a dose of 2 μ g/loop in a volume of 0.2 ml [43].

The aqueous suspensions of Crim_04 and Smecta® at a concentration of 50 mg/ml, 100 mg/ml, 200 mg/ml were introduced into the lumen of the intestine loops with a toxin in a volume of 0.2 ml. The concentration of enterosorbents in the

suspension was determined empirically, given the permeability of the suspension through the needle of the insulin syringe. In the control group, 2 µg of enterotoxin and 0.2 ml of 0.9% sodium chloride solution. Water suspensions of enterosorbents and 0.9% solution of sodium chloride were introduced into the lumen of the gut simultaneously with *E.coli* enterotoxin. The anterior abdominal wall was sutured layer by layer. Animals were placed in individual cells. After 4 hours, the animals were removed from the experiment. The degree of toxin exposure was assessed by the dilatation index, which was calculated from formula (3).

$$ID = \frac{M}{L} \quad (3),$$

where M is the weight of the loop in mg, L is its length in cm.

The severity of the inhibition of fluid outflow into the lumen of the intestinal loops was determined by the formula (4).

$$SI = \left[\frac{(\Delta 2 - \Delta 1)}{\Delta 2} \right] \times 100\% \quad (4),$$

where $\Delta 1$ is the difference between the loop ID with toxin, and the control loops in the experimental animals; $\Delta 2$ – the same in the control group. At the same time, the fluid outlet level in the control loops was assumed to be 100%. For the morphological study, the sites of the small intestine were taken from the animals.

To model acute toxic damage to the liver, male rats were intraventricularly injected with tetrachloromethane in an oily solution at a concentration of 1:1 at a dose of 0.5 ml / kg of active ingredient daily for six days ($n=20$ animals). On the seventh day, animals were intraperitoneally injected with *S.thyphi* lipopolysaccharide at a dose of 20 µg/kg [44].

To simulate chronic toxic damage to the liver intragastrically male rats were injected with carbon tetrachloride (CTC) in oily solution at a dose of 0.5 ml/kg of active ingredient daily for 20 days ($n=20$ animals). At 6, 13, and 20 days, animals were intraperitoneally injected with *S.thyphi* lipopolysaccharide (LPS) at a dose of 20 µg/kg [44]. Animals in intact groups received 0.9% solution of sodium chloride orally ($n=20$ animals).

On the third day, the animals of the experimental groups received the enterosorbent

under the Crim_04 cipher at doses of 385 mg/kg, 770 mg/kg and 1500 mg/kg and the Smecta preparation at a dose of 770 mg/kg as aqueous suspensions intragastrically 12 hours after the administration of carbon tetrachloride ($n=20$ animals).

In both series of experiments, the physical condition of the animals was monitored. After excretion, the animals were taken blood for biochemical studies and liver tissue for morphological investigation. The activity of alanine aminotransferase (AlAT, U/l), aspartate aminotransferase (AsAT, U/l), alkaline phosphatase (AP, U/l), urea (mmol/l), total bilirubin (µmol/L), creatinine (µmol/L) in the blood serum.

Experimental acute pancreatitis was modeled by intraperitoneal single administration of a solution of L-arginine in phosphate buffer (pH = 6.8) at a dose of 1,5 g/kg ($n=20$ animals) to male rats. In the intact group, the animals received a 0.9% solution of sodium chloride orally ($n=20$ animals). Enteric sorbent under the laboratory cipher of Crim_04 in doses of 385 mg/kg, 770 mg/kg, 1500 mg/kg in the form of aqueous suspensions were administered concomitantly with L-arginine and then every 4 hours, 4 times in total to rats intragastrically ($n=20$ animals). The rats in the control group were intragastrically injected with an equivalent volume of 0.9% sodium chloride solution.

The mortality and survival of animals in groups for the first 24 hours was assessed. Animals were withdrawn from the experiment under anesthesia 72 hours after the induction of acute pancreatitis. Animals were collected blood for biochemical research. The following parameters were studied: serum amylase activity (U/l), aspartate aminotransferase (AsAT, U/l), alanine aminotransferase (AlAT, U/l), glucose content (mmol/l), triglycerides (TG, mmol/l). Pancreatic tissue was taken for pathomorphological examination [45].

Biochemical research

When breeding animals from the experiment, the blood was taken into test tubes with sodium heparin as an anticoagulant for. Biochemical indicators were determined using standard reagent kits of Diakon JSC (Russia) on a biochemical analyzer URIT-800 Vet (URIT Medical Electronic Co., Ltd., China).

Morphological investigation

The tissues were fixed in a 10% solution of neutral formalin, followed by pouring into paraffin. From the resulting blocks, sections of 5-7 microns thick were prepared. The staining was performed with hematoxylin-eosin. Microscopic examination was carried out on a micrometer "Mikmed-6" (LOMO, St. Petersburg), image analysis was carried out using the program "Micro-analysis Pro" (LLC LOMO-Microsystems, St. Petersburg).

Statistical analysis

Statistical processing of data was carried out using the Microsoft Excel 2010 and STATISTIKA 6.0 software packages for Windows. The average values of the indicators studied are given in the form ($M \pm m$), where M is

the arithmetic mean and m is the standard error of the mean. To analyze the differences between the groups, the t-test of the Student was used. The difference of the compared indicators for $p < 0.05$ was considered reliable.

Results and discussion

Investigation of the dose-dependent nature of antidiarrhoeal activity of enterosorbent based montmorillonite under the laboratory code Crim_04 on various models of acute diarrhea.

It was found that an intraperitoneal injection of serotonin hydrochloride at a dose of 0.32 mg / kg causes diarrhea in 100% of the animals for 15 minutes, which is manifested by a significant increase in the number of defecations with a predominance of watery stools.

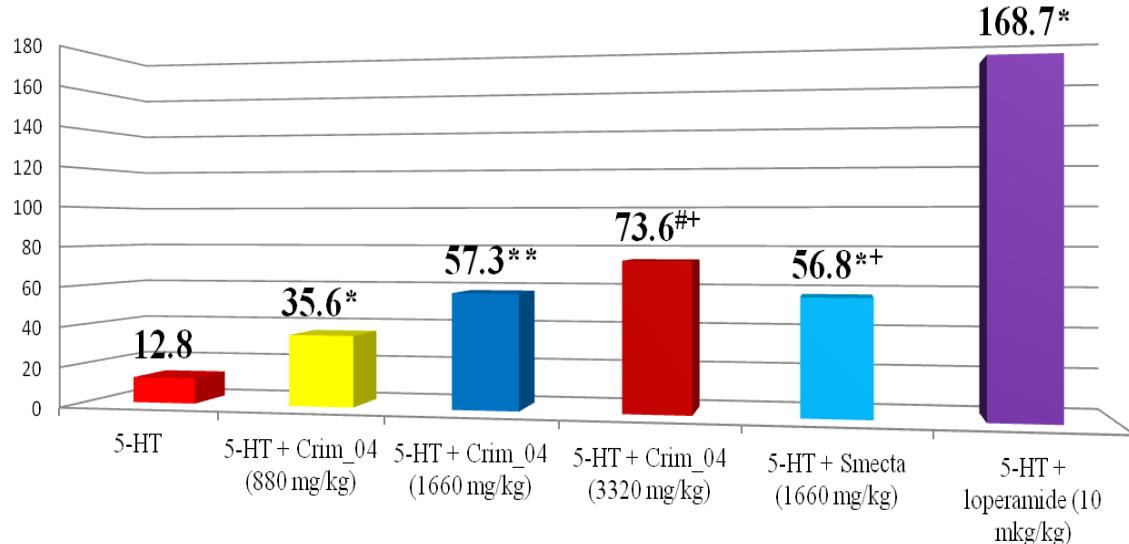


Fig. 1A. Dose-dependent influence of enterosorbent under the Crim_04 cipher for the time of onset of serotonin-induced diarrhea in mice (minutes).

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

As can be seen from Fig. 1A the use of enterosorbent under the code Crim_04 significantly increased the onset of diarrhea. Diarrhea occurred more than 5.5 times later than in the control group. This effect was most pronounced when using enterosorbent in a dose of 3320 mg/kg.

The use of enterosorbent under the Crim_04 cipher reduced the amount of defecations in mice when modeling serotonin-induced diarrhea. It was found that the use of enterosorbent Crim_04

at a dose of 3320 mg/kg caused an inhibition of the development of diarrhea by 52.1%, which is reflected in a decrease in the number of wet and liquid defecations in comparison with the control group. This result is significantly higher than in groups where the enterosorbent was used at doses of 880 mg/kg and 1660 mg/kg (26.7% and 35.9%, respectively). For Smekta and loperamide, this indicator was 34.8% and 73.8%, respectively (Fig. 1B).

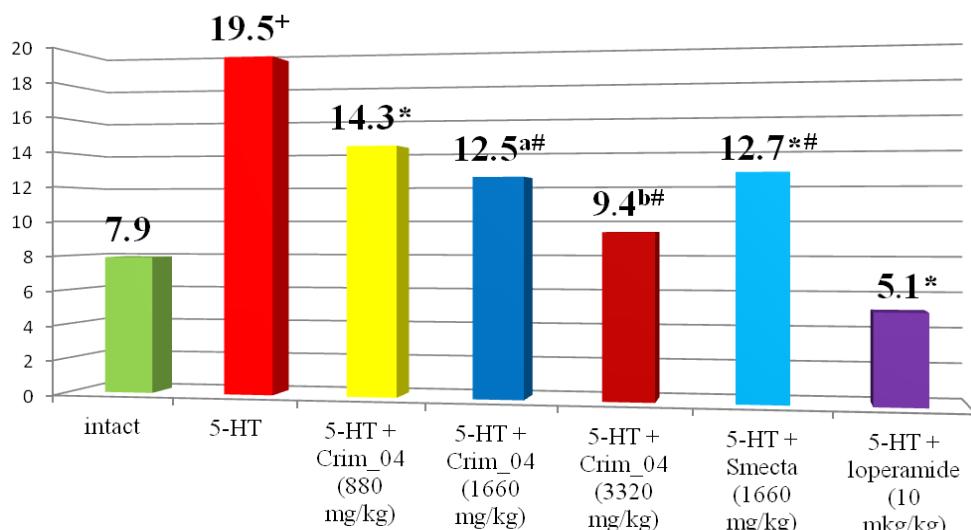


Fig. 1B. Dose-dependent influence of enterosorbent under the code Crim_04 on the number of defecations in serotonin-induced diarrhea in mice (number).

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

With intragastric administration of enterosorbent under the code Crim_04, a decrease in the fluid content in the feces was observed, defecation was predominantly moist. Most of this

effect is expressed in groups of animals receiving the enterosorbent Crim_04 at a dose of 3320 mg/kg (Figure 1C).

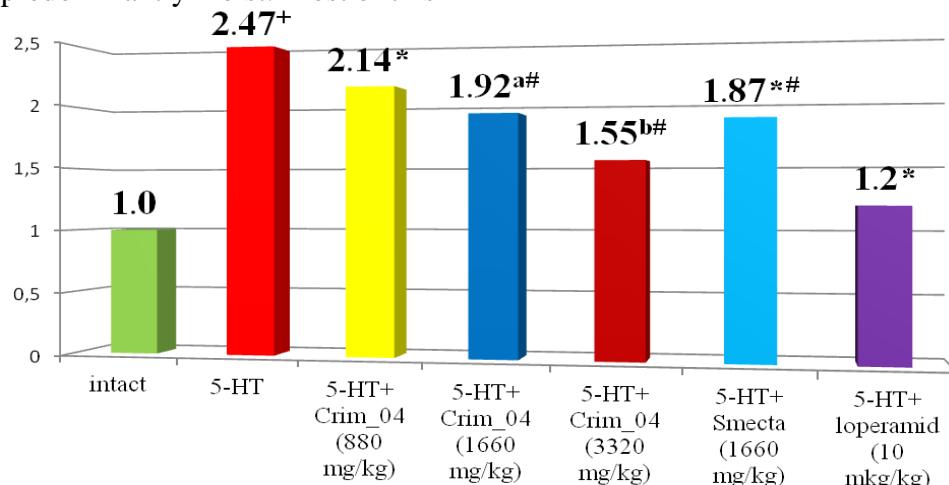


Fig. 1C. Dose-dependent influence of enterosorbent under the code Crim_04 on the consistency of feces in serotonin-induced diarrhea in mice (cond.

Note: + – for $p < 0.05$ in comparison with the group of intact animals; * – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg; b – $p < 0.05$ in comparison with the group Crim_04 in a dose of 1660 mg/kg; # – $p < 0.05$ in comparison with the group of loperamide

The indices of the enterosorbent under the code Crim_04 in the average therapeutic dose of 1660 mg/kg did not significantly differ from the effect of dioctahedral smectite in the same dose. At the same time, the enterosorbent under the code number Crim_04 and the "Smecta" preparation were significantly inferior to

loperamide by the time of onset of diarrhea, the total number of defecations and the fluid content in the stool.

Morphological study revealed that in the animals of the control group macroscopically the intestinal mucosa is swollen, edematous, pink-gray in color, and is hyperemic in separate areas. The

surface of the mucous membrane is covered with a slightly turbid, semi-liquid mucus, which is well washed away with water.

Microscopically the mucosa is edematous. Defined shortening and deformation of the villi. At the ends of some villi, the epithelium is squashed, exposing its own plate of mucosa. Defined hypertrophy of crypts is determined. The blood vessels of the mucosa and submucosa are full-blooded. Muscular and serous intestinal membranes are unchanged. In the lumen of the intestine, a large amount of mucus (Fig. 2B). These changes were not characteristic of intact animals (Fig. 2A).

In groups of animals that received enterosorbent under the laboratory cipher of Crim_04 at doses of 3320 mg/kg, the pathological changes in the small intestine consisted of a slight edema of the mucous membrane. Plots of hyperemia of the mucosa was not observed. The phenomena of slimming of the epithelium were minimal, there were no sites of exposure of the lamina propria of the mucous membrane. The blood supply to the vessels of the mucosa and the submucosal layer was moderate. Muscular layer and serosa without pathological changes (Fig. 2D).

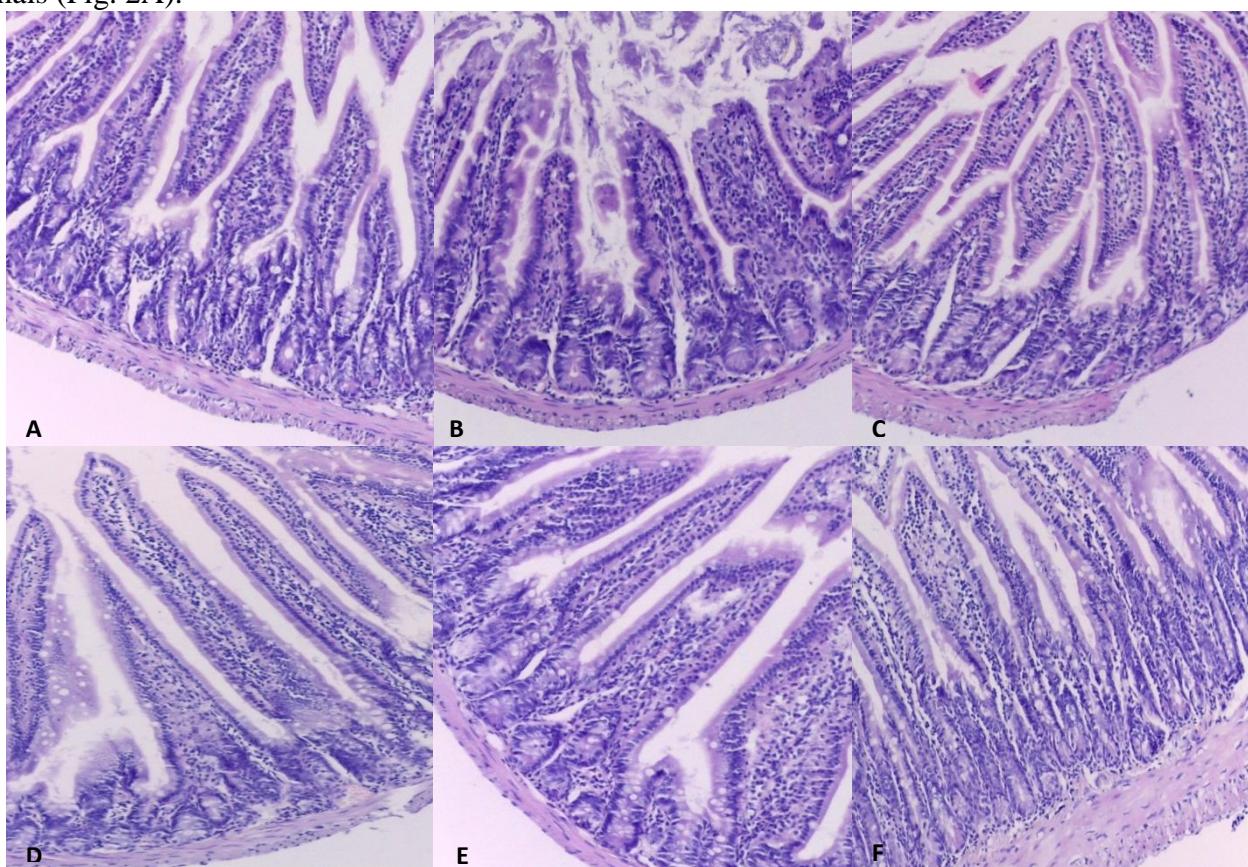


Fig. 2. Histological structure of the ileum in mice against the background of modeling serotonin-induced diarrhea (microphot $\times 100$).

Note: A – intact animals; B – control group; C – enterosorbent Crim_04 in a dose of 1660 mg/kg; D – enterosorbent Crim_04 in a dose of 3320 mg/kg; E – Enterosorbent "Smecta" 1660 mg/kg; F – loperamide 10 μ g/kg. Okr. hematoxylin and eosin

In the control group with simulation of serotonin-induced diarrhea, a decrease in the height of the villi was 1.4 times, an increase in the width of the villi at the base by 1.4 times and the depth of the crypts by 1.2 times in comparison with intact animals. The use of enterosorbent

under the code Crim_04 at a dose of 3320 mg/kg significantly improved the histological picture of the small intestine, bringing the morphometric parameters closer to the level of intact animals (Fig. 3).

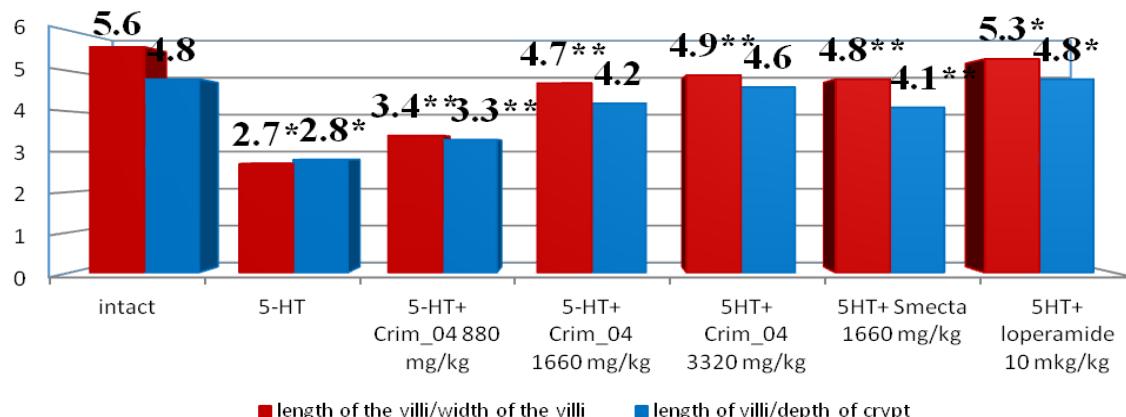


Fig. 3. Influence of the enterosorbent under the Crim_04 cipher on the ratio of the length of the villi to the width of the villi and the length of villi to the depth of crypt in the small intestine of mice when simulating serotonin-induced diarrhea (conv. Units).

Note: * – for $p < 0.05$ in comparison with the group of intact animals; ** – at $p < 0.05$ in comparison with the control group; a – $p < 0.05$ in comparison with the group Crim_04 in a dose of 880 mg/kg

Similar results were obtained in the modeling of castor-induced diarrhea and MgSO₄-induced diarrhea. So the use of the enterosorbent Crim_04 in a dose of 3320 mg/kg on the model of diarrhea induced by castor oil increased the time of onset of diarrhea from 42.7 ± 2.9 minutes in the control group to 124.2 ± 5.4 minutes, reduced the total number of defecations by 64.4%, and significantly affected the consistency of defecations, significantly increasing their density from 2.58 ± 0.02 to 1.54 ± 0.01 points.

With the application of enterosorbent Crim_04 at a dose of 3320 mg/kg on the model of MgSO₄-induced diarrhea, the time of onset of diarrhea increased from 62.8 ± 1.2 minutes in the control group to 126.8 ± 4.7 minutes, reducing the

total number of defecations by 50.5 % and significantly affected the consistency of defecations, significantly increasing their density from 2.79 ± 0.04 to 1.81 ± 0.04 points.

Investigation of the sorption activity of enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 on the model of an isolated loop of the small intestine.

The dose-dependent nature of the sorption activity of enterosorbent based on montmorillonite under the laboratory cipher of Crim_04 with respect to the thermolabile cholera-like enterotoxin *E.coli* in comparison with the "Smecta" preparation on the isolated loop model in rats is shown in Fig. 4.

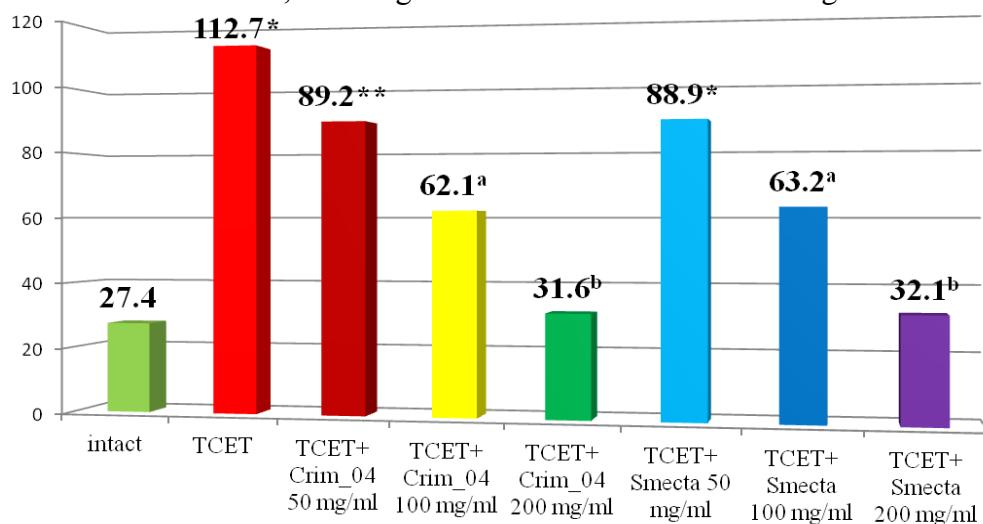


Fig. 4. Influence of the enterosorbent under the Crim_04 cipher in comparison with the Smecta preparation on the dilatation index on the isolated bowel loop model (mg/cm).

Note: * – for $p < 0.05$ in comparison with the control group; ** – at $p < 0.05$ in comparison with TCET; a – at $p < 0.05$ in comparison with enterosorbents in a dose of 50 mg/ml; b – at $p < 0.05$ in comparison with enterosorbents in a dose of 100 mg/ml

It is established that the enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 has a high sorption activity with respect to the thermolabile cholera-like

E.coli enterotoxin on the model of an isolated intestinal loop. This action is manifested by preventing the development of increased fluid formation in the lumen of the gut (Fig. 5).

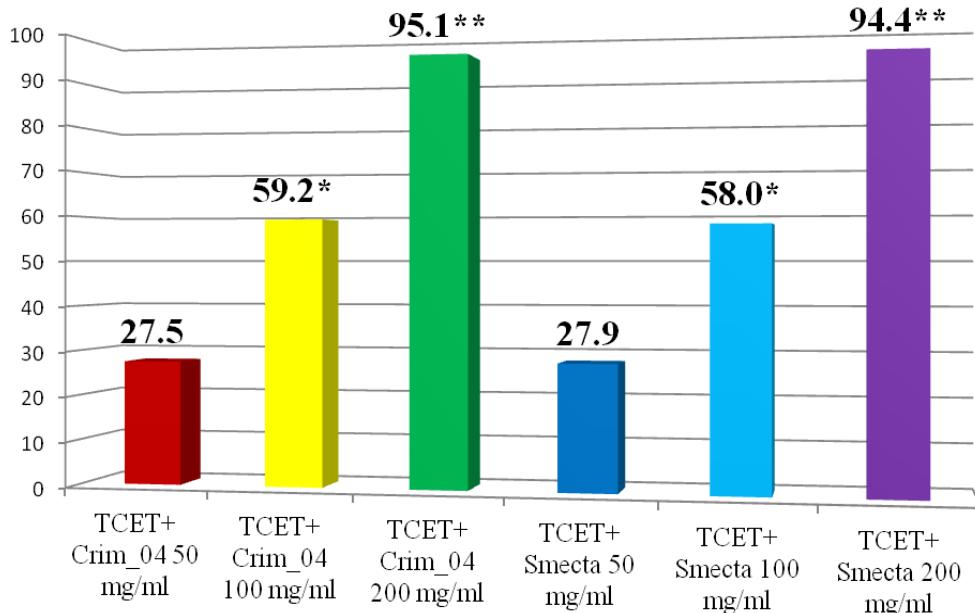


Fig. 5. The dose-dependent effect of the enterosorbent under the Crim_04 cipher in comparison with the Smecta preparation on the severity of the inhibition of fluid flow into the lumen of the gut on the model of the isolated bowel loop (%).

Note: * – at $p < 0.05$ in comparison with enterosorbents in a dose of 50 mg/ml; ** – at $p < 0.05$ in comparison with enterosorbents in a dose of 100 mg/ml

In this case, a clear dose-dependent effect is established, most pronounced when using an aqueous suspension of enterosorbent at a concentration of 200 mg/ml. This is also manifested by an improvement in the morphological pattern of the small intestine. The use of enterosorbent under the cipher of Crim_04 prevents the development of hypertrophy of crypts, swelling of the villi and increased mucosal epilation of the mucous membrane. Morphometric indices when using enterosorbent under the Crim_04 cipher are close to the level of intact loops.

The study of the detoxification activity of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 on models of acute and chronic toxic damage of the liver.

The dose-dependent character of the influence of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 on the activity of liver enzymes, biochemical parameters of blood serum in comparison with the "Smecta" preparation for modeling acute toxic damage of the liver is shown in Fig. 6A, 6B, 6C, 6D, 6E.

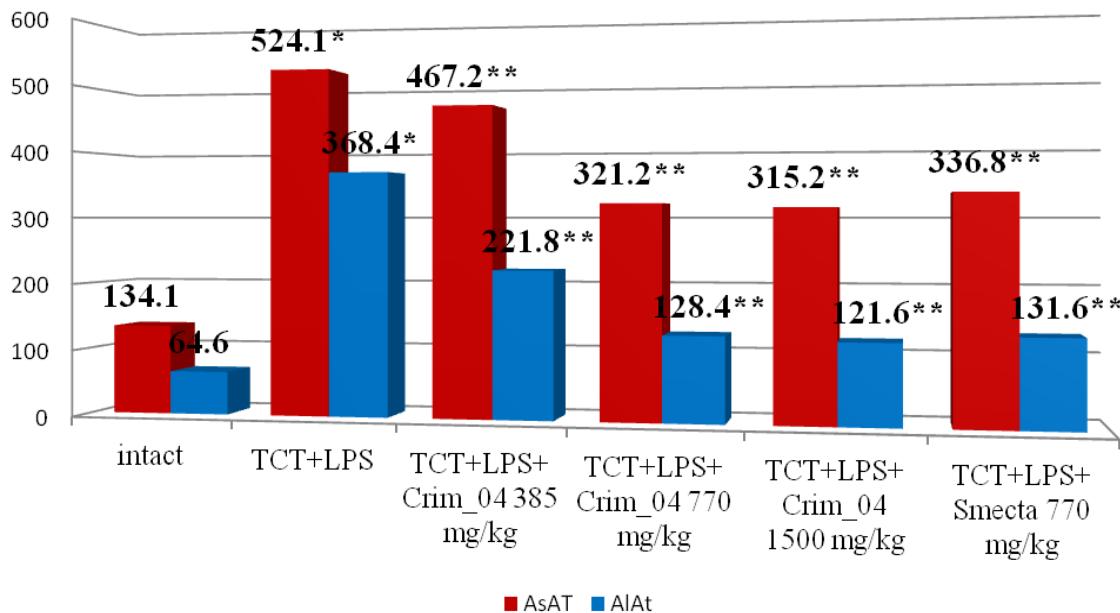


Fig. 6A. Influence of application of enterosorbent under the code Crim_04 on the activity of AsAT and ALAT in modeling acute toxic liver damage in rats (U/I).

Note: ** – p <0.05 – in comparison with the control; * – p <0.05 – in comparison with intact

When using enterosorbent under the laboratory cipher of Crim_04 on the model of acute CTC-induced toxic liver damage, the activity of AsAT and ALAT in the blood plasma

of animals decreased, the de Ritis coefficient decreased from 1.4 in the negative control group to 2.5 in the group using the enterosorbent Crim_04 (Fig. 6A).

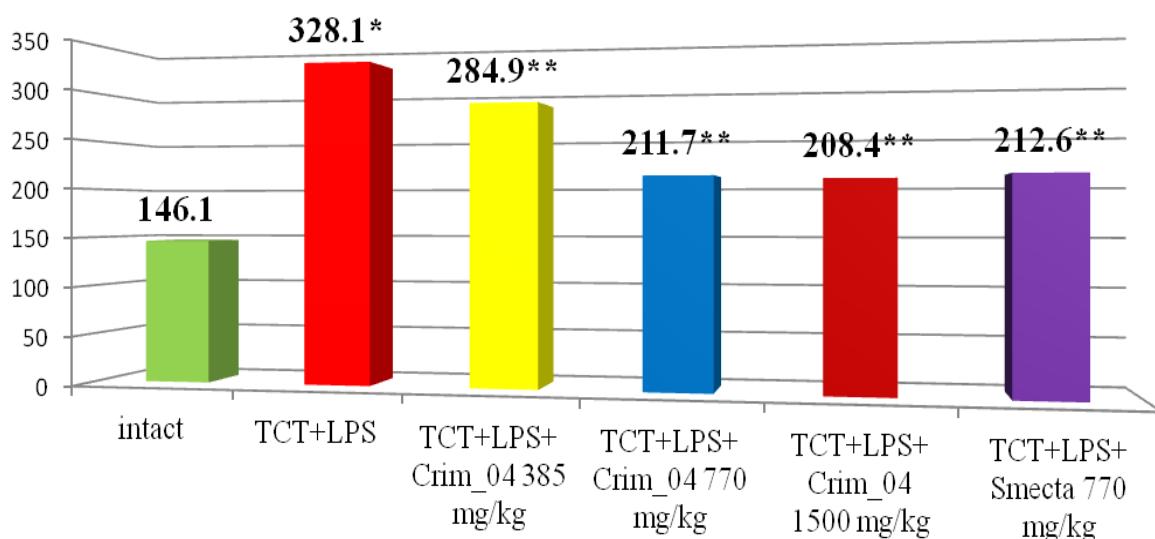


Fig. 6B. Effect of the use of enterosorbent under the code Crim_04 on the activity of alkaline phosphatase in the blood of rats when modeling acute toxic damage to the liver (U/I).

Note: ** – p <0.05 – in comparison with the control; * – p <0.05 – in comparison with intact

Also, the introduction of an enterosorbent under the Crim_04 cipher caused a decrease in the activity of alkaline phosphatase (Figure 6B),

levels of urea (Figure 6C), creatinine (Figure 6D) and total bilirubin (Figure 6E) in the plasma of experimental animals.

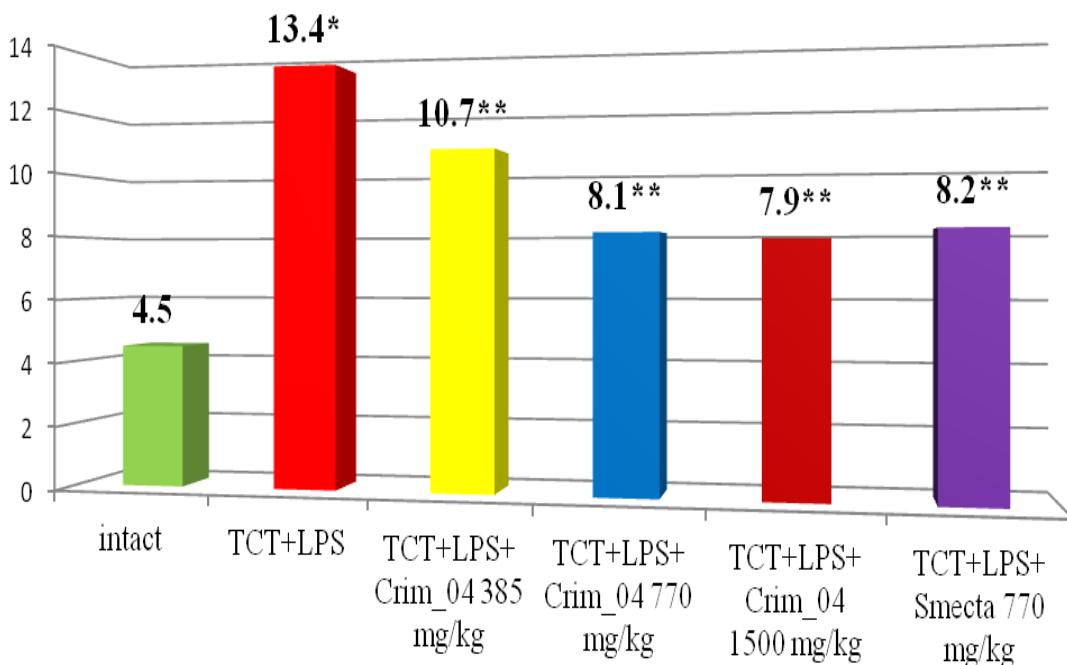


Fig. 6C. Effect of application of enterosorbent under the code Crim_04 on the level of urea in the blood of rats when modeling acute toxic damage to the liver (mmol/l).

Note: ** – p <0.05 – in comparison with the control; * – p <0.05 – in comparison with intact

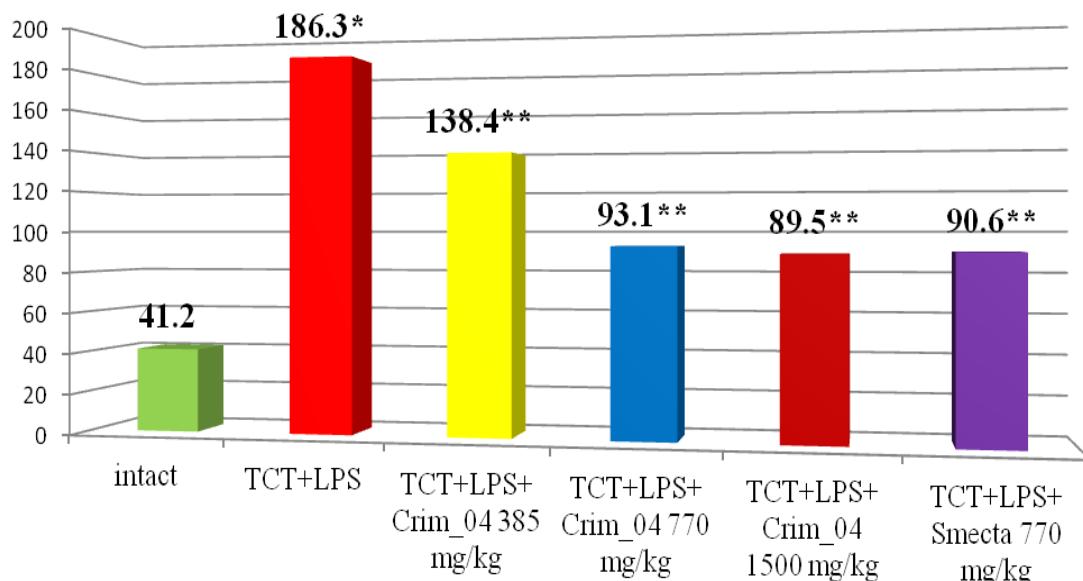


Fig. 6D. Effect of the use of enterosorbent under the code Crim_04 on the level of creatinine in the blood of rats when modeling acute toxic damage to the liver (μmol/l).

Note: ** – p <0.05 – in comparison with the control; * – p <0.05 – in comparison with intact

This effect of enterosorbent Crim_04 was dose-dependent. The enterosorbent Crim_04 showed the highest result in doses of 770 mg/kg and 1500 mg/kg without increasing the effect with further dose build-up. Biochemical indices of

experimental animals when using enterosorbent under the Crim_04 cipher are not significantly different from those in the group of animals that received the Smecta preparation.

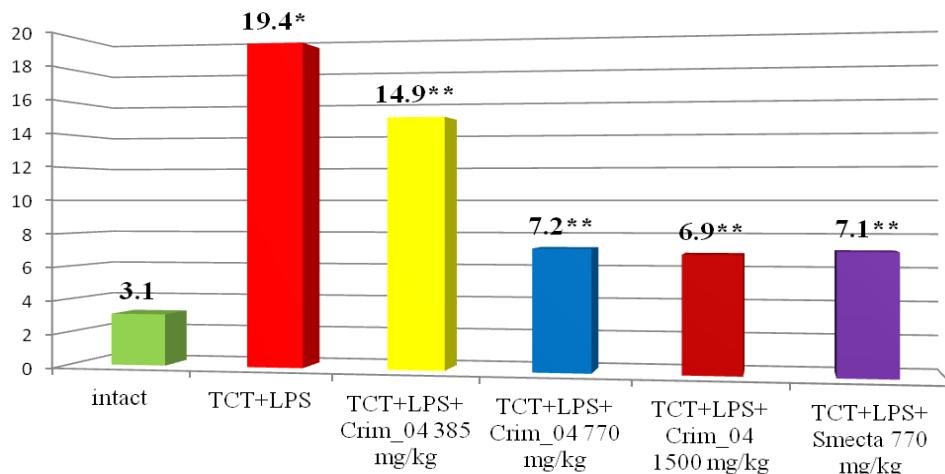


Fig. 6E. Effect of application of enterosorbent under the code Crim_04 on the level of total bilirubin in the blood of rats when modeling acute toxic damage to the liver ($\mu\text{mol/l}$).

Note: ** – $p < 0.05$ – in comparison with the control; * – $p < 0.05$ – in comparison with intact

A normal population of hepatic cells and tissue homogeneity are shown in a typical liver sample from a group of intact animals (Fig. 7A). In liver preparations of rats receiving CTC and LPS, the presence of small-focal necrosis of hepatocytes, signs of large droplet fatty

hepatocyte, granular dystrophy of hepatocytes, some hepatocytes had the appearance of cricoid cells. The fullness of the blood vessels of the liver, moderate expansion of the veins of the portal tracts was registered (Fig. 7B).

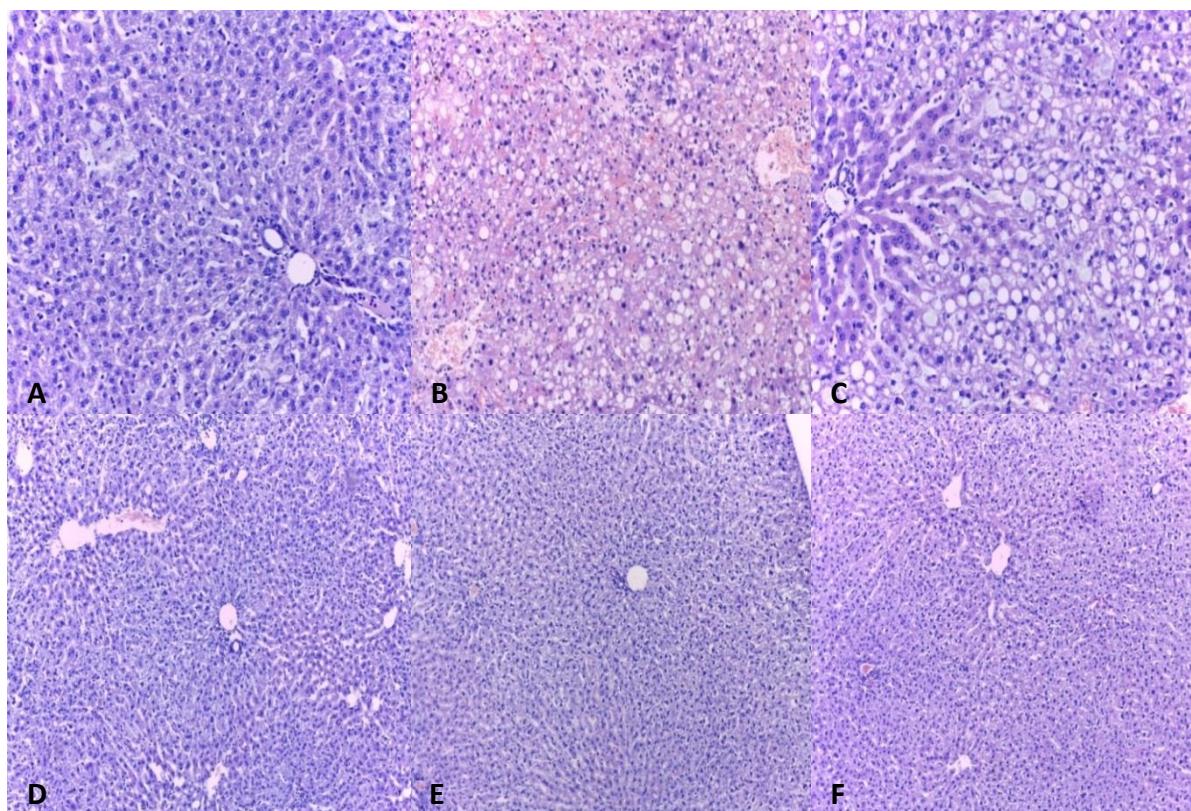


Fig. 7. Morphological examination of liver tissue (microphotography $\times 100$).

Note: A – group of intact animals; B – control group; C – enterosorbent under the cipher of Crim_04 in a dose of 385 mg/kg; D – enterosorbent under the code number Crim_04 in a dose of 770 mg/kg; E – enterosorbent under the cipher of Crim_04 in a dose of 1500 mg/kg; F – the drug "Smecta" in a dose of 770 mg/kg

In groups of animals treated with montmorillonite-based enterosorbent under the laboratory code Crim_04 at doses of 770 mg/kg and 1500 mg/kg, necrotic changes were not revealed. The structure of the liver retained normal histoarchitectonics. Hepatocytes are located typically. In some preparations, signs of small droplet fatty degeneration of hepatocytes. Determined moderate plethora of blood vessels of the liver. Portal tracts are not expanded (Fig. 7D, 7E).

In the morphometric analysis of liver tissue, it was found that when modeling acute toxic damage to the liver with 50% oil carbon tetrachloride, the volume of hepatocytes increased due to large-droplet fatty degeneration, as a result of which the nuclear-cytoplasmic index (NCI) in the control group decreased significantly. Thus, in the control group, the NCI was 0.14 ± 0.001 vs. 0.32 ± 0.001 for the group of intact animals. When the enterosorbent is used under the Crim_04 cipher, there is an increase in the parameters of the NCI, reaching a level of 0.28 ± 0.001 in the group of animals taking Crim_04 at a dose of 1500 mg/kg.

When using enterosorbent under the laboratory code Crim_04 on the model of chronic CTC-induced toxic liver damage, the activity of AsAT and AlAT in the blood plasma of animals decreased, the de Ritis coefficient increased from 1.6 to 2.3. Similarly, the introduction of enterosorbent under the code Crim_04 caused a

decrease in the activity of alkaline phosphatase, levels of urea, creatinine and total bilirubin in the blood plasma of experimental animals. This effect of enterosorbent Crim_04 was dose-dependent. The enterosorbent Crim_04 showed the highest result in doses of 770 mg/kg and 1500 mg/kg without increasing the effect with further dose build-up. Biochemical indices of experimental animals when using enterosorbent under the Crim_04 cipher are not significantly different from those in the group of animals that received the Smecta preparation.

The study of the dose-dependent nature of the effectiveness of the use of enterosorbent on the basis of montmorillonite under the laboratory code Crim_04 in the simulation of acute L-arginine-induced pancreatitis.

The dose-dependent nature of the influence of enterosorbent on the basis of montmorillonite under the laboratory cipher of Crim_04 on the biochemical parameters of blood serum of rats in comparison with the "Smecta" preparation against the background of modeling of acute L-arginine-induced pancreatitis is presented in the table 1. It has been established that the montmorillonite-based enterosorbent under the laboratory cipher Crim_04 has a dose-dependent effect when modeling acute L-arginine-induced pancreatitis in rats. This is expressed in a decrease in the activity of amylase, AsAt, AlAT, a decrease in serum triglyceride levels and glucose in rats.

Table 1

Influence of enterosorbent under the cipher of Crim_04 in comparison with the "Smecta" preparation on the biochemical parameters of blood of rats against the background of modeling of acute L-arginine-induced pancreatitis ($M \pm m$; $n=10$)

A drug	Amylase (U/l)	AsAt (U/l)	AlAt (U/l)	TG (mmol/l)	Glucose (mmol/l)
Intact	215 ± 3	121 ± 1	$60 \pm 0,9$	1.0 ± 0.1	5.3 ± 0.7
L-arginine	$1834 \pm 12^*$	$521 \pm 6^*$	$115 \pm 2^*$	$2.9 \pm 0.3^*$	$13.5 \pm 1.0^*$
L-arginine + Crim_04 385 mg / kg	$1320 \pm 14^{**}$	$325 \pm 5^{**}$	$94 \pm 4^{**}$	$2.1 \pm 0.1^{**}$	$10.9 \pm 2.2^{**}$
L-arginine + Crim_04 770 mg / kg	836 ± 4^a	182 ± 4^a	79 ± 2^a	1.4 ± 0.2^a	8.5 ± 1.1^a
L-arginine + Crim_04 1500 mg / kg	801 ± 3^a	171 ± 2^a	76 ± 2^a	1.2 ± 0.1^a	7.9 ± 1.2^a
L-arginine + Smecta 770 mg / kg	$852 \pm 5^{**}$	$189 \pm 4^{**}$	$77 \pm 3^{**}$	$1.3 \pm 0.1^{**}$	$8.8 \pm 1.4^{**}$

Note: * – for $p < 0.05$ in comparison with the intact group; ** – at $p < 0.05$ in comparison with the control group; a – at $p < 0.05$ in comparison with Crim_04 in a dose of 880 mg/kg.

This indicates a decrease in inflammation of the pancreas. In this case, the effect of enterosorbent based on montmorillonite under the Crim_04 cipher was dose-dependent, most

pronounced at a dose of 770 mg/kg without improvement in the parameters with further dose build-up. This is also confirmed by histological examination (Fig. 8).

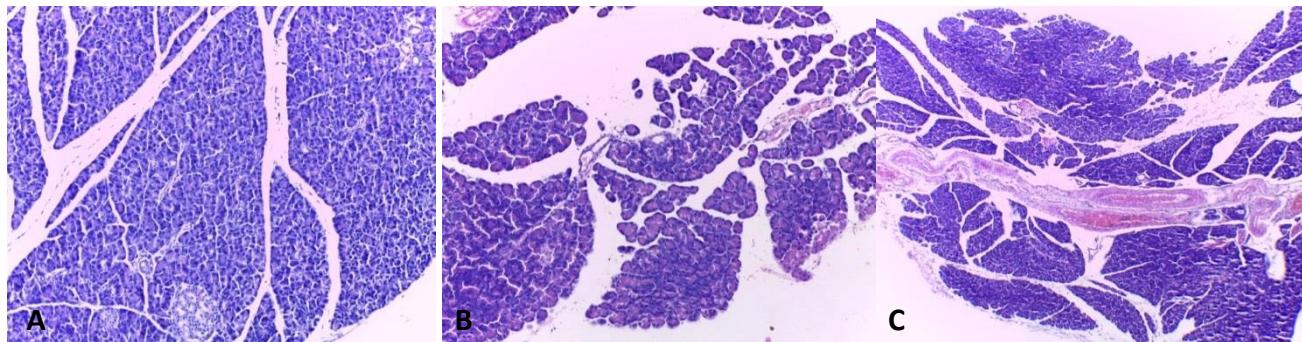


Fig. 8. Morphological examination of pancreatic tissue (microphoto $\times 100$).

Note: A – group of intact animals; B – control group; C – enterosorbent under the cipher of Crim_04 in a dose of 1500 mg/kg

In histological study, it was found that with the induction of acute pancreatitis by intraperitoneal administration of L-arginine at a dose of 1.5 mg / kg in pancreatic tissue, pronounced edema, focal necrosis of the acinocytes, islets of Langerhans of different calibers (Fig. 8B). In the group of intact animals, the pancreas retained a typical structure (Fig. 8A).

When using enterosorbent under the code Crim_04 at a dose of 385 mg / kg pancreas was moderately edematous, small foci of necrosis of acinocytes, islets of Langerhans of different calibers are determined.

In doses of 770 mg / kg and 1500 mg / kg enterosorbent under the cipher of Crim_04 prevented necrosis of the acinocytes, small puffiness of the pancreatic tissue, the islets of Langerhans of the usual structure is determined (Fig. 8C).

Discussion

In carrying out complex studies of the pharmacological activity of enterosorbent under the laboratory cipher of Crim_04, in the modeling of serotonin-induced diarrhea, MgSO₄-induced diarrhea, diarrhea induced by castor oil, isolated gut loop, acute and chronic liver damage, and L-arginine induced acute pancreatitis, that the enterosorbent under the cipher Crim_04 has a dose-dependent antidiarrheal, detoxification activity, a high sorption for enterotoxin of *E.coli* activity w, high therapeutic efficacy in experimental pancreatitis, most pronounced at a dose of 3320 mg/kg.

Taking into account the results obtained in the work, it is possible to determine the recommendations and outline the prospects for further work in this area.

In the course of the study, it was shown that the enterosorbent based on montmorillonite Crim_04 has a high antidiarrheal, detoxification activity, therapeutic efficacy in experimental acute pancreatitis, showing a clear dose-dependent effect.

The data obtained in the course of the study on the pharmacological activity of the enterosorbent under the laboratory cipher of Crim_04 gives broad prospects for further study of this pharmacological agent. Enterosorbent based on montmorillonite Crim_04 can be recommended for further clinical effectiveness and safety for the treatment of acute diarrhea of various etiologies, diseases accompanied by endotoxicosis, exogenous intoxications.

The data obtained in the course of the study on the pharmacological activity of the enterosorbent under the laboratory cipher of Crim_04 gives broad prospects for further study of this pharmacological agent. Enterosorbent Crim_04 can be recommended for further preclinical study of pharmacological activity in models of acute gastric ulcers, Crohn's disease, acute and chronic intoxications with salts of heavy metals, intoxications with mycotoxins and bacterial enterotoxins. Enterosorbent based on montmorillonite Crim_04 can be recommended for further clinical effectiveness and safety for the

treatment of acute diarrhea of various etiologies, diseases accompanied by endotoxicosis, exogenous intoxications.

It is promising to conduct further preclinical studies of enterosorbent based on montmorillonite under the laboratory code Crim_04 in modeling acute gastric ulcers, Crohn's disease, acute intoxications with heavy metal salts, mycotoxins, bacterial toxins, as well as complex preclinical toxicological safety tests and clinical studies of enterosorbent based on montmorillonite under the laboratory code Crim_04.

Conclusions

1. Enterosorbent based on montmorillonite under laboratory code Crim_04 on the results of the study of acute toxicity is low toxic pharmacological agent and refers to substances hazard class IV. At a dose of 8500 mg/kg, the enterosorbent does not cause lethality and changes in the functional state in mice.

2. Enterosorbent based on montmorillonite under laboratory code Crim_04 possesses a dose-dependent Antidiarrhoeal activity in the simulation serotonin-induced diarrhea, MgSO₄-induced diarrhea, diarrhea induced by castor oil. This is expressed in an increase in the onset of diarrhea, a decrease in the number of bowel movements and a decrease in the amount of fluid in the stool. At the same time, the prevention of the development of mucosal villus hypotrophy and crypt hypertrophy was detected morphometrically.

3. In the simulation, the isolated loop intestine enterosorbent based on montmorillonite possesses a cipher Crim_04 dose dependent sorption activity in relation to the heat labile enterotoxin of *E.coli* cholera, which is manifested in the prevention of exposure to toxin intestinal wall and, consequently, inhibition of fluid exudation into the gut lumen.

4. Enterosorbent based on montmorillonite under laboratory code Crim_04 disintoxicational possesses a dose-dependent effect in models of acute and chronic liver injury, which manifests itself in significant reduction in the activity of the liver enzymes, as well as levels of urea, creatinine, and total bilirubin. The activity of enterosorbent is confirmed by morphological examination of liver tissue.

5. Enterosorbent based on montmorillonite under cipher Crim_04 possesses a dose-

dependent therapeutic efficacy in experimental L-arginine-induced pancreatitis, it reduces the activity of amylase, transaminases, triglycerides, blood glucose serum. The efficacy of enterosorbent was confirmed by histological examination of pancreatic tissue.

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Conflicts of interest

The authors have no conflict of interest to declare.

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UDC: 615.065**DOI:** 10.18413/2313-8971-2017-3-3-55-70**Kholodov D.B.¹,**
Nikolaevsky V.A.²,
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Buzlama A.V.²**NEW APPROACHES TO PREVENTION OF NSAID-GASTROPATHY**¹LLC «MedicaSnab», 29b, Taranchenko Street, building 2, office 20, Voronezh, 394036, Russia²Voronezh State University, 1, University Square, Voronezh, 394018, Russia.³Voronezh N.N. Burdenko State Medical University, 10, Studentcheskaya Street, Voronezh, 394036, Russia.Corresponding author, ¹e-mail: dima1985otrchr@yandex.ru**Abstract****Introduction:** At present, the issue of gastric mucosal damage, induced by the use of non-steroidal anti-inflammatory drugs, remains unresolved.**Objectives:** The development of new methods of prevention of NSAID-gastropathies with oral coursework use of taurine and procaine, as well as the study of cellular mechanisms of the damaging effect of diclofenac sodium and Ketorolac tromethamine.**Methods:** The methodological approach was based on a range of theoretical, pharmacological, histological, statistical, biophysical methods.**Results and discussion:** Diclofenac sodium and ketorolac tromethamine, being in direct contact with cell membranes, cause a change in the structural and functional properties that present in the defect formation. This resulted in a decrease in the acid and hypo-osmotic resistance of model cells due to the broken or weakened bonds stabilizing the proteins molecules in membrane (which is associated with the dissociation of NH⁺-groups of the imidazole ring of histidine, the terminal α-amino groups (not less than 10.5% relative to the control), sulphydryl groups of cysteine, phenolic groups of tyrosine, ε-amino groups of lysine (not less than 8.7%)). In experiments *in vitro* and *in vivo* procaine reduces the damaging effect of Ketorolac trometamina 28% and 19.7%, respectively, the formation of hidden defects reduced by 69% when taurine cellular damage was reduced by 54% and 19.7% of the latent defects is less than 74%.**Conclusion:** Prophylactic intragastric administration of procaine or taurine for 7 days before ketorolac tromethamine administration significantly reduces the amount of erosive and ulcerative defects (87% and 90%, respectively).**Keywords:** Procaine, taurine, nonsteroidal anti-inflammatory drugs, membrane damage, prophylaxis, NSAID-gastropathy.**Introduction**

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used in medical practice [1, 2, 3]. However, the issue of tolerability and safety of NSAIDs is particularly urgent [1, 4]. Non-steroidal anti-inflammatory drug gastropathy (NSAID-gastropathy) is a specific syndrome, mainly presented by the gastric mucosal lesions with the development of erythema, erosions and/or ulcers, and is recognized as one of the most

common serious complications of NSAID therapy. Currently, research is being conducted to identify unknown mechanisms of NSAID-gastropathy and to develop new drugs for the treatment and prevention of complications [5, 6].

To prevent and treat NSAID-dependent gastropathies, proton pump inhibitors, histamine H₂-receptor antagonists, antacids, mechanical protectors for the erosive-ulcerative lesion, as well as new drugs, e.g. rebamipide

gastroprotector – synthetic analogue of prostaglandin E2 – are used [7, 8]. However, histamine H₂-receptor inhibitors, local bismuth preparations and antacids have been established to be ineffective for the treatment and prevention of gastric ulcers in patients taking non-steroidal anti-inflammatory drugs for a long time [9, 10].

Of all these drugs, proton pump inhibitors are considered to be the most effective. However, their efficiency is reduced by localization of lesions in the gastric mucosa, prolonged use of NSAIDs and the absence of *H. pylori* [11]. In addition, long-term use of drugs reducing the acidity of gastric juice (histamine H₂-receptor blockers, proton pump inhibitors, antacids) increases intragastric pH and is capable of causing digestion disorders, which is manifested by the clinical picture of dyspeptic syndrome. On the one hand, a prolonged increase in pH significantly weakens the barrier to pathogenic and potentially pathogenic flora entering the gastrointestinal tract. On the other hand, the persistent suppression of gastric acid secretion causes hypergastrinemia which is fraught with development of dis- and metaplastic processes in the gastric epithelium (against a background of chronic inflammation) [5, 12]. Therefore, the development of new ways of preventing NSAID-gastropathy and clarifying the mechanisms of the damaging effect of NSAIDs at the cellular level are topical issues [13, 14].

Materials and methods

The drugs used in the study are Diclofenac (Lotus Laboratories Pvt. Ltd., India), Ketorol (Dr. Reddy's Laboratories Ltd., India), procaine substances (No. LS-000007, 2010-01- 18 HubeiMaxpharmIndustriesCo, China) and taurine (FS.2.1.0039.15; CAS: 107-35-7, CJSC "Vekton", Russia). Experimental studies were conducted on 503 white mongrel male rats with an initial weight of 200-250 g and 225 white mongrel mice of both sexes weighing 20-25 g, obtained from the vivarium of the Voronezh N.N. Burdenko State Medical University. Animals were kept in vivarium conditions, T = 17-24 °C, under natural light conditions, 50-70% humidity, and were fed with conventional combined fodder [15].

Study of the ulcerogenic effect of ketorolac tromethamine

The experiments were carried out in accordance with the recommendations of the

"Guidelines for experimental (preclinical) study of new pharmacological substances" [16], using the "Biomed-1" microscope (research and production company Delta Trans LLC, Russia). The design of the study is shown in the figure (Fig. 1)

Study of the blood coagulation system

Coagulograms were recorded on the automated coagulation analyzer (model "H 334", JSC "Krasnodar ZIP", Russia) [17], the design and doses of the experiment being similar to the design of the ulcerogenic experiments.

Study of the structural and functional properties of erythrocyte membranes.

Spectrophotometric method was used ("PE 5400 VI" spectrophotometer, "Ekohim" Ltd., Russia) for the registration of hypo-osmotic acid resistance of erythrocytes placed in the hypo-osmotic solution of sodium chloride (0.55%) [18], or by adding of 0,1M hydrochloric acid solution to 5 ml of erythrocyte suspension [19]. The main analyzed erythrogram indices calculated by the equation [18] were as follows: 1) K_{max} – constant maximum speed of erythrocyte hemolysis (relative units), G_{sph} – relative amount of spherocytes (%), G₁₂₀ – hemolyzed erythrocytes in the hypo-osmotic environment for 120 seconds (%). The blood of 230 white mongrel rats was used. The design of the experiments is shown in the figure (Fig.1).

Study of the optical properties of proteins

Spectrophotometric method was used ("PE 5400 VI" spectrophotometer, "Ekohim" Ltd., Russia) [20], oxyhemoglobin solutions were prepared of the blood of 95 white mongrel male rats, the concentration was monitored spectrophotometrically (D = 0.8). The design of the study is shown in the figure (Fig. 1).

Study of alkaline buffer capacity of proteins with acid-base titration. The degree of modifier effect to physicochemical properties of the protein was evaluated according to the change in the alkaline buffer capacity of aqueous solutions of the protein using hemoglobin as an example. Oxyhemoglobin solutions at a concentration of 5 • 10⁻⁵ mol/L were used. Oxyhemoglobin solutions were made by osmotic hemolysis of red blood cells of white mongrel male rats weighing 180-240 g (n = 95). The amount of hemoglobin was monitored spectrophotometrically.

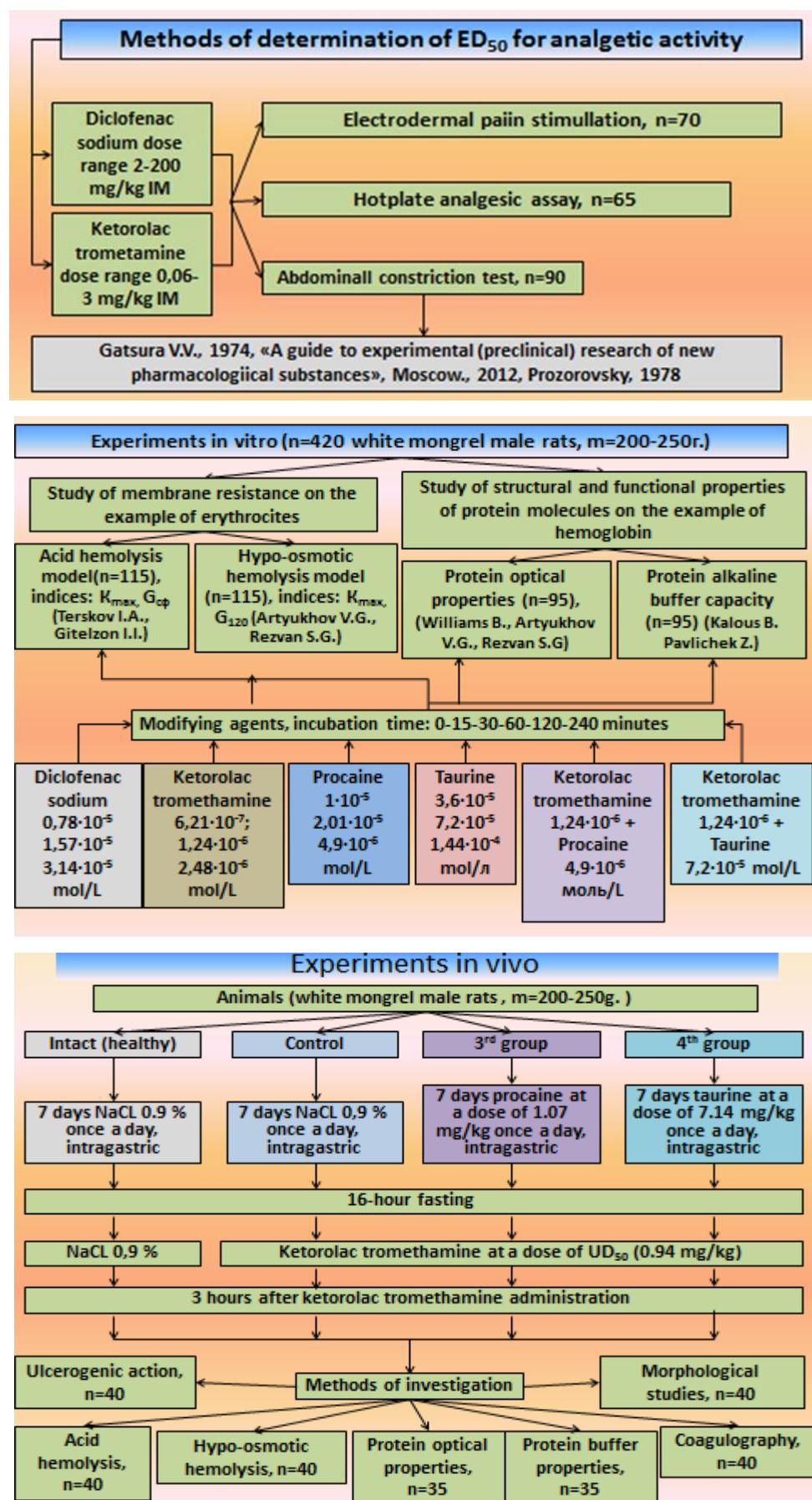


Fig. 1. The study design

The kinetics of the buffering capacity of hemoglobin was evaluated by means of "pH-150M" pH-metering device (RUE "Gomel Plant of Measuring Devices", Belarus), "TS-1/80SPU" electric dry-air thermostat, JSC "Smolenskoye SKTB SPU", Russia), "OPN-8" laboratory medical centrifuge ("TNK Dostan", OJSC, Kyrgyz Republic), "MS-01" magnetic stirrer, "ELMI" Ltd., Latvia), titrating a solution of intact or modified hemoglobin 0,1 M NaOH in the pH range 3.0-11.0 [21]. Substance concentrations in vitro experiments, use pattern and dosing regimen in vivo experiments, as well as the number of animal series correspond to the method of studying the structural and functional properties of erythrocyte membranes.

Pathomorphological studies

The number of series and animals in series, medical regimen, dosage regimen of procaine, taurine, ketorolac tromethamine are similar to the method used for studying ulcerogenic effects [16]. Internal organs (stomach, liver, kidneys) were taken to prepare histological preparations [22].

Reliability of the results obtained is based on the use of modern methods, instruments and devices, a sufficient number of experiments performed, representativeness of sample (728 specimens of laboratory animals, no less than 6 specimens per group) and adequate statistical processing of the information obtained using parametric criteria (Student's t-test) and nonparametric criteria (the Wilcoxon test and the Mann-Whitney U test).

Results

Study of the analgesic activity. The calculated effective dose (ED50) values required for further studies were 4.5 mg/kg for diclofenac

sodium and 0.375 mg/kg for ketorolac tromethamine, according to abdominal constriction test.

Study of the efficacy of procaine and taurine for the prevention of ketorolac tromethamine ulcerogenic effect

It has been established that prophylactic intragastric administration of procaine at a dose of 1.07 mg/kg and taurine at a dose of 7.14 mg/kg for 7 days before administration of ketorolac tromethamine at an ulcerogenic dose UD50 (0.94 mg/kg) decreased the number of erosive ulcerative lesions of the stomach by 87.4% and 89.96%, respectively, compared to ketorolac tromethamine administered alone. Omeprazole provided no cases of ulcers in animals, but the long-term use of proton pump inhibitors (PPI) is characterized by a number of side effects and firstly by digestive disorders, which was confirmed by a decrease in feed intake and a consequent decrease in body weight by 10% in animals of this experimental group.

Pathomorphological studies

It has been established that the use of ketorolac tromethamine at a UD50 dose (0.94 mg/kg) after a 16-hour fasting was accompanied by multiple small necrotic foci in the apical part of the gastric glands, as well as foci of ulceration, destruction with deeper damage to the gastric mucosa and a decrease in the mucous layer density (increased amount of thinned areas). These changes may be induced by the main mechanism of ketorolac tromethamine action (blocking COX isoforms) and direct damage to the cell membranes of gastric mucosa.

Table 1

Study of the efficiency of procaine and taurine for the prevention of ketorolac tromethamine ulcerogenic effects ($M \pm m$)

Animal group	Number of animals in the group	Number of gastric mucosal ulcers, pcs.
Control	10	0
Ketorolac tromethamine 0.94 mg/kg	10	$13.25 \pm 6.67^*$
Ketorolac tromethamine 0.94 mg/kg + Procaine 1.07 mg/kg	10	$1.67 \pm 1.5^*$
Ketorolac tromethamine 0.94 mg/kg + Taurine 7.14 mg/kg	10	$1.33 \pm 1.03^*$
Omeprazole 111.43 mg/kg	10	0

Note: * – the differences are statistically significant at $p < 0.05$

After the prophylactic taurine administration to animals at a dose of 7.14 mg/kg or procaine (1.07 mg/kg) before ketorolac tromethamine administration at a dose of UD50, the gastric mucosa was better preserved and the defects were much less pronounced; there were insignificant isolated foci of desquamation of the apical part of

the glands and dystrophy of the mucous membrane covering layer. Along with this, the gland mucous layer density compared to ketorolac tromethamine alone increased and approached the control value. Structural organization of the liver and kidneys in all groups remains within the norm.

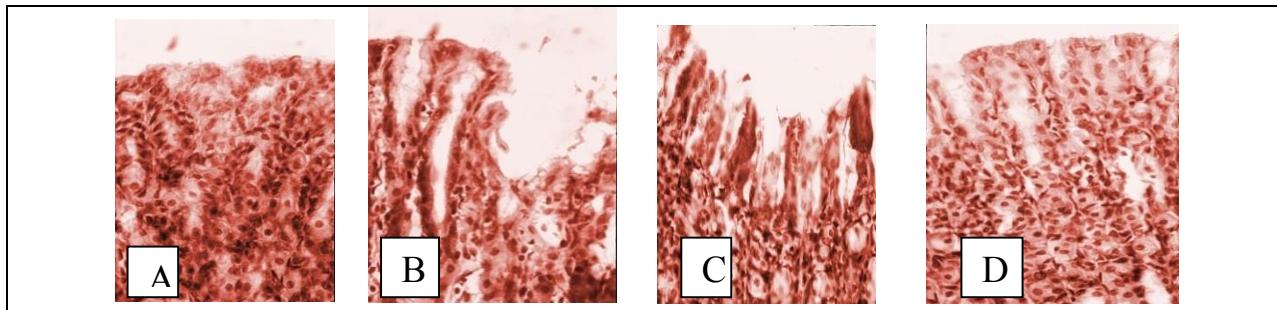


Fig. 2. Histoarchitecture of the gastric mucosa in the prevention of ketorolac tromethamine-induced NSAID-gastropathy by taurine and procaine

Note: A) control; B) ketorolac tromethamine; C) ketorolac tromethamine + taurine; D) ketorolac tromethamine + procaine (Color. gem-eosin. Magnification, appr. 7, v. 40)

Study of structural and functional properties of membranes for erythrocytes modified with sodium diclofenac and ketorolac tromethamine. Review of K_{max} for acid hemolysis (Table 2, 3), which characterizes the hemolytic sensitivity of mid-resistant erythrocytes, showed that sodium diclofenac at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L with incubation for 0, 15, 30, 60, 120 and 240 minutes in experiments *in vitro* results in dose-dependent increase (compared to the control) in the amount of erythrocytes concurrently entering the hemolysis stage. Thereafter they increase the hemolysis rate by 56.6% -1226.9% with the use of diclofenac sodium and by 27% -132% when introducing ketorolac tromethamine into the erythrocyte suspension, as a result of the formation of structural defects in cell membranes. Along with this, erythrocyte subpopulations have been conditionally classified under low-mid-high- and super-resistant subpopulations, which is represented by a change of modification processes for a destructive process, manifested in the presence of maximal and minimal values of K_{max} . Increase in the concentration of sodium diclofenac and ketorolac tromethamine shortens the period of

modification and subsequent destruction of erythrocyte membranes. This action of diclofenac sodium and ketorolac tromethamine is obviously associated with chemical binding to protein-lipid complexes, which leads to the membrane damage and a decrease in the H^+ ion permeability threshold compared to the control value.

The proportion of spherocytes (G_{sph} index reflecting the structural and functional properties of a low-resistant population of erythrocytes) modified by diclofenac sodium at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L was lower than those in the control group by 100% -14.7% and 100% -17.6%, respectively, depending on the concentration and incubation time (see tables 2, 3). This indicates that the low-resistant population of erythrocytes also shows greater sensitivity to the action of these modifiers. However, in separate incubation regimens, the proportion of spherocytes exceeds the control value by 33% -140%, and in half the cases, when erythrocytes were modified by ketorolac tromethamine, spherocytosis was not recorded. This points to a conditional division of low-resistant erythrocytes into subpopulations according to their resistance to acid hemolytic.

Table 2

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with sodium diclofenac depending on incubation time ($M \pm m$)

Concentrations, mol/L	K_{max} of acid hemolysis					
	Incubation time, min					
	0	15	30	60	120	240
Control	0.709±0.07	0.71±0.08	0.705±0.06	0.704±0.13	0.703±0.13	0.717±0.13
$0.78 \cdot 10^{-5}$	1.11±0.1*	1.88±0.07*	2.25±0.11*	3.27±0.13*	1.80±0.2*	1.483±0.25*
$1.57 \cdot 10^{-5}$	2.747±0.1*	3.08±0.08*	4.71±0.13*	4.35±0.2*	3.73±0.13*	3.487±0.19*
$3.14 \cdot 10^{-5}$	3.732±0.1*	6.314±0.1*	5.671±0.2*	5.15±0.25*	5.15±0.38*	9.514±0.5*
G_{sph} (%) of acid hemolysis						
Control	4.0±0.32	3.4±0.2	3.6±0.22	3.8±0.2	3.9±0.25	3.6±0.45
$0.78 \cdot 10^{-5}$	7.6±0.35*	2.6±0.2*	2.3±0.19*	1.8±0.32*	5.2±0.26*	3.6±0.45
$1.57 \cdot 10^{-5}$	0*	0.3±0.06*	0.6±0.13*	1.9±0.32*	2.5±0.25*	0.8±0.13*
$3.14 \cdot 10^{-5}$	0*	2.9±0.13*	2.9±0.13*	3.1±0.1*	1.7±0.13*	2.6±0.19*
K_{max} (relative units) of hypo-osmotic hemolysis						
Control	4.7±0.27	4.7±0.36	5.1±0.3	4.7±0.36	5.1±0.34	4.7±0.36
$0.78 \cdot 10^{-5}$	5.1±0.36*	7.1±0.4**	9.5±0.55**	8.1±0.45**	8.1±0.45**	8.1±0.45**
$1.57 \cdot 10^{-5}$	7.1±0.19**	9.5±1.0**	9.5±0.55**	8.2±0.4**	8.1±0.45**	8.1±0.45**
$3.14 \cdot 10^{-5}$	7.1±0.19**	14.3±1.2**	9.5±0.55**	14.3±1.25**	14.3±1.2**	19.1±1.7**
G_{120} (%) of hypo-osmotic hemolysis						
Control	11.64±1.5	11.64±1.5	11.66±1.9	11.64±2.1	11.66±1.95	11.64±1.5
$0.78 \cdot 10^{-5}$	25.11±2.3**	28.64±2.5**	32.01±3.1**	33.0±3.1**	33.5±3.2**	33.7±3.5**
$1.57 \cdot 10^{-5}$	29.21±2.1**	33.13±3.1**	34.25±3.5**	34.42±3.5**	36.45±3.7**	40.9±3.8**
$3.14 \cdot 10^{-5}$	34.67±2.5**	35.48±2.5**	37.41±3.2**	41.05±3.0**	43.52±3.5**	48.9±4.0**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

Table 3

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with ketorolac tromethamine depending on incubation time ($M \pm m$)

Concentrations, mol/L	Incubation time, min					
	0	15	30	60	120	240
	K_{max} of acid hemolysis, relative units					
Control	3.3±0.16	2.2±0.15	2.4±0.15	3.5±0.17	3.7±0.18	3.7±0.18
$6.21 \cdot 10^{-7}$	1.9±0.1**	5.1±0.2**	4.3±0.2**	6.3±0.3**	5.1±0.2**	5.8±0.3**
$1.24 \cdot 10^{-6}$	2.5±0.1**	4.7±0.2**	4.3±0.2**	7.1±0.3**	5.1±0.3**	6.3±0.3**
$2.48 \cdot 10^{-6}$	2.6±0.1**	5.1±0.2**	4.3±0.2**	5.8±0.3**	4.7±0.3**	2.9±0.2**
G_{sph} of acid hemolysis, %						
Control	-1.5±0.1	-2.7±0.2	-3.4±0.2	-3.6±0.2	-2.7±0.14	-1.4±0.11
$6.21 \cdot 10^{-7}$	0**	0.6±0.03**	1.2±0.1**	0.3±0.01**	1.0±0.1**	1.7±0.1**
$1.24 \cdot 10^{-6}$	-2.0±0.1**	0.2±0.1**	-0.5±0.15**	0.8±0.1**	0.6±0.03**	0.8±0.05**
$2.48 \cdot 10^{-6}$	-3.6±0.2**	-3.6±0.2**	-2.8±0.14**	-1.7±0.1**	-2.0±0.1**	-2.8±0.2**
K_{max} of hypo-osmotic hemolysis, relative units						
Control	6.314±0.32	6.314±0.32	6.314±0.35	7.115±0.36	7.115±0.36	7.115±0.4
$6.21 \cdot 10^{-7}$	7.115±0.4**	11.43±0.6**	9.5±0.5**	7.115±0.41	11.43±0.5**	11.4±0.6**
$1.24 \cdot 10^{-6}$	9.514±0.5**	11.43±0.5**	9.514±0.5**	8.1±0.5**	9.5±0.48**	9.5±0.48**
$2.48 \cdot 10^{-6}$	11.43±0.5**	11.43±0.5**	9.514±0.5**	11.43±0.5**	9.514±0.4**	8.144±0.4**
G_{120} (%) of hypo-osmotic hemolysis						
Control	24.2±2.1	26.0±1.4	27.0±1.3	29.0±1.51	29.0±2.0	29.0±1.7
$6.21 \cdot 10^{-7}$	24.4±1.5	29.8±1.5**	27.0±1.4	21.8±1.8**	22.8±1.1**	25.1±1.3**
$1.24 \cdot 10^{-6}$	29.7±1.5**	38.4±2.0**	34.6±1.8**	32.4±1.6*	27.0±1.4	31.0±1.6
$2.48 \cdot 10^{-6}$	39.4±2.5**	37.5±1.9**	37.3±1.9**	35.5±2.0**	31.4±1.8	31.3±1.8**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

Analysis of the kinetics of erythrocyte hypo-osmotic hemolysis (in *in vitro* experiments) showed that addition of diclofenac sodium at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L and ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L to the incubation medium increases dose-dependently the number of latent defects of erythrocyte membranes, which is confirmed by an increase in the values of K_{max} for hypo-osmotic hemolysis by 8.5% -306.4% and 12.7% -81%, respectively, compared to the control (Tables 1, 2). This is confirmed by a dose-dependent increase in the proportion of hemolyzed erythrocytes by 115.7% -320.8% compared to the control using diclofenac sodium at the concentrations specified when observed for 120 seconds of the experiment (G_{120}) (Table 1). At concentrations of $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L G_{120} values (Table 2) exceed the control level by 8% -62.8%, and at ketorolac tromethamine concentrations of $6.21 \cdot 10^{-7}$ mol/L they either exceed the control level by 14.6% or they are less than the control level up to 24.8%. This indicates the manifestation of modifying reactions for the more resistant red blood cells compared to the control. As incubation time and/or dose increase, modifying reactions are replaced by the

prevalence of destructive processes. These changes probably indicate the interaction of sodium diclofenac and ketorolac tromethamine with spectrin-actin and lipo-stromatin complexes, i.e. the cytoskeleton of the membrane, which presents in a decrease in the erythrocyte hypo-osmotic resistance by means of membrane destruction and increased permeability for water and ions.

Study of the structural and functional properties of membranes for erythrocyte modified with procaine and taurine

The values of the main indices of acid and hypo-osmotic hemolysis erythrograms are presented in Tables 4-5. The amount (%) of erythrocytes modified with procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L, simultaneously entering the stage of proper acid hemolysis (K_{max}) in all cases is less than the control by 6.6% -38.5% or equal to it (Table 3). Analysis of spherocytosis for erythrocytes, modified with procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L with incubation time 0 to 240 minutes, showed that the spherocyte amount (G_{sph}) in the vast majority of cases is more than the control by 15-170%, but in a number of experiments it is equal to or less than the control (Table 3).

Table 4

Indices of acid and hypo-osmotic hemolysis for erythrocytes modified by procaine depending on incubation time (M±m)

Concentrations, mol/L	K_{max} of acid hemolysis, relative units						
	Incubation time, min.						
0	15	30	60	120	240		
Control	3.7±0.19	3.7±0.27	3.7±0.21	3.5±0.18	3.5±0.19	3.5±0.19	
$2.01 \cdot 10^{-5}$	3.7±0.2	2.7±0.15**	2.7±0.18**	3.1±0.16*	2.9±0.16*	2.9±0.15*	
$1.0 \cdot 10^{-5}$	3.5±0.18	2.7±0.14**	2.7±0.15**	2.6±0.2**	2.5±0.13**	2.5±0.15**	
$4.9 \cdot 10^{-6}$	3.5±0.21**	2.7±0.2**	2.4±0.15**	2.1±0.1**	2.6±0.22**	2.6±0.15**	
G_{sph} of acid hemolysis, %							
Control	-1.9±0.12	-1.7±0.1	-1.1±0.11	-1.2±0.1	-1.0±0.09	-1.0±0.1	
$2.01 \cdot 10^{-5}$	-2.6±0.2**	-1.3±0.13*	-2.0±0.1**	-2.2±0.14**	-4.0±0.17**	-4.0±0.2**	
$1.0 \cdot 10^{-5}$	-2.2±0.12*	-0.8±0.1**	-1.4±0.1*	-2.4±0.12**	-3.0±0.15**	-3.1±0.2**	
$4.9 \cdot 10^{-6}$	-1.8±0.1	-1.2±0.1**	-1.6±0.12**	-1.2±0.13	-2.50.13**	-2.7±0.14**	
G_{120} (%) of hypo-osmotic hemolysis							
Control	44.1±2.5	35.4±2.1	37.1±1.9	36.6±1.6	40.4±1.9	40.4±2.1	
$2.01 \cdot 10^{-5}$	32.6±1.7**	40.7±2.0*	38.4±2.3	40.8±2.1*	39.3±2.0	39.1±2.0	
$1.0 \cdot 10^{-5}$	37.4±2.5**	40.0±2.3*	38.6±1.8	35.9±1.8*	32.8±1.6**	32.5±1.6**	
$4.9 \cdot 10^{-6}$	44.3±3.0	36.2±1.9	36.4±2.2	35.0±1.8	38.9±1.9	39.0±2.0	

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

Table 5

Indices of acid and hypo-osmotic hemolysis for erythrocytes modified with taurine depending on incubation time ($M \pm m$)

Concentration, mol/L	Incubation time, min.				
	0	15	30	60	120
K_{max} of acid hemolysis, relative units					
Control	3.732±0.21	3.732±0.19	3.732±0.18	3.732±0.18	3.732±0.2
$1.44 \cdot 10^{-4}$	3.732±0.18	3.732±0.2	3.732±0.21	3.732±0.18	$2.904 \pm 0.14^*$
$7.2 \cdot 10^{-5}$	3.078±0.17*	3.078±0.16*	2.605±0.14**	2.1±0.11**	2.05±0.13**
$3.6 \cdot 10^{-5}$	3.078±0.16*	3.078±0.16*	2.246±0.11**	1.963±0.11**	2.904±0.14**
G_{sph} of acid hemolysis, %					
Control	-1.94±0.18	-1.70±0.17	-1.12±0.15	-1.17±0.16	-1.02±0.12
$1.44 \cdot 10^{-4}$	-2.9±0.15**	-3.4±0.21**	-3.4±0.17**	-5.2±0.25**	$5.26 \pm 0.25^{**}$
$7.2 \cdot 10^{-5}$	-4.4±0.27**	-4.9±0.25**	-3.69±0.19**	-	$6.38 \pm 0.39^{**}$
$3.6 \cdot 10^{-5}$	-5.41±0.3**	-5.90±0.3**	-6.20±0.31**	-	$6.44 \pm 0.35^{**}$
K_{max} of hypo-osmotic hemolysis, relative units					
Control	6.3±0.31	6.314±0.32	6.314±0.29	6.314±0.43	6.314±0.35
$1.44 \cdot 10^{-4}$	2.9±0.16**	3.5±0.17**	3.7±0.18**	1.4±0.1**	$1.2 \pm 0.11^{**}$
$7.2 \cdot 10^{-5}$	2.9±0.14**	2.1±0.15**	1.9±0.1**	2.1±0.11**	$0.7 \pm 0.1^{**}$
$3.6 \cdot 10^{-5}$	4.7±0.23**	4.0±0.24**	2.475±0.15**	1.7±0.13**	$3.1 \pm 0.16^{**}$
G_{120} (%) of hypo-osmotic hemolysis					
Control	44.1±2.72	30.2±1.91	30.8±2.13	30.9±1.5	31.8±1.64
$1.44 \cdot 10^{-4}$	16.4±1.5**	12.8±1.53**	14.3±0.74**	7.2±1.11**	$4.3 \pm 0.56^{**}$
$7.2 \cdot 10^{-5}$	15.4±0.81**	14.0±0.77**	12.4±0.88**	9.4±0.54**	$5.7 \pm 0.47^{**}$
$3.6 \cdot 10^{-5}$	15.3±0.13**	11.4±0.57**	8.7±0.45**	4.3±0.22**	$4.9 \pm 0.25^{**}$

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

At the same time, more intensive development of the prehemolytic stage indicates an increased sensitivity of the "low-resistant" erythrocyte population to acid hemolitics. From this it follows that procaine increases the permeability of "low-resistant" erythrocytes membranes for H^+ ions, for "mid-" and "high-resistant" erythrocytes the use of procaine leads to modification, which is performed by increasing the barrier of permeability for H^+ ions.

When using taurine at concentrations of $3.6 \cdot 10^{-5}$; $7.2 \cdot 10^{-5}$; $1.44 \cdot 10^{-4}$ mol/L, prehemolytic phase is within 120 seconds, while for the control group it is in the range of 30-60 seconds, which indicates a certain delay in the development of the phase of RBC hemolysis proper. The number of erythrocytes simultaneously entering the proper hemolysis stage (K_{max}) did not exceed the control or was less than the control by 17.5% -47.4% (Table 4). At the same time, the amount of spherocytes against the background of taurine administration exceeded

the control by 48.5% -563.7% (Table 4). These changes indicate an increase in the acid resistance of erythrocytes, apparently due to the interaction of taurine with protein-lipid membrane complexes and its antioxidant, membrane-stabilizing action.

Addition of procaine at concentrations of $4.9 \cdot 10^{-6}$; $1 \cdot 10^{-5}$; $2.01 \cdot 10^{-5}$ mol/L to the suspension of erythrocytes, in conditions of hypo-osmotic environment, does not affect the rate of hypo-osmotic hemolysis, and taurine at concentrations of $3.6 \cdot 10^{-5}$; $7.2 \cdot 10^{-5}$; $1.44 \cdot 10^{-4}$ mol/L reduces it by 25.5% -88.1% (Table 3, 4). However, the structural and functional state of erythrocytes more resistant to the hypo-osmotic environment (G_{120}) is characterized by the dose-dependent interaction of procaine with the spectrin-actin and lipo-stromatin membrane complexes. And with an increase in the dose of procaine to $1.0 \cdot 10^{-5}$ mol/L; $2.01 \cdot 10^{-5}$ mol/L and the incubation time, possibly due to the formation of a larger number of complexes, some degradation of the

cytoskeleton of erythrocyte membranes is observed, that is, formation of latent membrane defects. At the procaine concentration of $4.9 \cdot 10^{-6}$ mol/L, such changes were not observed and the amount of hemolyzed erythrocytes within 120 seconds of the experiment did not exceed the control level (Table 3). In case of using taurine at the indicated concentrations, in all experiments, the G_{120} values were less than the control by 63%-91.5% (Table 4).

Study of structural and functional properties of membranes for erythrocytes modified with ketorolac tromethamine in combinations with procaine and taurine

In experiments *in vivo*, animals were prophylactically given intragastric procaine or taurine at doses of 1.07 mg/kg and 7.14 mg/kg for 7 days, and ketorolac tromethamine at a dose of UD50 was administered on day 8. The results are presented in Tables 6, 7.

Table 6

Indices of acid and hypo-osmotic hemolysis of erythrocytes modified with ketorolac tromethamine in combination with procaine and taurine ($M \pm m$)

Group	K_{max} of acid hemolysis, relative units					
	Incubation time, min.					
	0	15	30	60	120	240
Control	4.011±0.2	4.011±0.23	4.011±0.21	3.732±0.19	3.732±0.22	3.732±0.18
Taurine + Ketorolac tromethamine	4.011±0.24	4.011±0.2	3.271±0.18**	4.331±0.23*	4.331±0.21*	4.331±0.25*
Procaine + Ketorolac tromethamine	5.2±0.25**	5.2±0.25**	5.145±0.28**	5.671±0.27**	5.671±0.29**	6.314±0.32**
G_{sph} of acid hemolysis, %						
Control	-1.936±0.11	-1.698±0.11	-1.121±0.1	-1.166±0.12	-1.016±0.1	-1.016±0.07
Taurine + Ketorolac tromethamine	-3.7±0.19**	-4.5±0.22**	-4.6±0.23**	-5.1±0.21**	-5.7±0.25**	-5.4±0.27**
Procaine + Ketorolac tromethamine	-2.2±0.11	-1.3±0.1*	-1.5±0.14*	-2.9±0.16**	-2.56±0.13**	-4.35±0.18**
K_{max} of hypo-osmotic hemolysis, relative units						
Control	5.145±0.25	4.705±0.23	4.705±0.24	4.705±0.23	4.705±0.23	4.705±0.23
Taurine + Ketorolac tromethamine	4.7±0.25	3.7±0.19**	3.7±0.2**	2.9±0.17**	3.7±0.21**	3.7±0.18**
Procaine + Ketorolac tromethamine	3.5±0.18**	4.7±0.24	5.1±0.26	5.1±0.25	5.7±0.29**	6.3±0.32**
G_{120} (%) of hypo-osmotic hemolysis						
Control	22.2±1.51	22.9±1.15	21.8±1.14	23.6±1.25	23.4±1.17	22.7±1.14
Taurine + Ketorolac tromethamine	19.8±1.13	15.1±1.21**	13.0±1.43**	10.9±0.94**	9.2±1.11**	7.2±0.73**
Procaine + Ketorolac tromethamine	15.8±0.82**	11.8±1.32**	12.3±1.15**	11.4±0.87**	9.1±0.57**	9.1±0.57**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$

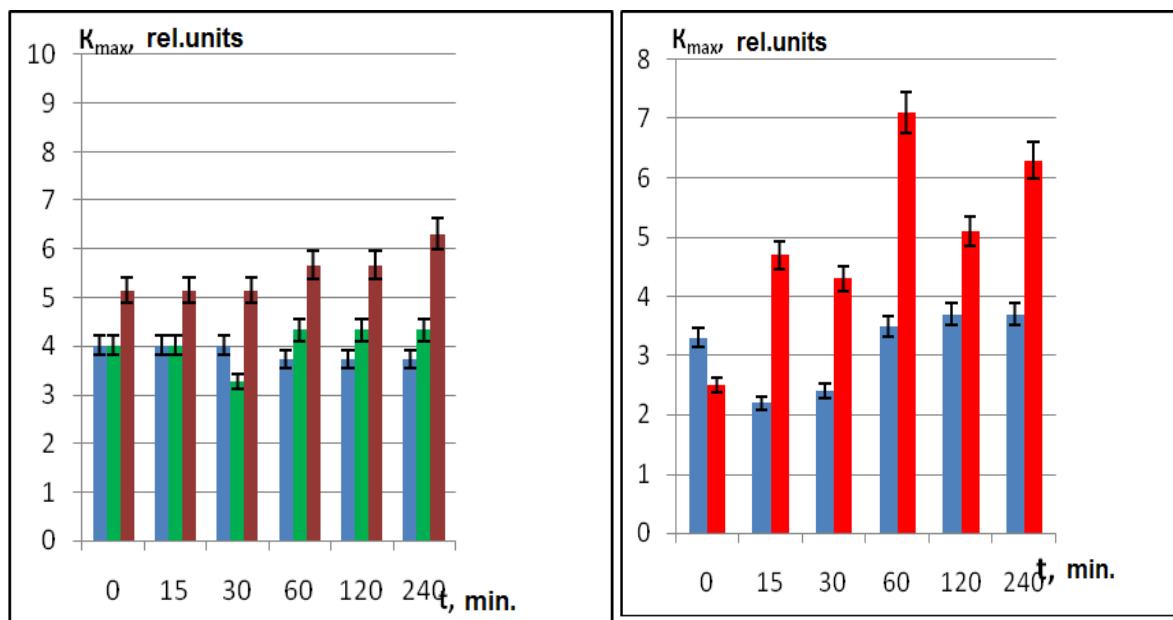


Fig. 3. The values of K_{max} (relative units) of erythrocyte acid hemolysis against the background of the use of ketorolac tromethamine and its combination with taurine and procaine.

Note: control ■ ; taurine + ketorolac tromethamine ■; procaine + ketorolac tromethamine ■; ketorolac tromethamine ■

Analysis of the combined use (in *in vitro* experiments) of taurine (7.2×10^{-5} mol/L) and procaine (4.9×10^{-6} mol/L) with ketorolac tromethamine (1.24×10^{-6} mol/L) allow us to conclude that procaine and, to a greater extent, taurine, prevent ketorolac tromethamine-induced damage to erythrocyte membranes. This is confirmed by a significant decrease in the rate of acid hemolysis (up to 57%) for the main mid-

resistant erythrocyte population, a significant increase in the amount (%) of erythrocytes that are simultaneously in the stage of spherulation (maximum by 100%), and the duration of the prehemolytic stage of hemolysis (maximum by 100%) against the background of combined use of these modifiers compared to the use of ketorolac tromethamine alone.

Table 7

Values of K_{max} , G_{sph} and G_{120} indices of acid and hypo-osmotic hemolysis against the combination of ketorolac with procaine and taurine *in vivo* ($M \pm m$)

Name	Acid hemolysis		Hypo-osmotic hemolysis	
	K _{max} , relative units	G _{sph} , %	K _{max} , relative units	G ₁₂₀ , %
Control	4.3±0.25	-0.9±0.05	5.7±0.29	42.9±2.15
Ketorolac tromethamine	7.1±0.35**	-1.1±0.06*	19.1±0.75**	62.3±3.12**
Ketorolac tromethamine + Taurine	5.7±0.28**	-2.4±0.12**	4.3±0.27*	25.8±1.97**
Ketorolac tromethamine + Procaine	5.7±0.31**	-2.2±0.11**	5.7±0.36	30.6±2.57**

Note: ** – the differences are statistically significant at $p < 0.001$, * – the differences are statistically significant at $p < 0.05$.

Analysis of the hypo-osmotic erythrolytic fainders reveals that the introduction of procaine and taurine into the suspension of erythrocytes prevents the formation of latent defects induced by ketorolac tromethamine. This is reflected in a decrease in the rate of erythrocyte hypo-osmotic hemolysis when combined with taurine by 8.6% –

38.3% compared to the control. In case of combined use of ketorolac tromethamine with procaine, the hemolysis rate was less than or equal to the control during incubation up to 30 min, and it increased by 9.4% -34.2% in the period of 30-240 minutes compared to the control. When ketorolac tromethamine used alone, K_{max}

exceeded the control by 14.5% -81%. Besides, G₁₂₀ values for hypo-osmotic hemolysis against the combined use of procaine and taurine with ketorolac tromethamine with an incubation time of 0-240 minutes decreased by 10.8% -68.1% compared to the control. While for the use of ketorolac tromethamine alone, the G₁₂₀ values exceeded the control by 6.9% -47.7%.

Analysis of the results of the *in vivo* experiments (Table 6) showed that the preliminary intragastric administration of taurine (7.14 mg / kg) and procaine (1.07 mg / kg) for 7 days reduces the damaging effect of ketorolac tromethamine at a UD50 level (0.94 mg/kg) for a population of mid-resistant red blood cells under conditions of acid hemolysis. This is confirmed by the fact that the acid resistance of the main erythrocyte population when using ketorolac tromethamine at a dose of UD50 is reduced by 64.3% relative to control, and with the preventive administration of taurine and procaine – by 30.9%. At the same time, against the background of combined use of taurine and procaine with ketorolac tromethamine, the proportion of erythrocytes which are simultaneously at the stage of spherulation is increased by 115.99% and 93.2% compared to the use of ketorolac tromethamine alone. Also in both cases of combined use, the erythrogram fold area is reduced which indicates that the final level of hemolysis has been reached for a longer period of time.

Comparing the data of hypo-osmotic erythrograms (*in vivo*), it can be concluded that the prophylactic use of taurine (7.14 mg / kg) and procaine (1.07 mg / kg) before administration of ketorolac tromethamine at a dose of UD50 (0.94 mg / kg) decreases the number of latent defects in erythrocyte membranes, as evidenced by a decrease in the rate of hypo-osmotic hemolysis with prophylactic administration of taurine and procaine by 77.3% -70.3% and G₁₂₀ by 58.58% -50.9%, respectively, compared to the use of ketorolac tromethamine alone.

Study of the effect of diclofenac sodium, ketorolac tromethamine, procaine, taurine and their combinations on the optical properties of proteins

In experiments *in vitro*, diclofenac sodium was injected into the hemoglobin solution at concentrations of 0.78×10^{-5} ; $1.57 \cdot 10^{-5}$; $3.14 \cdot$

10^{-5} mol/L and ketorolac tromethamine ($6.21 \cdot 10^{-7}$, $1.24 \cdot 10^{-6}$, $2.48 \cdot 10^{-6}$ mol/L) against the background of a 30-minute incubation at 55 ° C promotes an increase in light absorbance, and hence the denaturation depth by 15.1%; 17.82%; 42.15% and 14.35%; 19.4%; 35.65% respectively, compared to the control. These changes are evidently caused by a decrease in the number or weakening of intramolecular bonds (hydrogen bonds) that stabilize the space structure of the protein, which manifest themselves as a dose-dependent increase in the denaturation level under conditions of thermal incubation compared to the control. The introduction of procaine into the solution of oxyhemoglobin at concentrations of $4.9 \cdot 10^{-6}$ mol/L; $1 \cdot 10^{-5}$ mol/L; $2.01 \cdot 10^{-5}$ mol/L was accompanied by changes in the light absorbance, which nature was opposite to the experiments with sodium diclofenac and ketorolac tromethamine, which was reflected in a decrease in the denaturation depth by 2.95%; 9.44% 17.6%, respectively, compared to the control. These changes may indicate some consolidation of the protein molecule under these conditions. A single application of taurine does not cause a change in the optical properties of this biopolymer under conditions of thermal incubation compared to the control. The combined use (in *in vitro* experiments) of procaine (4.9×10^{-6} mol/L) and taurine (7.2×10^{-5} mol/L) with ketorolac tromethamine (1.24×10^{-6} mol/L) demonstrated the denaturation depth to exceed the control by 7.43% -15.1%, respectively. When ketorolac was used at this dose alone, the light absorbance exceeded the control by 19.44%. This indicates a decrease in the denaturation depth for oxyhemoglobin under the influence of procaine in combination with ketorolac tromethamine compared to the use of the latter alone. The results of studying the optical properties of oxyhemoglobin, modified by ketorolac tromethamine and its combined use with procaine and taurine in *in vivo* experiments were fairly close to the control and showed no statistically reliable differences from the control, but a tendency to decrease the degree of denaturation against the background of preliminary use of procaine and taurine was observed.

Study of the effect of sodium diclofenac, ketorolac tromethamine, procaine, taurine and

their combinations on the buffer properties of proteins

In experiments *in vitro*, sodium diclofenac at concentrations of $0.78 \cdot 10^{-5}$; $1.57 \cdot 10^{-5}$; $3.14 \cdot 10^{-5}$ mol/L was found to cause conformational changes in the oxyhemoglobin molecule, which are accompanied by an increase in the buffer capacity of this protein due to the dissociation of H⁺. When using ketorolac tromethamine at concentrations of $6.21 \cdot 10^{-7}$; $1.24 \cdot 10^{-6}$; $2.48 \cdot 10^{-6}$ mol/L, a similar situation persisted, which was manifested by an increase in the alkaline buffer capacity mainly due to an increase in the dissociation of the NH⁺ groups of the imidazole ring of histidine, terminal α-amino groups (by 10.5%, 13.13%; 17.2% compared to the control, respectively) and dissociation of sulphydryl groups of cysteine, phenolic tyrosine groups, and ε-amino groups of lysine (by 8.7%, 11.76%, and 13.8% compared to the control, respectively). The use of procaine at concentrations of $4.9 \cdot 10^{-6}$ mol/L; $1 \cdot 10^{-5}$ mol/L; $2.01 \cdot 10^{-5}$ mol/L was accompanied by a decrease in the buffer capacity of hemoglobin molecules mainly due to a decrease in the dissociation of the NH⁺ groups of the imidazole ring of histidine, terminal α-amino groups (by 3.7%, 14.35%, 19.5%) and dissociation of sulphydryl groups of cysteine, phenolic tyrosine groups, ε-amino groups of lysine (by 5.7%, 7.7%, 10.8%) compared to the control. Hemoglobin modification with taurine solution at concentrations of $3.6 \cdot 10^{-5}$; $7.2 \cdot 10^{-5}$; $1.44 \cdot 10^{-4}$ mol/L was similar to the use of procaine (a decrease in the dissociation of NH⁺ groups of the imidazole ring of histidine, terminal α-amino groups by 15.4%, 18.8%, 22.6% and sulphydryl groups of cysteine, phenolic tyrosine groups, ε-amino groups of lysine by 1.9%, 4.2%, 5.51% compared to the control, respectively). These indices indicate conformational changes

caused by the use of procaine and taurine at various concentrations, which are accompanied by protein molecule consolidation and / or a decrease in the number of ionic groups available for titration, possibly due to the procaine (taurine) binding to the corresponding functional groups, which leads to a decrease in the number of ionogenic groups determined in the appropriate ranges of pH.

In combination with *in vitro* (Table 8) of ketorolac tromethamine (1.24×10^{-6} mol/L) with procaine (4.9×10^{-6} mol/L) and taurine (7.2×10^{-5} mol/L), an increase in the alkaline buffer capacity was recorded by 5.95% and 8.3%, respectively, compared to the control, mainly due to an increase in the degree of dissociation of sulphydryl groups of cysteine, phenolic tyrosine groups, and ε-amino groups of lysine, which is less than values of corresponding changes for ketorolac tromethamine alone. Therefore, it can be assumed that the combined use *in vitro* of ketorolac tromethamine with procaine and taurine to some extent prevents conformational changes in the hemoglobin molecule, which manifest themselves as molecule unfolding and increase in the number of ionic groups available for titration.

In experiments *in vivo* ketorolac tromethamine at a dose of UD₅₀ was found to cause a change in the structural and functional properties of hemoglobin due to conformational transformation of the protein space structure, possibly due to molecule unfolding caused by breaking or weakening of bonds stabilizing it. This results in an increase in the buffer capacity, while procaine and taurine in these conditions *in vivo* do not affect the hemoglobin properties specified, however, there is a tendency to weaken the ketorolac tromethamine action on hemoglobin.

Table 8

Oxyhemoglobin buffer properties under the action of ketorolac tromethamine in combination with procaine and taurine (M±m)

Name, concentration, mol/L	pH /VNaOH intervals, μL		
	3 – 5	5 – 9	9 – 11
Control	422±8.37	101±8.22	605±19.40
Ketorolac tromethamine $1.24 \cdot 10^{-6}$ + Procaine $4.9 \cdot 10^{-6}$	424±11.40	104±8.22	641±23.02*
Ketorolac tromethamine $1.24 \cdot 10^{-6}$ + Taurine $7.2 \cdot 10^{-5}$	420±15.81	109±7.42	655±15.81*

Note: * – the differences are statistically significant at p <0.05

Study of the blood coagulation system.
When studying the effect of ketorolac

tromethamine and its combined use with procaine and taurine on the coagulation system of blood, no significant changes were detected.

Discussion

Long-term administration of currently used marketed drugs that reduce the acidity of gastric juice (histamine H₂-receptor blockers, proton pump inhibitors, antacids), increase intragastric pH and are capable of causing digestive disorders, which present in the clinical picture of dyspeptic syndrome. A prolonged increase in pH, on the one hand, significantly weakens the barrier to pathogenic and opportunistic flora entering the gastrointestinal tract. Persistent suppression of gastric secretion, on the other hand, causes hypergastrinemia, which is fraught with the development of dis- and metaplastic processes in the gastric epithelium (against the background of chronic inflammation) [12].

It is known that the enteral use of procaine in gastroduodenal ulcer at doses corresponding to the therapeutic range (0.25-0.5% solution up to 30-50 ml 2-3 times a day) is well tolerated and is characterized by the absence of side effects, in particular, due to the fact that it poorly penetrates through the mucous membranes [23]. In addition, procaine has a local anesthetic effect, providing a reduction in the severity of epigastric pain. It should be emphasized that procaine blocks the ion channels of the cell membrane and does not affect the acidity of the gastric juice.

Taurine is characterized by almost complete absence of side effects. Taurine has membrane-protective properties, normalizes the ratio of cell membrane phospholipids, regulates oxidative processes and exhibits antioxidant properties, reduces the degree of apoptosis for endothelial cells, prevents excessive calcium release from cells, has a number of cardiovascular benefits, has cardio-radio- and hepatoprotective properties, participates in the conjugation of bile acids and prevents cholestasis, participates in the regulation of GABA secretion, has hypoglycemic and hypolipidemic properties [24, 25].

Nevertheless, despite a number of properties that suggest the presence of a gastroprotective effect, procaine and taurine are not currently used in the clinical practice for the prevention of NSAID-induced damage to the gastric mucosa.

New components of the mechanism of the NSAIDs damaging effect on cell membrane and

gastric mucosa have been revealed for the first time on the example of diclofenac sodium and ketorolac tromethamine in effective analgesic doses determined experimentally (diclofenac sodium 2.25 mg/kg – 9 mg/kg, ketorolac tromethamine 0.1875 mg/kg 0.75 mg/kg). These components involves changed structural and functional properties of protein molecules, which leads to a decrease in acid and hypo-osmotic resistance of cells. It has been found that procaine and taurine exhibit a membrane-protective effect, since they increase the acid and hypo-osmotic cell resistance to the action of damaging factors in *in vitro* and *in vivo* experiments. It is observed both in their use alone and in their combination with ketorolac tromethamine.

Conclusion

The mechanism of the diclofenac sodium and ketorolac tromethamine damaging effect on the gastric mucosa has been clarified, and a direct damaging effect on cell membranes has been revealed. It has been established that the use of procaine and taurine increases the resistance of cell membranes in conditions of acidic and hypo-osmotic environment, and also reduces damage to cell membranes caused by ketorolac tromethamine. The findings of the *in vitro* experiments are confirmed by *in vivo* experiments, demonstrating ability of procaine and taurine in their preventive administration to reduce the number of experimental erosive ulcers and changes of the gastric mucosal morphological structure induced by ketorolac tromethamine use. Obviously, the revealed efficiency of procaine in treating NSAID-gastropathy is associated with the ability to interact with membrane proteins and protein-lipid membrane complexes, causing changes in their conformation, and thereby changing the structural and functional properties of cell membranes, which leads to an increase in the permeability threshold for H⁺. The ability of taurine to inhibit ketorolac tromethamine-induced ulceration is possibly associated with its proven antioxidant and membrane-stabilizing properties, as well as the ability to normalize the ratio of phospholipids to cell membranes and the ratio of cholesterol to phospholipids. It follows that the prophylactic use of taurine (7.14 mg/kg) and procaine (1.07 mg/kg) before administration of ketorolac tromethamine (0.94 mg/kg) leads to an increase in gastric mucosal resistance. On this

evidence, original methods of protecting the gastric mucosa from the damaging effect of NSAIDs are proposed and consist of 7 days of prophylactic taurine (7.14 mg/kg) or procaine (1.07 mg/kg) dose. [26, 27, 28].

Besides, it was found that, both for the administration of ketorolac tromethamine alone and for its combined use with procaine or taurine, there were no changes in the structural organization of the liver and kidneys, the coagulogram findings did not differ from the control, indicating no hepatotoxic, nephrotoxic and hematotoxic effects.

Conclusions

1. New components of the mechanism of the damaging effect of sodium diclofenac and ketorolac tromethamine on cell membranes have been revealed; they influence the ability to induce weakening or breaking of intramolecular bonds stabilizing the protein molecule, which is associated with the dissociation of NH⁺ groups of the imidazole ring of histidine, terminal α-amino groups (not less than by 10.5% compared to the control), sulphydryl cysteine groups, tyrosine phenolic groups, and ε-amino groups of lysine (by at least 8.7%), and presents in an increase in the denaturation depth.

2. Procaine and taurine *in vitro* increase the resistance of cells to the effects of damaging factors. This is proved by an increase in: a) acid resistance of red blood cells with the addition of procaine by no more than 38.5%; b) acid and hypo-osmotic resistance of erythrocytes with taurine use by no more than 47% and 88%, respectively.

3. The experiments *in vitro* and *in vivo* have shown that procaine and taurine reduce the damage to cell membranes caused by ketorolac tromethamine at a concentration equivalent to ED₅₀ and a dose equal to UD₅₀: a) procaine at a concentration equivalent to a minimum therapeutic dose of 1.07 mg / kg – by 28% and 19.7%, respectively, and reduces the formation of latent membrane defects by no less than 69%; b) taurine at a concentration equivalent to an average therapeutic dose of 7.14 mg / kg – by 54% and 19.7%, respectively, and reduces the formation of latent defects by at least 74%; c) oral prophylactic procaine administration to rats at a dose of 1.07 mg / kg and taurine at a dose of 7.14 mg / kg reduces the number of erosive and ulcerative

defects caused by ketorolac tromethamine at a dose equal to UD₅₀ by 87% and 90%, respectively, and also prevents changes in the gastric mucosal morphological structure according to the histological studies.

4. Oral prophylactic procaine (at a dose of 1.07 mg / kg) and taurine (7.14 mg / kg) administration before the damaging action of ketorolac tromethamine (0.94 mg / kg dose) is associated with no changes in the liver and kidney histoarchitecture, which is indicative of no hepato- and nephrotoxic effects.

Conflicts of interest

The authors have no conflict of interest to declare.

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**EVALUATION OF THE INFLUENCE OF COMBINATIONS OF DRUGS FOR GENERAL ANESTHESIA ON CHANGE OF ACTIVITY OF STRESS-LIMITING AND STRESS-REALIZING LINKS
ON THE CLINICAL MODEL OF ACUTE STRESS DAMAGE**

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Abstract

Introduction: When a person is in a state of anesthesia – sedation, the realization of the stress reaction is carried out through the mesocortical – limbic system, while performing intensive therapy outside sedation – through the amygdala and the hippocampus. In this regard, the response of the stress system under anesthesia and outside it will be different and, consequently, the evaluation of reactions during anesthesia is extremely interesting and necessary for targeted (individual) choice of combinations of drugs for anesthesia, depending on their effect on the links of the stress system. The more interesting is the response of the stress system in the conditions of the existing pathology, which in itself is accompanied by a stressful response.

Objectives: Evaluation of the reaction of the stress system (with the identification of age-specific features), determining the response characteristics of the stress-realizing and stress-limiting links, determining the total response of the stress system, depending on the combination of drugs used for general anesthesia on the clinical model of general surgical acute stress (adults and children).

Methods: Multicentre open clinical trial (2003-2015). This article presents the data of the group without the syndrome of intracranial hypertension (WSICH) – a general surgical group: 78 adults and 87 children. The group without SICH was interesting, as a group of "typical response" to stress in the form of trauma, emotional stress, pain factor. Two types of total intravenous anesthesia were used: standard (SA) – fentanyl, propofol and modified (MA) – fentanyl, propofol, ketamine, sodium oxybate. The markers of activity of the stress system (cortisol, corticotropin releasing factor (CTRF), β -endorphin, serotonin, histamine) were studied, the concentrations of interleukins in the blood serum (IL-4, IL-6, TNFa, IL-1 β , IL-2). The study was conducted in the preoperative period and on the 5th-7th day after the operation. Statistical processing of the results was carried out.

Results and discussion: The normal initial reaction of the stress-realizing-link (SR) of the stress system was determined (the normal stress-typical stress response in adults and children) on preoperative (psycho-emotional) and pain (traumatic) stress, which is expressed in: normal level of cortisol (adrenal level); normal level of CTRF (hypothalamic level); increased level of histamine; The normal level of IL1; normal level of IL6; increased IL2; decrease in the level of TNFa. The normal initial reaction of the stress-limiting-link (SL) of the stress system is determined which is expressed in: normal serotonin level in children and increase in 30% of adults (a typical pathophysiological reaction associated with age, which demonstrates the "vulnerability" of the SL link in adults); Reduction of β -endorphin level (a discussion on the need to enhance opioid exposure in order to prevent

inadequate anesthesia); An increase in IL4 mean values. The typical reaction of the SR and SL – stress system links to the surgical intervention (surgical stress) using standard (group SA) and modified (group MA) variants of TIA was revealed. For the SR-link, increased cortisol levels, increased CTRF level, normalization of histamine level, normalization of IL1 indices, a decrease in IL6 level, an increased level of IL2, an increase in the level of TNF α . For the SL-link, serotonin level was decreased (decrease of the SL potential and the need for additional activation via NMDA receptors), increased β -endorphin level (adequacy of opioid stimulation of the SL-link), an increase in IL4 in 75% of adults and 80% of children Need for additional stimulation via GABA receptors).

Conclusions: The "norm of pathology" (or "stress-norm") of the reaction of the SL-link (activation of stress-limitation), which can be estimated as normal when it is identical to the stimulation of the SR-link, for leveling the destabilizing influence of SR-stimulation, that is, Vegetative stabilization is achieved. In contrast to the SR-link, the possibility of combinations of drugs for general anesthesia to the activity of the SL-link receptors was revealed, the "point of application" for drugs was shown, with the prospect of developing techniques (new combinations) that could affect serotonin metabolism in the brain. It is possible to introduce a new concept – stress-limiting anesthesia, which is necessary, due to the received data that "depth of anesthesia" and "adequacy of anesthesia" are not identical concepts. Preparations for general anesthesia affect, first of all, the activity of the SL-link of the stress system and it is due to their combinations based on the initial activity of the stress system that it is possible to achieve adequate stress-limiting anesthesia.

Keywords: pharmacological action, surgical stress, hormones, cytokines, anesthesia.

Introduction

The stress system is a complex regulatory complex that helps coordinate homeostasis under normal conditions and plays a key role in realizing and coordinating all changes in the body that make up an adaptive response to stressors [1]. In general, the stress system receives information from the environment and the body through a variety of sensory systems and blood flow, from the "waking" brain – through the amygdala and the hippocampus and from the "emotional" brain – through the mesocortico-limbic system [2, 3, 4]. Thus, when a person is in the state of anesthesia-narcosis-sedation, the realization of the stress response is carried out through the mesocortical-limbic system, while performing intensive therapy outside sedation through the amygdala and the hippocampus. In this regard, the response of the stress system under anesthesia and outside it will be different and, consequently, the evaluation of reactions during anesthesia is extremely interesting and necessary for targeted (individual) choice of combinations of drugs for anesthesia, depending on their effect on the links of the stress system. The more interesting is the response of the stress system in the conditions of the existing pathology, which in itself is accompanied by a stressful response.

It is enough conditionally to separate neuropeptides, mediators and cytokines according to their role in stress reactions. So, the stress-realizing part of the stress system [2, 5, 6, 7, 8, 9, 10] will include: hypothalamic hormones – corticotropin hormone (corticotropin releasing factor, CTRF), vasopressin, oxytocin, histamine, neuropeptide Y, Adrenal hormones (epinephrine, norepinephrine, dopamine), cortisol, IL 1, IL 2, IL 6 and TNF. In the implementation of the stress response, a special role is played by the "pro-inflammatory" cytokine IL 1b [11, 12, 13], which consists not only in the mediation of inflammation (produced mainly by activated macrophages, enhances the production of IL2, IL6, TNF, CRP, proliferation and B- Lymphocytes, NK cells), and the "inclusion" of the hypothalamic-pituitary system itself with a characteristic hyperthermia reaction and a cascade of stress response (stimulation of production of IL 6, CTRF). Only IL 1b [11] can penetrate through blood-brain barrier (BBB), directly stimulating the hypothalamus. Particular attention should be paid to the involvement of proinflammatory cytokines (IL 1b, TNF, IL2) in the pathogenesis of neurodegeneration, due to the latter's violation of serotonin metabolism in the brain [12, 13]. The

level of IL6 is interesting, as a cytokine realizing the stress-realizing link (SR) of the stress system [14, 15, 16] (hypothalamic-pituitary-adrenal system by increasing the level of ACTH and cortisol) and participation in stress-induced suppression of inflammatory reactions, by Inhibition of the formation of TNF and IL 1b. IL2 is one of the cytokines of the "cohort" of stress-realizing [14, 15, 16], the mechanism of action is neurodegenerative – IL2 increases the activity of tryptophan and serotonin-degrading enzyme – indolamine-2,3-dioxygenase. An increase in the activity of this enzyme contributes to a decrease in the level of both tryptophan and serotonin in the brain and this is accompanied by a weakening of serotonergic brain mechanisms. TNF (TNF) – is one of three "classical pro-inflammatory" cytokines [14, 15, 16], stimulating the stress response (stress-realizing link). Enhances the production of IL1, IL6, enhances the reuptake of serotonin [17].

The level of cortisol is interesting as the level of the resultant effect of stress-realizing mechanisms [7, 9, 10] on the one hand (adrenal level), and as the most important hormone affecting immune responses that inhibits most aspects of the immune response, including proliferation of lymphocytes, production Immunoglobulins, cytokines and inflammatory mediators and cellular toxicity, including the production of inflammatory leukotrienes. Corticotropin hormone (CTRF), one of the most interesting stress-realizing hormones (neurotransmitters) [12], responsible for the actual triggering of the central link of the stress system (hypothalamic level), is stimulated by IL 6, after activation of the last IL1. Histamine is produced in a number of organs and tissues [6, 7, 8, 9, 14], it is a broad-spectrum biologically active substance widely distributed in the CNS synapses of the parasympathetic nervous system, so in our opinion it can be used to assess the adequacy of the parasympathetic Systems for the determination of sympathetic-parasympathetic equilibrium in the work of a stress-realizing system (as a serotonin antagonist in terms of activation of NMDA receptors and development of neurodegeneration) [14, 17].

The stress-limiting part is [2, 5, 6, 7, 8, 9, 10] opioid peptides (enkephalins, endorphins, β -endorphins); GABA (GABA); Serotonin; IL 4, NO; Substance P; Acetylcholine; Hormones of the posterior hypothalamus; Hormones of the adrenal glands (A, NA, GCS, DOFA). In this case, serotonin and IL4, can be attributed to mediators (neuro-immune-endocrine), and β -endorphin [6] already to the effector part. The stress-limiting system as a system of "tight control" from the destructive effects of stress is protected by evolutionary mechanisms, many of which have not yet been studied (activated mainly by the gamma-aminobutyric acid system (GABA), opioid peptides) [15, 16, 18], but The changes revealed in it are the most dangerous for the body and therefore are most interesting from the point of view of the impact on them (including the drugs for anesthesia), because the brain's response to cytokines and neurotransmitters can be both beneficial and destructive. IL4 is a "classic" anti-inflammatory cytokine, which, according to its mechanism of action in the neuroendocrine system, is stress-limiting by suppressing production of IL-1, TNF, IL 6 [5, 12, 14, 19].

One of the most promising ways of solving the problems of inadequacy of anesthesia in anesthetics may be the study of the involvement of neuroregulatory stress-limiting systems of the brain in the implementation of compensatory and sanogenetic processes under the influence of drugs for anesthesia and without them. And also in the formation of optimal for the brain and organism of the patient level of neurovegetative stabilization during surgical intervention [3, 4, 5, 6, 7, 8, 9].

Objectives

The purpose of the study was to evaluate the reaction of the stress system (with the identification of age-related features), to determine the characteristics of the response of the stress-realizing and stress-limiting elements, to determine the total response of the stress system, depending on the combination of drugs used for general anesthesia on the clinical model of the general surgical Acute stressful effects (adults and children).

Methods

Multicentre open clinical trial. The research in 2003-2009. Was conducted in the Donetsk Regional Clinical Territorial Association: in the Neurosurgery Clinic, Neuroreanimation Unit and Anesthesiology Department, in 2009-2015. In

"Clinical Rudnichnaya Hospital": in the departments of anesthesiology and intensive care, neurosurgery. In this article, the data of the group without the syndrome of intracranial hypertension (WSICH) – a general surgical group: 78 adults and 87 children (Table 1).

Table 1

Separation of patients by subgroup (anesthetic components) in study groups

Characteristic of subgroups	Study groups (adults, n = 78)	
	I	
The type of anesthesia*	I.1	I.2
Amount of patients	31	47
Age, years (M±m)	52,2±3,3	51,7±2,6
Sex (%)		
male	52,4	32,7
female	48,6	67,3
Characteristic of subgroups	Study groups (children, n = 87)	
Amount of patients	24	63
Age, years (M±m)	12,1±4,3	11,7±3,7
Sex (%)		
male	44,4	49,7
female	55,6	50,3

Note: * – (1 – standard anesthesia, 2 – modified anesthesia)

The group without ICH was interesting, as a group of "typical response" to stress in the form of trauma, emotional stress, pain factor. This group includes patients who underwent primary surgical treatment of splinter wounds after a mine explosion injury, tangential bullet wounds, reposition of the facial skeleton, surgical treatment of diseases of the spine, etc.

The group with standard anesthesia (SA) was initial in the study (2003-2006), the patients of the group with modified anesthesia (MA) were included in the study as the regularities were revealed, so the group was recruited from 2006 to 2015. In two clinical institutions.

Anesthesia Scheme in the Standard Anesthesia Group (SA). The patients of the CA group underwent standard total intravenous anesthesia (TIA). Introductory anesthesia was carried out with propofol 1% – 1.5-4.5 mg / kg,

fentanyl 0.005% – 5-7 µg / kg. The basis of anesthesia included propofol 1% (1.5-2.5 mg / kg / h) in combination with fentanyl (5-7 µg / kg / h). The drugs were injected with a perfusor, if necessary, the rate of administration changed. The main goal was the performance of BIS 40-45. The ventilation was performed in the normoventilation mode (up to 5-7 ml / kg, pETCO2 38-42, FiO2 40%).

Scheme of the multicomponent modification of TIA (MA). Patients of the general surgical group (WSICH) were characterized as a group forming the concept of "stress-norm" or "norm-pathology" (explanation in the text, section "results and discussion").

Adult patients. Considering the normal activity of the SR-link, benzodiazepines were added to the premedication schedule (sibazone 0.5% 0.2-0.5 mg / kg). An additional anesthetic

regimen included ketamine 0.5% to 0.5-1.0 mg / kg, propofol 1% 1.5-2.5 mg / kg, fentanyl 0.005% 1.5-2.5 µg / Kg (up to 5 µg / kg). The basis of anesthesia included: continuous administration of propofol 1% – 1.5-2.5 µg / kg / h, fentanyl 0.005% – 3-5 µg / kg / h, ketamine 0.5% – 0.05 mg / kg / Hour (with the restriction of the total dose to 125 mg). The target is BIS 40-45.

Children's contingent of patients. Priority was the additional activation of the SL-link. In the premedication scheme included benzodiazepines (sibazone 0.5% 0.2-0.5 mg / kg). In the introductory anesthesia scheme, sodium oxybate was intravenously administered 20% to 5-10 mg / kg, propofol 1% 1.5-2.5 mg / kg, fentanyl 0.005% 1.5-2.5 µg / kg (up to 5 Mkg / kg). The basis of anesthesia included: continuous administration of propofol 1% 1.5-2.5 µg / kg / h, fentanyl 0.005% – 1-3 µg / kg / h, sodium oxybate 5-10 mg / kg / h. The target is BIS 40-45.

Laboratory methods of research. In this study, blood sampling was carried out in two stages: at the stage of preparing the patient for surgical intervention – with the placement of a venous catheter and at 5-7 days after surgery, taking into account the features of the immune system's biomechanisms with the development of the maximum immune response, which is not required Less than 5 days. Blood sampling, for immuno-biochemical analyzes characterizing the work of the stress system (cortisol, corticotropin releasing factor, β-endorphin) was performed in the morning hours (6.00-7.00), according to the circadian rhythm.

Given pleiotropic effects of cytokines, their activity was taken into account both for assessing the performance of the immune system and for assessing the activity of the stress system. Interleukin concentrations in the blood serum were determined on ELISA test systems-IL-4, IL-6, TNFa, IL-lβ, IL-2 (manufactured by OOO Protein Contour, St. Petersburg, by solid-phase ELISA method).

Stress-realizing (SR) link of the stress system: the level of cortisol was studied by an enzyme immunoassay (a set of reagents of the firm "AlcorBio" (Russia, St. Petersburg)), in the laboratory of the Department of Microbiology of DonNMU. M. Gorky; The level of corticotropin releasing factor (KTPF, KRG) was studied by the methods of MKA and ELISA, in accordance with

the standard protocol of analysis, which is attached to the reagent kit (IBL ACTH ELISA kit); The level of histamine was determined in the urine (the device for determining – Spectrofluorometer, JASCO, FP-770, Japan), a biochemical method in the laboratory DDC Doktmo.

Stress-limiting (SL) link of the stress-system unit: the level of β-endorphin was studied by the methods of MKA and ELISA, according to the standard protocol of analysis that goes in the kit of reagents with commercial sets of Bachem Peninsula Laboratories, Inc.; The level of serotonin was determined in urine (the device for determination – Spectrofluorometer, JASCO, FP-770, Japan), biochemical method in the laboratory DDC Doktmo.

Statistical processing of data. The obtained data were subjected to statistical processing using the program STATISTICA 6.0. An estimation of the quantitative indices for the normality of the distribution was carried out using the Kolmogorov-Smirnov agreement criterion (with the Lilliefors correction) [20]. The quantitative indicators are presented in the form $M \pm sd$, where M is the arithmetic mean, sd is the standard deviation, the median (Me), minimum and maximum values were also determined. Since the distribution law of the quantitative indicators studied differed from the normal one, the statistical significance of the differences was checked using the Kruskall-Wallis criterion (in the case of multiple independent samples). This criterion is intended to assess differences between three or more samples at the level of a feature and can be considered as a nonparametric analog of the method of dispersion single-factor analysis for unrelated samples. In the case of dependent populations, the Wilcoxon W-test was used [21]. To estimate the difference between groups, a method was used to calculate the frequency of deviation from the norm. For the indicators characterizing the qualitative characteristics, the absolute number and the relative frequency in percent (P%) with the representation error (m) were indicated, and 95% confidence interval (95% CI) of the relative value was also calculated. To test the statistical hypotheses about the differences in relative frequencies, proportions, and ratios in two independent samples, the Pearson χ^2 criterion (with the Yates correction),

the 95% CI difference, and the Fisher * ϕ criterion (the Fisher angular conversion), which is designed to compare two samples On the frequency of occurrence of the effect (sign). Fisher's angular transformation makes it possible to estimate the significance of the differences between the fractions of two samples in which the effect (feature) is registered [22]. In all statistical analysis procedures, the achieved significance level (p) was calculated, while the critical level of significance was assumed to be 0.05 [23].

Results and discussion

Comparative data of the initial change of mediators responsible for realization of a stress response (SR-link).

The level of *IL 1b* (Fig. 1) in both children and adults was within the age limit, the mean values were: 5.43 (4.4) Me = 4.1 (2.9-6.2) in adults And 5.2 (4.24) Me = 3.6 (2.7-4.7) in children. Within the age range was $74.4 \pm 4.9\%$ of adults and $75.9 \pm 4.6\%$ of children; The excess to 1.2-2.0 norms was detected in $10.3 \pm 3.4\%$ of adults and $10.3 \pm 3.3\%$ of children; The excess to 2.1-4.0 norms in $21.8 \pm 4.7\%$ of adults and $13.8 \pm 6.2\%$ of children. Its increase in 25% of both adults and children can be considered a normal typical response to a stressful situation.

The mean values of *IL 6* (Fig. 1) in this group were within the normal range and were 4.2 (2.13) Me 3.8 (2.2-6.0) in adults and 4.25 (2.15) Me 3.8 (2.2-6.0) in children. In assessing the distribution, features were revealed. So within the limits of the age norm, the indices were in $75.6 \pm 4.9\%$ of adults and $55.2 \pm 5.3\%$ of children, in $17.2 \pm 4.0\%$ of adults and $29.9 \pm 4.9\%$ of children there was an increase To 1.6-3.0 norms (which corresponded to an increase in the level of *IL 1b*, due to mediated stimulation). It was interesting to reduce this indicator to 0.8-0.9 norms in $10.3 \pm 3.3\%$ of children, and to 0.5-0.7 norms in $4.6 \pm 2.2\%$ of adults and $4.6 \pm 2.2\%$ of children. This fact spoke of a possible defect in the response of the sympathoadrenal response in 15% of children, with violation of CA activation.

The mean values of *IL2* (Fig. 1) were 0.33 (0.12) Me = 0.33 (0.25-0.37) and children 0.32 (0.13) Me = 0.33 (0.23-0.37), which significantly exceeded the norm in both adults and children. Normal indicators were detected only in $10.3 \pm 3.4\%$ of adults. In $46.2 \pm 5.6\%$ of adults and $5.7 \pm 2.5\%$ of children, the *IL2* level exceeded

4.1-6.5 norms, in $10.3 \pm 3.4\%$ of adults and 49.4 $\pm 5.4\%$ Of children exceeded 2.6-4.0 norms and in $33.3 \pm 5.3\%$ of adults and $44.8 \pm 5.3\%$ of children there was an excess of up to 2.5 norms. The increase in *IL2* levels can be attributed to the typical reaction of the body to a stressful situation, requiring the activation of immune defense systems. Short-term reaction, perhaps, is the key to the absence of adverse reactions on the part of organs and systems, and the central nervous system, which, probably, for an acute stress response just does not need a "soothing" serotonin action.

The mean indices of *TNF* (Fig. 1) were: 3.44 (0.61) Me = 1.25 (0.74-2.22) in adults and 2.19 (1.18) Me = 1.5 (0.74-2.22) and did not differ significantly from age-related indicators, although they tended to increase. The most interesting was the evaluation of the intra-group distribution of this indicator, characterizing the entire heterogeneity of the response to stress. So, within the norm, the *TNF* (*TNF*) indicator was diagnosed only in $30.8 \pm 5.2\%$ of adults and $16.1 \pm 3.9\%$ of children. Exceeding more than 10 norms was revealed in $5.1 \pm 2.5\%$ of adults and $4.6 \pm 2.2\%$ of children, exceeding to 2.1-3.7 norms in $5.1 \pm 2.5\%$ of adults, exceeding to 1 , 6-1,7 norms in $10.3 \pm 3.4\%$ of adults and $13.8 \pm 3.7\%$ of children, exceeding to 1,1-1,5 norms in $9.2 \pm 3.1\%$ of children. In this case, a decrease to 0.1-0.2 norms in $5.1 \pm 2.5\%$ of adults and $4.6 \pm 2.2\%$ of children, a decrease to 0.3-0.4 in 14.1 ± 3 , 9% of adults and $14.9 \pm 3.8\%$ of children, a decrease to 0.5-0.7 rates in $19.2 \pm 4.5\%$ of adults and $18.4 \pm 4.2\%$ of children, a decrease to 0, 8-0.9 rates in 10.3 ± 3.45 adults and $16.1 \pm 3.9\%$ of children. Thus, up to 50% of adults and children had a decrease in the level of *TNF*, and up to 20% – an increase, which is interesting and perhaps suggests that in about 50% of cases there is a risk of inadequate stimulation of the SR-link.

The average level of *cortisol* (Fig. 1) was: 510 (260.5) Me = 541 (281-740) in adults and 505.99 (258) Me = 541 (281-740) in children. That did not significantly exceed the parameters of the control group. Within the limits of the norm, the level of cortisol was $79.5 \pm 4.6\%$ of adults and $90.8 \pm 3.1\%$ of children, an excess of up to 1.2-1.3 norms was found in $10.3 \pm 3.4\%$ of adults and 4 , 6 $\pm 2.2\%$ of children, an excess of 1.4-1.6 norms was found in $10.3 \pm 3.4\%$ of adults

and $4.6 \pm 2.2\%$ of children. Thus, for a normal stress reaction, the level of cortisol is within normal limits, with a moderate excess in 20% of adults and 10% of children.

The mean CTRF values (Fig. 1) were: 21.64 (11.01) Me = 18.35 (17-22) in adults and 21.37 (10.59) Me = 18.7 (16.0-22.0) in children. That did not differ significantly from the norm. Thus, within the age limit, the CTRF level was $98.9 \pm 1.2\%$ for both adults and children. Thus, a normal stress reaction is characterized by a normal KTPF level.

The average level of histamine (Fig. 1) was: 1157 (719) Me = 1154 (985-1280) in adults and 1175.3 (205.69) Me = 1170 (1085-1290), which significantly exceeded normal indices. At the same time, within the norm, the histamine index was in $25.6 \pm 4.9\%$ of adults and $23.0 \pm 4.5\%$ of children. Exceeding up to 1.2-1.4 norms in $56.4 \pm 5.5\%$ of adults and $56.3 \pm 5.3\%$ of children; The excess to 1.5-1.8 norms in $17.9 \pm 4.3\%$ of adults $20.7 \pm 4.3\%$ of children. Thus, a normal stress reaction is characterized by an increase in the level of histamine in 75% of both adults and children.

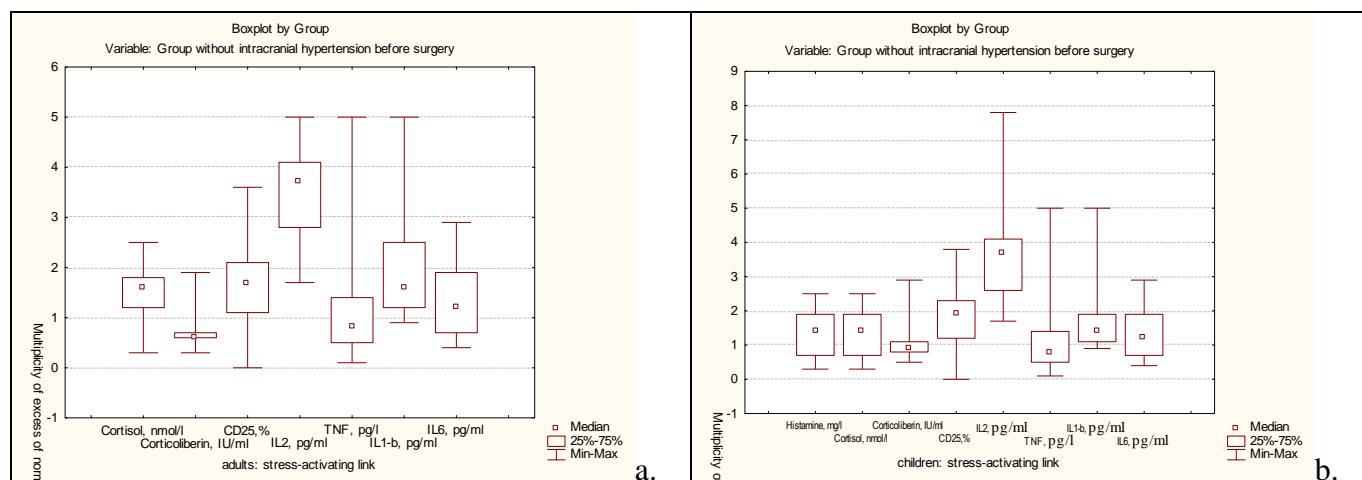


Fig. 1. The multiplicity of the excess of the stress-activating link of the stress system in adults and children
(a – adults, b – children)

Thus, the first result of the study was the determination of the normal reaction of the SR-link of the stress system (Fig. 1a, b) (estimated normal stress response in adults and children), which was expressed in: normal level of cortisol (adrenal level), with an excess of 20 % Of adults and 10% of children; Normal level of CTRF (hypothalamic level); An increased level of histamine in 75% of both adults and children; The normal level of IL1 (with a possible increase to 4.0 in 25% of patients); The normal level of IL6 (with a possible increase to 3.0 in 20% of patients); Increased level of IL2 (typical reaction of the body to a stressful situation, requiring the activation of immune defense systems); Decrease in the level of TNF (in 50% of adults and children).

Comparative data of changes in mediators responsible for realizing the stress response (SR-link) after operation using total intravenous

anesthesia (TIA) based on fentanyl and propofol (standard anesthesia group – SA).

Mean values of IL1 (Fig. 2a, 3a) in the postoperative period tended to decrease from preoperative indicators and were within the normal range. The mean values were 2.7 (1.16) Me = 2.9 (2.1-3.3) in adults and 2.64 (1.15) Me = 2.8 (2.1-3.25) In children. Within the norm, the indicator was $97.0 \pm 2.9\%$ of adults (more than 20% before the operation) and $96.2 \pm 3.7\%$ of children (also 20% higher than before the operation). Thus, surgery / anesthesia led to the normalization of IL1 values in 95% of both adults and children, demonstrating a decrease in the index compared with preoperative data in 20% of patients.

The mean values of IL6 (Fig. 2a, 3a) showed a decrease in the postoperative period, relative to preoperative data and relative to the norm. The mean IL6 was 3.9 (0.36) Me = 2.9 (2.1-4.4) in adults and 4.0 (0.4) Me = 2.8 (2.1-4.4) In

children. The majority of patients were patients with a decrease to 0.1-0.2 norms – $51.6 \pm 9.0\%$ in adults and $58.3 \pm 10.0\%$ in children, a decrease to 0.2-0.4 norms Revealed in $45.2 \pm 8.9\%$ of adults and $35.7 \pm 9.9\%$ of children. Thus, surgery / anesthesia resulted in a marked decrease in IL6 in 95% of both adults and children, with a decline in the relative preoperative data in 90% of adults and 70% of children.

Changes in IL2 (Fig. 2a, 3a) had a similar tendency to decrease with respect to preoperative data, but remained significantly higher than normal. The mean values were 0.28 (0.13) Me = 0.25 (0.19-0.35) in adults and 0.26 (0.14) Me = 0.24 (0.17-0.33) In children. Within the norm, the indicator was in $25.8 \pm 7.9\%$ of adults (more by 15% than before the operation) and $8.3 \pm 5.6\%$ of children (more by 5% than before the operation). The main group consisted of patients with an excess of 1.0-2.5 norms – $38.7 \pm 8.7\%$ of adults (more 5% than before the operation) and $62.5 \pm 9.9\%$ of children (20% more than Before the operation), exceeding to 2,6-4,0 norms in $9.7 \pm 5.3\%$ of adults and $25.0 \pm 8.8\%$ of children (less by 25% than before the operation), exceeding to 4,1- 6.5 norms in $25.8 \pm 7.9\%$ of adults (less by 20% than before the operation) and $4.2 \pm 4.1\%$ of children. Thus, in the postoperative period, the level of IL2 remained elevated, being within the normal range in 25% of adults (more by 15% than before the operation) and 8% of children (more by 8% than before the operation), on the whole, the indicator showed dynamics to decrease The degree of excess in comparison with preoperative data in 20% of adults and 25% of children.

Mean values of TNF α (Fig. 2a, 3a) were 8.18 (3.64) Me = 7.4 (5.4-10.10) in adults and 8.4 (3.93) Me = 7, 55 (5.4-10,25) in children. The majority of patients were patients with excess of normal indices up to 3.8-10 norms – $58.1 \pm 8.9\%$ of adults (more by 55% than before the operation) and 58.3 ± 10.1 children (55% more than Before the operation); With an excess of up to 2,1-3,7 norms – $38.7 \pm 8.7\%$ of adults (more by 30% than before the operation) and $33.3 \pm 9.6\%$ of children (more by 30% than before the operation). Thus, surgery / anesthesia in patients without ICH resulted in a significant increase in the level of TNF α in 95% of adults (more by 75% than before the operation) and 95% of children (more by 75% than before the operation), with the increase in the

indicator relative to preoperative data In 50% of adults and children.

The level of cortisol (Fig. 2a, 3a), as a characteristic of the "adrenal level" of the stress response, in the postoperative period showed a significant increase in comparison with preoperative data and norm data. The mean values of cortisol (nmol / L) were 966 (160.94) Me = 962.0 (884, -1100.0) in adults and 988.63 (166.5) Me = 962.0 (884.0-1117 , 5) in children. Within the limits of the norm, the indicator was in $19.4 \pm 7.1\%$ of adults (less by 60% than before the operation) and $12.5 \pm 6.8\%$ of children (less by 75% than before the operation), exceeding to 1,2- 1.3 norms were detected in $9.7 \pm 5.3\%$ of adults and $33.3 \pm 9.6\%$ of children (more by 30% than before the operation), exceeding to 1.4-1.6 norms in $58.1 \pm 8.9\%$ of adults (more by 45% than before the operation) and $33.3 \pm 9.6\%$ of children (more by 30% than before the operation), exceeding to 1.7-2.0 norms in 19.4 ± 7 , 1% of adults (15% more than before surgery) and $20.8 \pm 8.3\%$ of children (20% more than before surgery). Thus, surgery / anesthesia led to an increase in the level of cortisol in 80% of adults (more than 70% before the operation) and 85% of children (more by 80% than before the operation), with a corresponding increase in dynamics with respect to preoperative data.

The level of CTRF (Fig. 2a, 3a), characterizing the "hypothalamic level" of the stress response, significantly increased in the postoperative period both in relation to preoperative data and in relation to the norm. The mean values were 62.74 (17.84) Me = 68.0 (54.0-74.0) in adults and 62.88 (18.41) Me = 68.0 (48.0-74.0) In children. Within the norm, the indicator was $48.4 \pm 9.0\%$ of adults (less than 50% before the operation) and $45.8 \pm 10.2\%$ of children (less than 50% before the operation), exceeding the figure to 1, 2-1,5 norms were detected in $32.3 \pm 8.4\%$ of adults (more by 30% than before the operation) and $33.3 \pm 9.6\%$ of children (more by 30%), exceeding to 1,6-1 , 8 norms in $16.1 \pm 6.6\%$ of adults (more by 15% than before the operation) and $16.7 \pm 7.6\%$ of children (also more by 15%), exceeding to 1.9-2.5% In $3.2 \pm 2.2\%$ of adults and $4.2 \pm 4.1\%$ of children (an average of 5%). Thus, surgery / anesthesia resulted in a significant increase in the CTRF level in 50% of both adults

and children, with the same increase in preoperative data.

The level of *histamine* (Fig. 2a, 3a), as a characteristic of the parasympathetic link (sympathetic-parasympathetic balance), of the stress-realizing part of the stress system, showed a tendency to decrease in the postoperative period in comparison with preoperative data, but exceeded the norm, demonstrating "Movement towards the limits of the norm." The mean values were 693.32 (190.75) Me = 665.0 (478-870) in adults and 708.33 (190.4) Me = 704.50 (550.0-870.5) in children. Within the norm, the indicator was in $83.3 \pm 6.6\%$ of adults (more by 60% than before the operation) and $95.8 \pm 4.1\%$ of children (more by 70% than before the operation). Thus, surgery / anesthesia led to the normalization of the level of histamine in 80% of adults and 95% of children, with a positive trend of "striving for norm" and a decrease in the rate in 70% of adults and 75% of children.

Thus, the following result of the study was the evaluation of the reaction of the SR-link of the stress system (Fig. 2a, 3a) to the surgical

intervention (surgical stress) using standard TIA variants (group SA). It was revealed: an increase in the level of cortisol in 80% of adults (more by 70% than before the operation) and 85% of children (more by 80% than before the operation), with a corresponding increase in dynamics with respect to preoperative data; An increase in the CTRF level in 50% of both adults and children, with an increase in relative preoperative data; Normalization of histamine levels in 80% of adults and 95% of children, with a decrease in 70% of adults and 75% of children; Normalization of IL1 indices in 95% of both adults and children; A decrease in the IL6 level in 95% of both adults and children, with a decrease in the relative preoperative data in 90% of adults and 70% of children; Increased level of IL2, with dynamics to decrease in comparison with preoperative data in 20% of adults and 25% of children; An increase in TNF α in 95% of adults (75% more than before surgery) and 95% of children (75% more than before surgery), with an increase in the rate of preoperative data in 50% of adults and children.

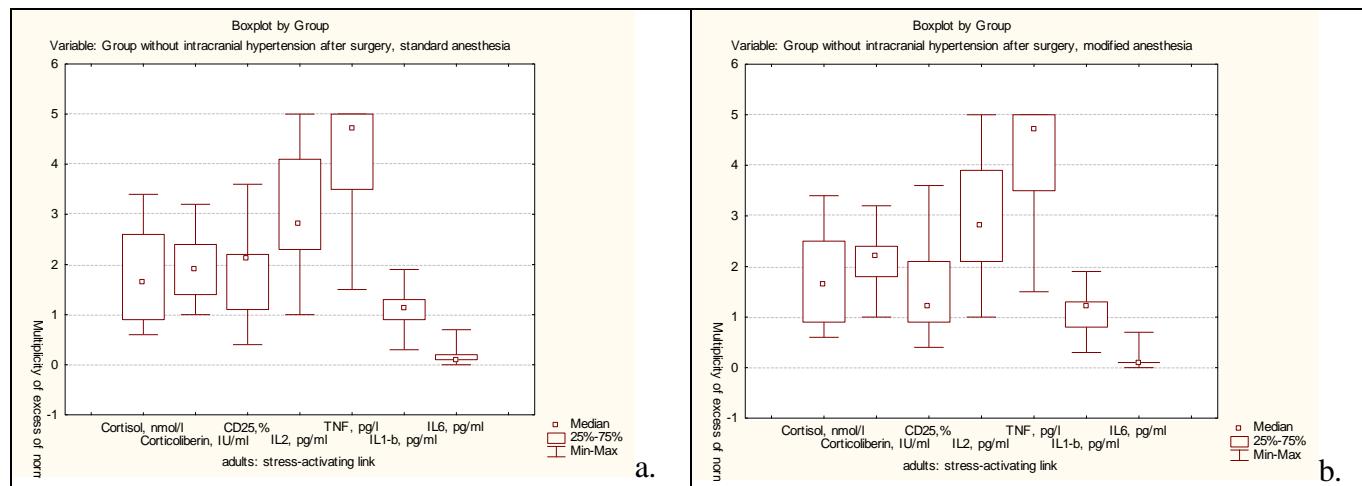


Fig. 2. Changes in the stress-activating link in adult patients of the general surgical profile (a – after surgery, standard anesthesia, b – after surgery, modified anesthesia, where 1 – average values of normal indicators)

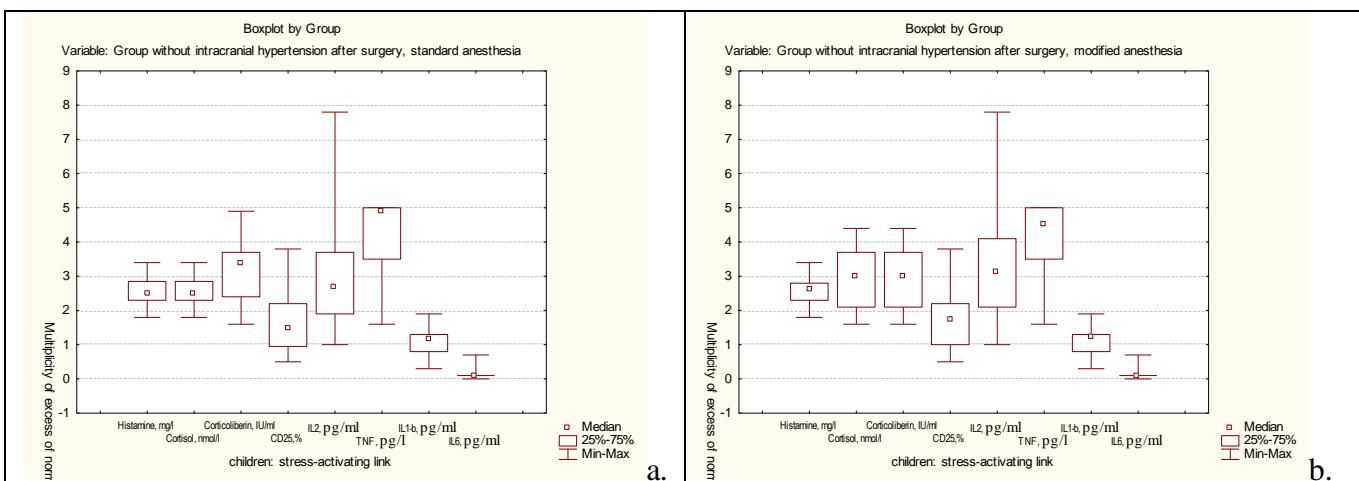


Fig. 3. Change in the indicators of stress-activating link in children of general surgical profile (a – after surgery, standard anesthesia, b – after surgery, modified anesthesia, where 1 – average values of normal indices)

Comparative data of changes in mediators responsible for realizing the stress response (SR-link) after surgery using modified anesthesia (TIA) based on fentanyl, ketamine, sodium oxybate and propofol (MA group).

In the adult group, the response of the SR-link of the stress-system unit was expressed in: the level of cortisol (Fig. 2b) was 1022.3 ± 171.1 ; Me = 998.0 (685.0 – 1325.0) nmol / L, with a significant increase in the content from preoperative data ($\chi^2 = 80.4$, df = 4, p <0.001), without significant difference with the comparison group (SA). The level of histamine (Fig. 2b) was 690.1 ± 196.4 ; Me = 665.0 (412.0 – 1085.0) mg / l, with a significant decrease to normal ($\chi^2 = 57.7$, df = 3, p <0.001), without significant difference with the comparison group (SA). The CTRF level (Fig. 2b) was 62.15 ± 19.83 ; Me = 59.0 (32.0 – 98.0) IU / ml, with a significant increase in the content from preoperative data ($\chi^2 = 44.3$, df = 3, p <0.001), without significant difference with the comparison group (SA). The level of IL2 (Fig. 2b) was 0.30 ± 0.14 ; Me = 0.25 (0.09-0.70) pg / ml, with a significant increase from the norm (p <0.001), without significant difference with preoperative data and the comparison group (SA). The level of TNF (Fig. 2b) was 8.13 ± 3.95 ; Me = 7.40 (2.40 – 17.70) pg / l, with a significant increase in the content from preoperative data ($\chi^2 = 99.8$, df = 8, p <0.001), without significant difference with the comparison group (SA). The level of IL1-b (Fig. 2b) was 2.70 ± 1.01 ; Me = 2.70 (0.70-4.70) pg / ml with a significant decrease to normal ($\chi^2 = 57.7$, df = 3, p <0.001),

without significant difference with the comparison group (SA). The level of IL6 (Fig. 2b) was 0.47 ± 0.47 ; Me = 0.33 (0.1-2.1) pg / ml with a significant decrease below the norm ($\chi^2 = 112.8$, df = 4, p <0.001), without significant difference with the comparison group (SA).

In the group of children, the reaction of the SR-link of the stress-system unit was expressed in: the level of cortisol (Fig. 3b) was 988.6 ± 166.5 ; Me = 962.0 (685.0 – 1325.0) nmol / L, with a significant increase in the content from preoperative data ($\chi^2 = 89.3$, df = 3, p <0.001), without significant difference with the comparison group (SA). The level of histamine (Fig. 3b) was 708.3 ± 190.4 ; Me = 704.5 (412.0 – 1085.0) mg / l, with a significant reduction to normal ($\chi^2 = 76.8$, df = 3, p <0.001), without significant difference with the comparison group (SA). The level of KTPF (Fig. 3b) was 62.88 ± 18.41 ; Me = 68.0 (32.0 – 98.0) IU / ml, with a significant increase in the content of preoperative data ($\chi^2 = 51.7$, df = 2, p <0.001), without significant difference with the comparison group (SA). The level of IL2 (Fig. 3b) was 0.26 ± 0.14 ; Me = 0.24 (0.09 – 0.70) pg / ml, with a significant increase from the norm and from preoperative data ($\chi^2 = 7.99$, df = 3, p = 0.046), without significant difference with the comparison group (SA). The level of TNF (Fig. 3b) was 8.40 ± 3.93 ; Me = 7.55 (2.40 – 17.70) pg / l, with a significant increase in the content from preoperative data ($\chi^2 = 134.0$, df = 9, p <0.001), without significant difference with the comparison group (SA). The level of IL1-b (Fig. 3b) was 2.64 ± 1.15 ; Me = 2.80 (0.70-4.70) pg / ml with a significant

decrease to normal ($\chi^2 = 17.7$, df = 2, p <0.001), without significant difference with the comparison group (SA). The level of IL6 (Fig. 3b) was 0.40 ± 0.40 ; Me = 0.28 (0.1-2.1) pg / ml with a significant decrease below the norm ($\chi^2 = 138.4$, df = 5, p <0.001), without significant difference with the comparison group (SA).

Thus, in spite of different numerical data, the reaction of the stress-realizing link of the stress system under the influence of a modified version of anesthesia (another combination of drugs for general anesthesia) under the conditions of identical surgical stress can be estimated as "typical", without significant differences in the response by age And the direction of changes in the average data, between the applied types of anesthesia (Fig. 4). This characterizes the reaction

as a stress-realizing (hyper-stress) – protective, which made it possible to determine the "norm of pathology" for the reaction of the SR-link of the stress system, starting from which it became possible to evaluate the reaction of the stress system in other pathologies and species (combinations) Anesthesia. It was proved that combinations of drugs for general anesthesia (in manufacturer's recommended dosages) could not influence the activity of the SR-link of the stress system in patients of the general surgical group.

For visual presentation of the changes, the coefficients of exceeding the stress-system indicators were summarized, calculating the difference in the changes from preoperative data (Δ before-SA and Δ before-MA) (Fig. 4).

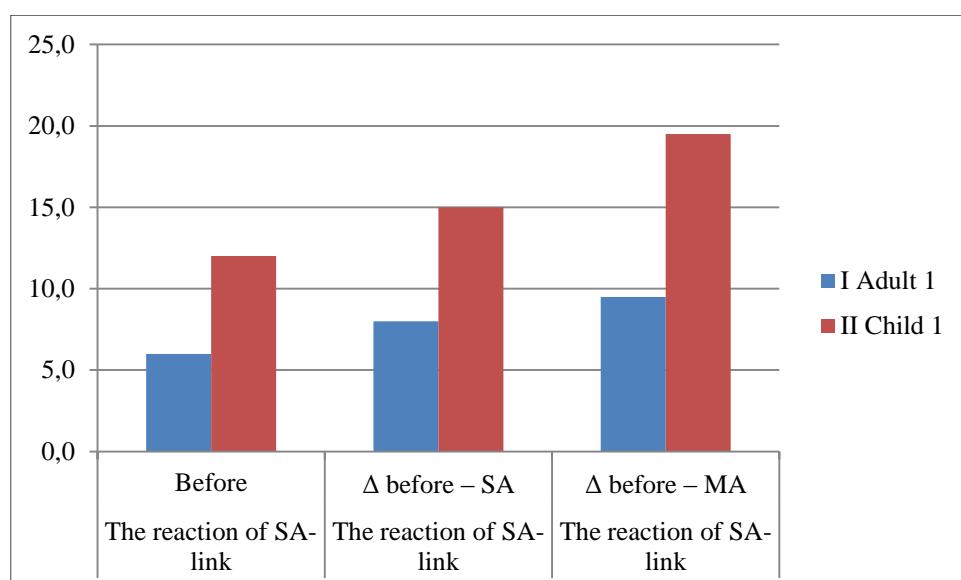


Fig. 4. The total typical evaluation of the reaction of the stress-activating -link of stress system («pathology norm») in adults and children in the preoperative period and after standard and modified anesthesia, where the indicators within the limits of the norm are taken as 1, the excess of the indicator (times deviations from the norm) – «+2, +4, etc.», decrease in the indicator (times by deviations from the norm) – «-2, -4, etc.», the value of the indicator, which has up to 50% of the results within the norm, And the remaining increase is «+0,5», the value of the indicator, which has up to 50% of the results of the decrease from the norm – «-0,5»

Comparative data of the initial change of mediators responsible for the limitation of stress response (SL-link).

The average level of serotonin (Fig. 5a, b), as a powerful endogenous antistress and neuroprotective factor (blockade of the glutamate cascade, due to interaction with NMDA receptors) was 1096.62 (30.9-14) Me = 999.0

(945 -1187.0) in adults and 1095.82 (302.6) Me = 999.0 (965-1200.0), which, although it tended to exceed, but within the norm was $70.5 \pm 5.2\%$ Of adults and $99.4 \pm 0.6\%$ of children. It is characteristic that 30% of adults noted an excess of this indicator (a feature associated with age, the course of a typical pathophysiological reaction). Thus, up to 1.4-1.8 serotonin levels were elevated

in $14.1 \pm 3.8\%$ of adults; Up to 1,1-1,3 norms in $15.4 \pm 4.1\%$ of adults.

β -endorphin (Fig. 5a, b), the mean β -endorphin values (pmol / L) were: 2.98 (0.97) Me = 3.0 (2.3-3.3) in adults and 2 , 94 (0.94) Me = 2.9 (2.2-3.2), which, on the whole, tended to decrease relative to the norm group. The level of β -endorphin was within the normal range in $65.4 \pm 5.4\%$ of adults and $64.4 \pm 5.1\%$ of children. Nevertheless, a decrease to 0.5-0.6 norms revealed $10.3 \pm 3.4\%$ of adults; A moderate decrease to 0.7-0.8 norms in $24.4 \pm 4.9\%$ of adults and $25.3 \pm 4.7\%$ of children. Thus, a moderate decrease in the level of β -endorphin detected in patients with a moderate "inhibition" of the central SL response may be a variant of the normal course of the stress response when stimulation of the stress-realizing link of the stress system is necessary for an adequate physiological response.

The mean values of IL4 (Fig. 5a, b) were: 5.23 (4.64), Me = 4.15 (1.99-6.60) in adults and 5.17 (4.41) Me = 4 , 2 (2,2-5,2) and significantly

exceeded the age norm, which can be considered a variant of a normal stress response, when along with stress-realizing mechanisms, stress-limiting, equalizing and regulating responses are triggered in strength. In $25.6 \pm 4.9\%$ of adults and $31.0 \pm 5.0\%$ of children, the indicator was normal. Thus, over 10 norms were diagnosed in $5.1 \pm 2.5\%$ of adults and $4.6 \pm 2.2\%$ of children; The excess to 6.0-10.0 norms in $10.3 \pm 3.4\%$ of adults and $4.6 \pm 2.2\%$ of children; Excess to 3.1-5.0 norms in $24.4 \pm 4.9\%$ of adults and $14.9 \pm 3.8\%$ of children; Excess to 1.5-3.0 norms in $34.6 \pm 5.4\%$ of adults and $42.5 \pm 5.3\%$ of children; The excess to 1.3-1.4 norms in $5.7 \pm 2.5\%$ of children. Thus, the excess of the IL4 index (in 70% of patients) can be considered as a prognostically favorable direction of the stress-limiting reaction of the stress system aimed at the regulation of the T and B immunity systems, monocyte-macrophage system (MMS). The level of increase, possibly, depends on the strength of aggressive influence, the question of the duration of the favorable influence of the revealed changes remains open.

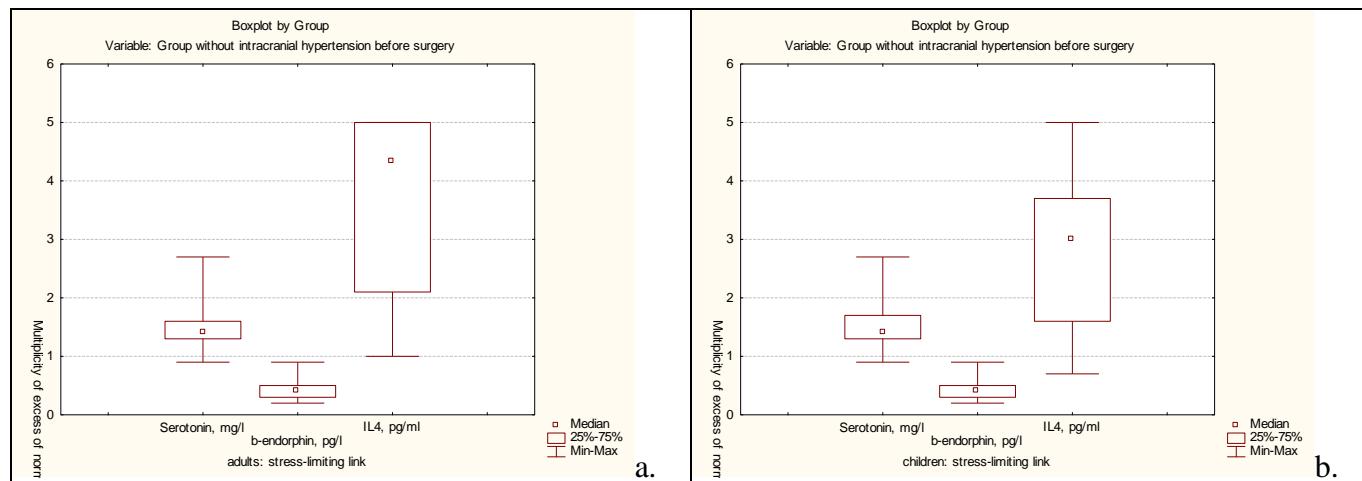


Fig. 5. Multiplicity of excessing indicators of the stress-limiting link of the stress system in adults and children
(a – adults, b – children)

Thus, the following result of the study was the determination of the normal response of the SL-link in the stress system (Fig. 5a, b) (estimated normal stress response in adults and children), which was expressed in: normal serotonin levels in children and an increase in 30% of adults A characteristic of a typical pathophysiological reaction associated with age, which demonstrates the "vulnerability" of the SL link in adults); Reduction of β -endorphin level (a discussion on

the need to enhance opioid exposure in order to prevent inadequate anesthesia); An increase in IL4 mean values.

Comparative data of changes in mediators responsible for the limitation of the stress response (SL-link) after operation using total intravenous anesthesia (TIA) based on fentanyl and propofol (standard anesthesia group – SA).

Mean values of *IL4* (Fig. 6a, 7a) were significantly elevated in the postoperative period both in relation to preoperative data and in relation to the norm, "balancing" the multidirectional changes in the system of stress-realizing / pro-inflammatory cytokines (see Fig. 2, 3). The mean IL4 was 4.62 (2.37) Me = 5.2 (2.2-5.89) in adults and 4.88 (2.17) Me = 5.2 (4.16-5.96) In children. Within the norm, the indicator was in $22.6 \pm 7.5\%$ of adults and $20.8 \pm 8.3\%$ of children (less by 10% than before the operation); The main group consisted of patients with excess to 3.1-5.0 norms – $58.1 \pm 8.9\%$ of adults (more by 30% than before the operation) and $16.7 \pm 7.6\%$ of children (without dynamics); And with an excess of 1.5-3.0 norms (more in children) – $9.7 \pm 5.3\%$ of adults (less than 25% before the operation) and $62.5 \pm 9.9\%$ of children (more by 20 % Than before the operation); The excess to 6.0-10.0 norms – in $9.7 \pm 5.3\%$ of adults. Thus, despite the increased average rates, in the postoperative period in patients of the SA group, the level of IL4 had a dynamics to decrease. Operation / anesthesia resulted in a significant increase in IL4 in 75% of adults and 80% in children (more than 10% before surgery) – a normal "protective" response of the SL link, with a decrease in the rate of preoperative data in 5% of adults and 10% Children – the prevalence of the destructive effect of SA activation on the SL reaction.

The serotonin level (Fig. 6a, 7a) was 670.94 (322.56) Me = 741.0 (254-947) in adults and 673.13 (311.24) Me = 471.0 (537.5- 947.0) in children. Within the norm, the indicator was in $74.2 \pm 7.9\%$ of adults and $79.2 \pm 8.3\%$ of children (less by 20% than before the operation); A decrease to 0.6-0.8 norms was found in $12.9 \pm 6.0\%$ of adults (more by 10% than before the operation) and $4.2 \pm 4.1\%$ of children; A decrease to 0.3-0.5 rates in $12.9 \pm 6.0\%$ of adults (20% more than before surgery) and $16.7 \pm 7.6\%$ of children (more by 15% than before surgery).

Thus, surgery / anesthesia led to a decrease in serotonin levels in 25% of adults and 20% of children, demonstrating a decline in the relative preoperative data in 55% of adults and 20% of children, which characterizes the decrease in the SL potential.

The level of β -endorphin (Fig. 6a, 7a) in the postoperative period was significantly increased in comparison with preoperative data and tended to exceed the norm values. The mean values were 12.52 (3.92) Me = 13.2 (9.2-16.2) in adults and 12.38 (4.04) Me = 13.65 (9.0-16.05) In children. Within the limits of the norm, the indicator was in $41.9 \pm 8.9\%$ of adults (less by 25% than before the operation) and $37.5 \pm 9.9\%$ of children (less by 25% than before the operation), exceeding to 1,2-1.5 norms were found in $45.2 \pm 8.9\%$ of adults (more by 45% than before the operation) and $50.0 \pm 10.2\%$ of children (more by 50% than before the operation), excess to 1.6- 2,0 norms in $12.9 \pm 6.0\%$ of adults (more by 10% than before the operation) and in $12.5 \pm 6.5\%$ of children (more by 10% than before the operation). Thus, the operation / anesthesia led to a significant increase in the level of β -endorphin in 55% of adults and 60% of children, which demonstrates the adequacy of the opioid effect on the receptors of the SL-link in the stress system.

Thus, the following result of the study was an evaluation of the response of the SL-link of the stress system (Fig. 6a, 7a) to the surgical intervention (surgical stress) using standard TIA variants (group SA). A decrease in serotonin levels in 25% of adults and 20% of children (decrease in the SL potential and the need for additional activation via NMDA receptors), an increase in β -endorphin in 55% of adults and 60% of children (adequacy of opioid stimulation of the SL link), An increase in IL4 in 75% of adults and 80% of children (with a decrease in 5% in adults and 10% in children, the need for additional stimulation via GABA receptors).

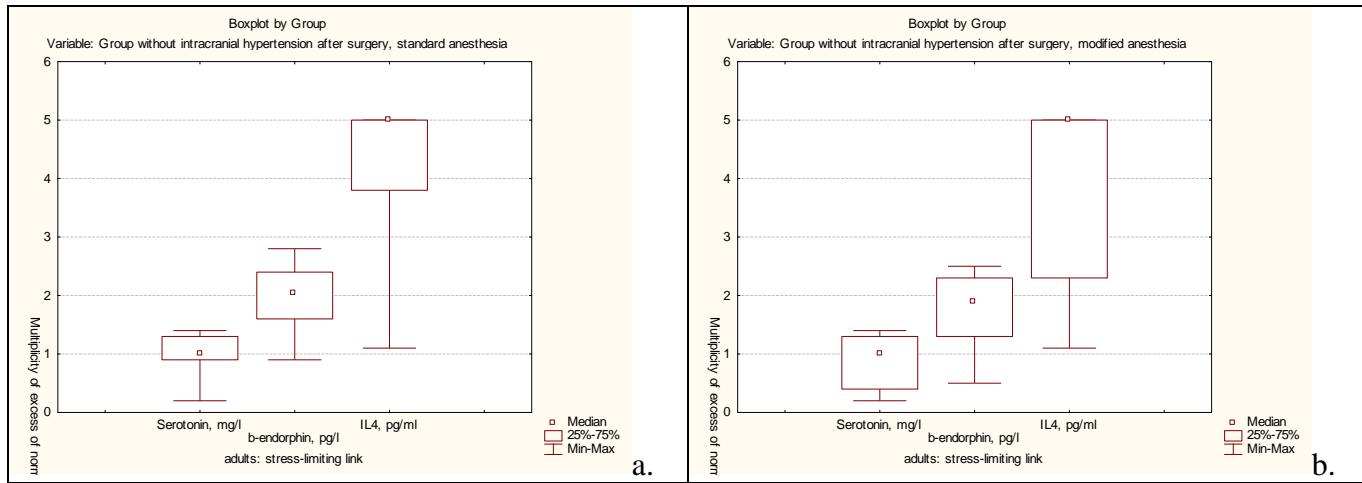


Fig. 6. Changes in the indicators of stress-limiting link in adult patients of general surgical profile
(a – after surgery, standard anesthesia, b – after surgery, modified anesthesia,
where 1 – average values of normal indices)

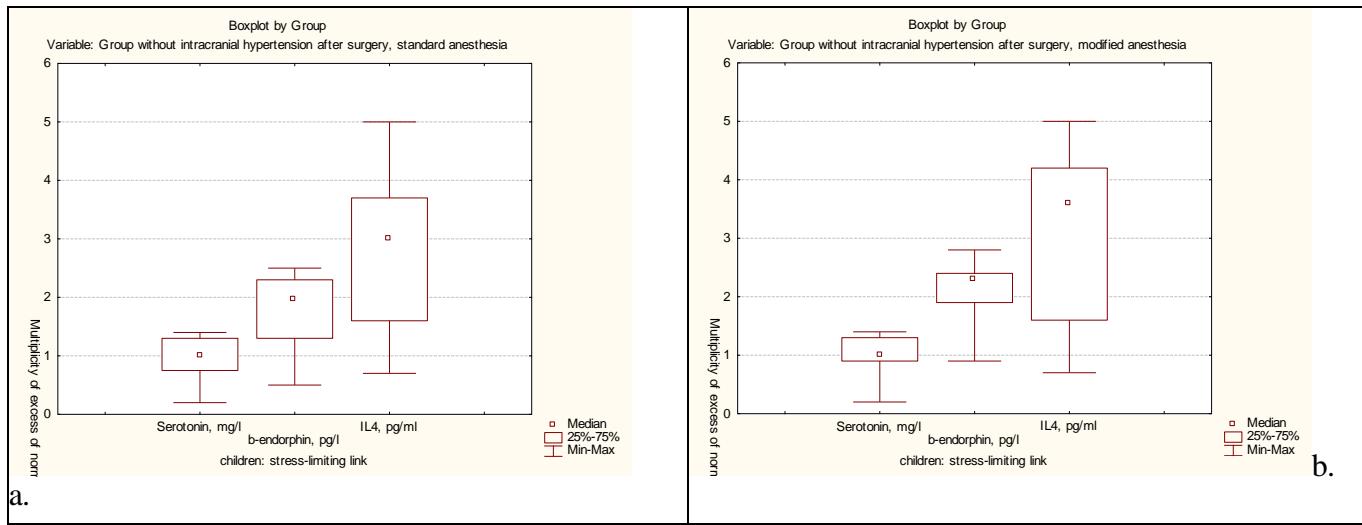


Fig. 7. Changes in the stress-limiting link in children of the general surgical profile (a – after surgery, standard anesthesia, b – after surgery, modified anesthesia, where 1 – mean values of normal indicators)

Comparative data of changes in mediators responsible for the limitation of the stress response (SL-link) after surgery using modified anesthesia (TIA) based on fentanyl, ketamine, sodium oxybate and propofol (MA group).

In the group of adult patients in the postoperative period (MA group), the reaction of the SL-link of the stress system was expressed in: a significant decrease to the norm of serotonin level (Fig. 6b) (mean values 709.8 ± 290.7 , Me = 747.0 (112 , 0 – 999.0)), compared with preoperative data ($\chi^2 = 27.7$, df = 4, p < 0.001), without significant difference with the comparison group (SA), with a tendency to decrease the degree of decline in 10% in Group MA (p = 0.064).

The level of β -endorphin (Fig. 6b) was 14.39 ± 3.90 ; Me = 14.90 (6.50 – 19.80) pmol / L, with a significant excess of preoperative data ($\chi^2 = 83.6$, df = 4, p < 0.001), without significant difference with the comparison group (SA).

The level of IL4 (Fig. 6b) was 4.96 ± 2.48 ; Me = 5.20 (1.04 – 9.80) pg / ml with a significant excess of preoperative data ($\chi^2 = 18.4$, df = 4, p = 0.001), without significant difference with the comparison group (SA).

In the group of children in the postoperative period (MA group), the response of the SL-link of the stress system was expressed in: a significant decrease in the level of serotonin (Fig. 7b) (mean values 673.1 ± 311.2 , Me = 741.0 (112.0 – 999.0)), compared with preoperative data

($\chi^2 = 23.0$, df = 2, p <0.001), without significant difference with the comparison group (SA).

The level of β -endorphin (Fig. 7b) was 12.38 ± 4.04 ; Me = 13.65 ($3.80 - 17.80$) pmol / l, with a significant excess of preoperative data ($\chi^2 = 115.1$, df = 4, p <0.001), without significant difference with the comparison group (SA), with The tendency to increase the degree of excess in 20% (p = 0.052) in the MA group.

The level of IL4 (Fig. 7b) was 4.88 ± 2.17 ; Me = 5.20 ($1.04 - 9.80$) pg / ml with a significant excess of preoperative data ($\chi^2 = 11.4$, df = 5, p = 0.044), without significant difference with the comparison group (SA).

Thus, as in the case of the SA-link reaction described above, the reaction of the stress-limiting (SL) – link of the stress system under the

influence of a modified variant of anesthesia (another combination of drugs for general anesthesia) under identical surgical stress can be estimated as " ", Without significant differences in the response by age and direction of changes in mean data, between the types of anesthesia used (Fig. 8). The reaction can be evaluated as stimulation of the SL-link, identical stimulation of the SR-link, for leveling the destabilizing effect of SR-stimulation. Unlike the SR-link, the tendency of combinations of drugs for general anesthesia to affect the activity of the SL-link receptors has been revealed, that is, the application point for drugs has been shown, with the prospect of developing techniques (new combinations) that can affect serotonin metabolism.

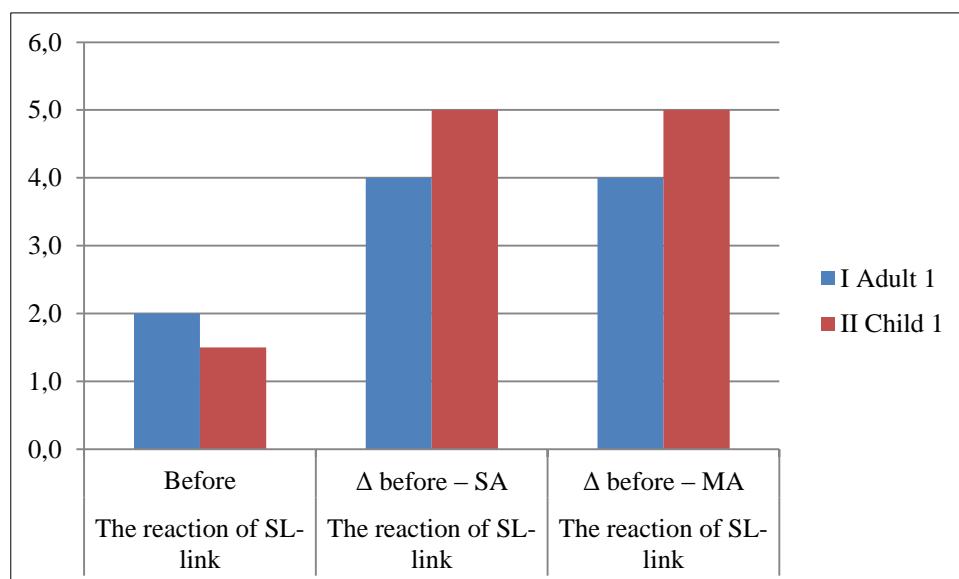


Fig. 8. The total typical evaluation of the response of the SL-link of the stress system (the «pathology norm») in adults and children in the preoperative period and after standard and modified anesthesia, where the parameters within the limits of the norm were taken as 1, the excess of the indicator (times deviations from the norm) – «+2, +4, etc.», decrease in the indicator (times by deviations from the norm) – «-2, -4, etc.», the value of the indicator, which has up to 50% of the results within the norm, And the remaining increase is «+0,5», the value of the indicator, which has up to 50% of the results of the decrease from the norm – «-0,5»

The main result of the study is that the data for the first time have been obtained that allow us to evaluate the normal reaction of the stress system links and the stress system as a whole

("stress rate" or "rate of pathology") for surgery (surgical stress) and combinations of drugs for general Anesthesia (Fig. 9).

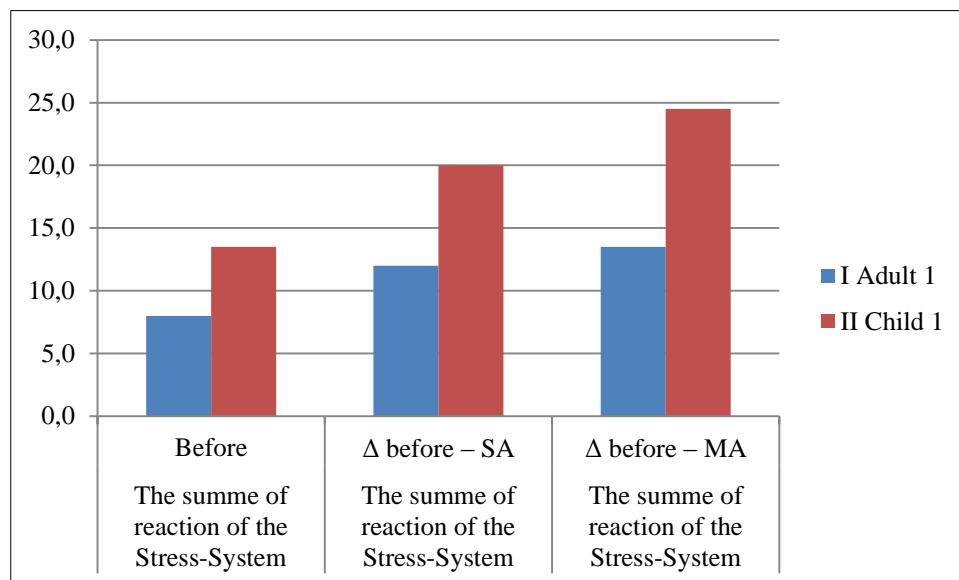


Fig. 9. The total typical evaluation of the reaction of the stress system in adults and children (the «pathology norm» or «stress norm») in the pre- and postoperative periods (SA and MA), where the indices within the norm are taken as 1, – deviation from the norm) – «+2, +4, etc.», decrease in the indicator (times by deviations from the norm) – «-2, -4, etc.», the value of the indicator, which has up to 50% Results within the limits of the norm, and the remaining increase is «+0,5», the value of the indicator, which has up to 50% of the results of the decrease from the norm – «-0,5»

According to the data obtained (see Fig. 9), the normal reaction of the stress system is characterized by synchronous activation of the SR and SL links, that is, the concept of "vegetative balance" is defined. Based on these data, it becomes possible to evaluate the reaction of the stress system in various clinical situations. It has been revealed that the point of application of preparations for general anesthesia is the SL-link of the stress system, which allows using the new term "stress-limiting" anesthesia, which differs from the term "general anesthesia" in assessing the adequacy of anesthesia, and not just "depth of anesthesia." The data obtained can be a platform for assessing the adequacy of new combinations of drugs for general anesthesia in various clinical situations associated with the initial disruption of the activity of the stress-system links.

Conclusions

1. The normal initial reaction of the SR-link of the stress system was determined (the normal stress-typical stress response in adults and children) on preoperative (psycho-emotional) and pain (traumatic) stress, which is expressed in: normal level of cortisol (adrenal level); Normal level of CTRF (hypothalamic level); Increased level of histamine; The normal level of IL1; The normal level of IL6; Increased IL2; A decrease in the level of TNF α .

2. The normal initial reaction of the SL-link of the stress system was determined which was expressed in: normal serotonin level in children and increase in 30% of adults (a typical pathophysiological reaction associated with age, which demonstrates the "vulnerability" of the SL-link in adults); Reduction of β -endorphin level (a discussion on the need to enhance opioid exposure in order to prevent inadequate anesthesia); An increase in IL4 mean values.

3. A typical reaction of the SR-link of the stress system to the surgical intervention (surgical stress) was carried out using standard variants of TIA (SA group) and modified variants (MA group). It was revealed: increased cortisol level, increased CTRF level, normalization of histamine level, normalization of IL1 indices, lowering of IL6 level, increased level of IL2, increase of TNF α level.

4. A typical reaction of the SL-link of the stress system to the surgical intervention (surgical stress) was carried out using standard TIA variants (group SA) and modified variants (MA group). A decrease in the level of serotonin (decrease in the SL potential and the need for additional activation via NMDA receptors), an increase in β -endorphin level (adequacy of opioid stimulation of the SL-link), an increase in IL4 in 75% of adults and 80% of children

(the need for additional stimulation through GABA receptors).

5. The "norm of pathology" (or "stress-norm") of the reaction of the SR-link was first defined-stress-realizing (hyper-stress) - protective, starting from which it became possible to evaluate the reaction of the stress system in another pathology and species (combinations) Anesthesia. It was proved that combinations of drugs for general anesthesia (in manufacturer's recommended dosages) could not influence the activity of the SR-link of the stress system in patients of the general surgical group.

6. For the first time, the "pathology norm" (or "stress rate") of the SL-link reaction (stress-restriction activation) was determined, which can be evaluated as normal when it is identical to the SR-stimulation, to level out the destabilizing effect of SR-stimulation. That is, autonomic stabilization is achieved. Unlike the SR-link, the tendency of the combination of drugs for general anesthesia to affect the activity of the SL-link receptors has been revealed, the "point of application" for drugs has been shown, with the prospect of developing techniques (new combinations) capable of affecting serotonin metabolism in the brain.

7. It is possible to introduce a new concept – stress-limiting anesthesia, which is necessary, due to the received data that "depth of anesthesia" and "adequacy of anesthesia" – these are not identical concepts. Preparations for general anesthesia affect, first of all, the activity of the SL-link of the stress system and it is due to their combinations based on the initial activity of the stress system that it is possible to achieve adequate stress-limiting anesthesia.

Conflicts of interest

The authors have no conflict of interest to declare.

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INTERLEUKIN-6 IS A POTENTIAL TARGET FOR A CORRECTION OF ENDOTHELIAL DYSFUNCTION ASSOCIATED WITH LOW-GRADE SYSTEMIC INFLAMMATION

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Abstract

Introduction: One of pathogenetic links of development of endothelial dysfunction, as an early marker of cardiovascular disease and comorbidity, is a low-grade systemic inflammation. In this regard, correction of cytokines imbalance is one of the possible ways of prevention and treatment of major chronic noninfectious diseases.

Aims: To study the endothelium protective effects of anti-IL-6 receptor monoclonal antibodies tocilizumab in experimental preclinical studies in a model of endothelial dysfunction associated with the low-grade systemic inflammation.

Methods: The study was performed with 40 white male Wistar rats. The endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress was produced by sequential administration of gentamicin, bacterial lipopolysaccharide and N-nitro-L-arginine methyl ester. The administration of tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) was performed at 6 day from the experiment start in doses of 4 mg/kg and 8 mg/kg once. The endothelium protective activity was studied at 34th day from the s the experiment start through the analysis of changes of pharmacological vascular tests and calculation of the endothelial dysfunction coefficient. The cytokine profile was assessed by measuring of serum concentrations of tumor necrosis factor- α , interleukin-6 and interleukin-10 by enzyme linked immunosorbent assay.

Results: In the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress, tocilizumab at the doses of 4 mg/kg and 8 mg/kg had the dose-dependent endothelium protective action that is logged to reduce of the endothelial dysfunction coefficient from 7.2 ± 0.6 to 2.8 ± 0.3 and 2.3 ± 0.2 , respectively. In addition, there was normalization of the cytokine profile in the form of lower serum concentrations of the tumor necrosis factor- α , interleukin-6 and interleukin-10.

Conclusion: Tocilizumab (at the doses of 4 mg/kg and 8 mg/kg) after one dose delivery has a pronounced endothelium protective activity in the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress and prevents of the cytokine profile imbalance.

Key words: endothelial dysfunction, low-grade systemic inflammation, interleukin-6, tocilizumab.

Introduction

Endothelial dysfunction is an early marker and predictor of cardiovascular diseases [1, 2, 3, 4, 5]. It is characterized by dysfunction of relaxation caused by nitric oxide (NO) and other vasodilatory compounds (such as prostacyclin),

whereas the production of endothelial vasoconstrictor factors increases [6, 7, 8]. Pro-oxidant and proinflammatory vascular environment is another endothelial dysfunction characteristic [8, 9].

Low-grade chronic inflammation has a close pathogenetic connection with the development of endothelial dysfunction. Key molecules that trigger the main pathogenetic links are C-reactive protein and proinflammatory cytokines such as tumor necrosis factor alpha, interleukins 1 β and 6 [1, 6, 10, 11]. The increase of their concentration in the serum may lead to reduction of NO production by endothelial nitric oxide synthase (eNOS) inhibiting [6, 12, 13], and the increasing of adhesion molecules expression, stimulating the migration of white blood cells and initiate the proliferation of smooth muscle cells of blood vessels [14, 15].

In this regard, the search and preclinical studies of drugs with endothelium protective properties aimed at correcting of the cytokines imbalance are one of the most promising areas of targeted therapy.

Materials and methods

Animals

The study was performed with 40 white male Wistar rats weighing 220±10 g. Animals were kept under controlled environmental conditions when the air temperature is +18-25°C, humidity is 40-60%, under a 12-hour lighting cycle. Plan and research protocol were approved by the local ethics committee (protocol №12-2016 from 21.11.2016). Studies were conducted in compliance with ethical norms and rules with regard to the requirements of the European Convention for the protection of vertebrate animals using for experimental and other scientific purposes (1986), principles of good laboratory practice, state standard 33044-2014 and the order of the Ministry of health of the Russian Federation No. 199n «On approval of Rules of good laboratory practice».

Experimental groups

All animals were divided into 4 groups of 10 animals each (table 1).

Table 1

The experimental groups of animals (n=10)

Group	Description
A	Intact animals
B	A control group with the modeling of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress
C	A group with tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) administration at the dose of 4 mg/kg on the background of endothelial dysfunction
D	A group with tocilizumab (Actemra®, F. Hoffmann-La Roche Ltd, Switzerland) administration at the dose of 8 mg/kg on the background of endothelial dysfunction

The endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress was modulated by the following way: gentamicin at a dose of 20 mg/kg was administered intraperitoneally once a day, for 5 days. After administration of gentamicin, there was started the administration of the *E. coli* lipopolysaccharide type O55:B5 at a dose of 1 mg/kg intraperitoneally once every week for three weeks. At the 27th day from the experiment start the animals of the experimental group were intraperitoneally injected with N-nitro-L-arginine methyl ester (L-NAME) at a dose of 25 mg/kg daily for week.

The administration of tocilizumab was performed intraperitoneally once at 6 day after the experiment start in doses of 4 and 8 mg/kg, respectively.

Evaluation of hemodynamic and pharmacological vascular tests

At the 34th day from the experiment start under anesthesia (chloral hydrate 300 mg/kg)

there was catheterized the left carotid artery for registration of hemodynamic parameters: systolic, mean and diastolic arterial pressure (SBP, MAP and DBP), measuring by the sensor TSD104A and hardware and software MP150 complex produced by «Biopac System inc.», the USA. There were conducted functional tests: endothelium-dependent vasodilatation (EDVD) after intravenous administration of acetylcholine (AC) at a dose of 40 mcg/kg and endothelium-independent vasodilatation (EIVD) after intravenous administration of nitroprusside sodium (NP) at a dose of 30 mg/kg.

To evaluate the severity of endothelial dysfunction there was used the endothelial dysfunction coefficient (EDC), calculated as the ratio of the areas of the triangles above the curve recovery of blood pressure after nitroprusside and acetylcholine administration.

The evaluation of cytokine profile

Blood samples from each rat was centrifuged at 3000 rpm for 15 minutes, the plasma was

placed in a plastic test tube type "Eppendorf" and frozen at -20°C until analyzed. Serum concentrations of TNF- α , IL-6 and IL-10 were determined by the enzyme linked immunosorbent assay. The results were expressed in pg/ml.

Statistical data processing

Statistical analysis of obtained data was performed in Microsoft Excel. "Descriptive statistics" was used to calculate the mean value (M) and standard error of the mean (m). "The two-sample t test with unequal variances" was used to compare the data of different groups of animals and determining the significance of differences between them. Statistically significant differences were considered when $p<0.05$.

Results

The results of the assessment of hemodynamic and vascular pharmacological tests

Modeling of the endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress, caused arterial

hypertension (SBP – 173.0±15.2, DBP – 134.0±3.9, MAP – 152.0±11.2 mm Hg) and led to a greater loss of the arterial blood pressure after the acetylcholine administration (SBP to 102.9±8.1, DBP to 66.9±8.0, MAP to 79.2±4.8 mm Hg) and less loss of it after the nitroprusside administration (SBP to 92.8±4.7, DBP to 47.1±4.2 and MAP to 62.0±2.9 mm Hg) in comparison with the intact animals.

Tocilizumab administration dose-dependently reduced the severity of hypertension, however, did not allow to achieve the values of the intact animals group (table 2). On the other hand, processing of the experimental data allowed to establish that tocilizumab has the strong endothelium protective action, expressed in a statistically significant decrease of the endothelial dysfunction coefficient to 2.8±0.3 and 2.3±0.2 in the animal groups receiving the drug at the doses of 4 and 8 mg/kg, respectively (table 3).

Table 2

Dynamics of hemodynamic parameters in the experimental animal groups ($M \pm m$; $n = 10$)

Group of animals	SBP, mm Hg	DBP, mm Hg	MAP, mm Hg
A (raw values)	137±3.7	101.9±4.3	115.0±1.9
EDVD	84.3±4.4	38.7±2.8	53.9±2.7
EIVD	83.0±3.7	42.1±4.4	55.7±3.5
B (raw values)	190.3±6.7*	145.0±3.9*	160.1±4.6*
EDVD	110.6±5.2*	82.8±6.6*	92.1±6.1*
EIVD	88.7±4.7*	50.8±4.2*	63.4±4.1*
C (raw values)	157.9±6.9**	133.0±4.1**	141.6±4.4
EDVD	86.1±3.8**	47.2±3.6**	59.0±3.2
EIVD	94.6±3.1**	43.9±0.9**	60.8±1.2
D (raw values)	153.9±4.5**	128.0±1.7**	138.2±2.0
EDVD	79.9±2.6**	56.4±2.1**	64.2±1.9
EIVD	105.5±3.7**	45.9±2.1**	65.8±1.8

Comment: SBP – systolic blood pressure; DBP – diastolic blood pressure; MAP – mean arterial pressure; EDVD – endothelium-dependent vasodilatation; EIVD – endothelium-independent vasodilatation; * – $p<0.05$ in comparison with the intact animals; ** – $p<0.05$ in comparison with the control group.

Table 3

The calculation of the endothelial dysfunction coefficient in the experimental animal groups ($M \pm m$; $n = 10$)

Group	Functional tests	The area of the vascular response (relative units, RU)	EDC
A	EDVD	1268.0±74.8	1.1±0.2
	EIVD	1375.3±93.7	
B	EDVD	814.2±78.0*	7.2±0.6*
	EIVD	5862.3±116.7*	
C	EDVD	1119.4±110.1**	2.8±0.3**
	EIVD	3006.5±232.5**	
D	EDVD	1253.3±71.5**	2.3±0.2**
	EIVD	2823.2±114.3**	

Comment: EDVD – endothelium-dependent vasodilatation; EIVD – endothelium-independent vasodilatation; EDC – endothelial dysfunction coefficient; * – $p<0.05$ in comparison with the intact animals; ** – $p<0.05$ in comparison with the control group.

The results of the evaluation of the cytokine profile

On the background of the modeling of endothelial dysfunction by the use of bacterial lipopolysaccharide, the concentrations of TNF- α , IL-6 and IL-10 increased several times and

reached 101.88 ± 6.87 PG/ml, 156.93 ± 7.02 PG/ml and 82.78 ± 577 PG/ml, respectively. The correction by tocilizumab resulted in a decrease in the concentration of these cytokines to the intact animals level at the dose of 4 mg/kg and their normalization at the dose of 8 mg/kg.

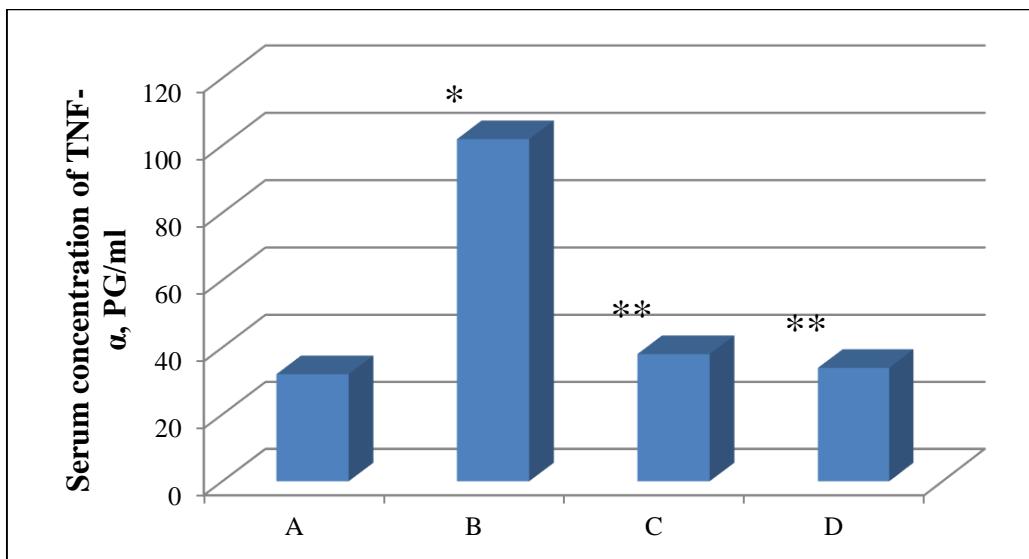


Fig. 1. The influence of the test compounds on the serum concentration of tumor necrosis factor- α . * – $p < 0.05$ in comparison with the intact animals; ** – $p < 0.05$ in comparison with the control group

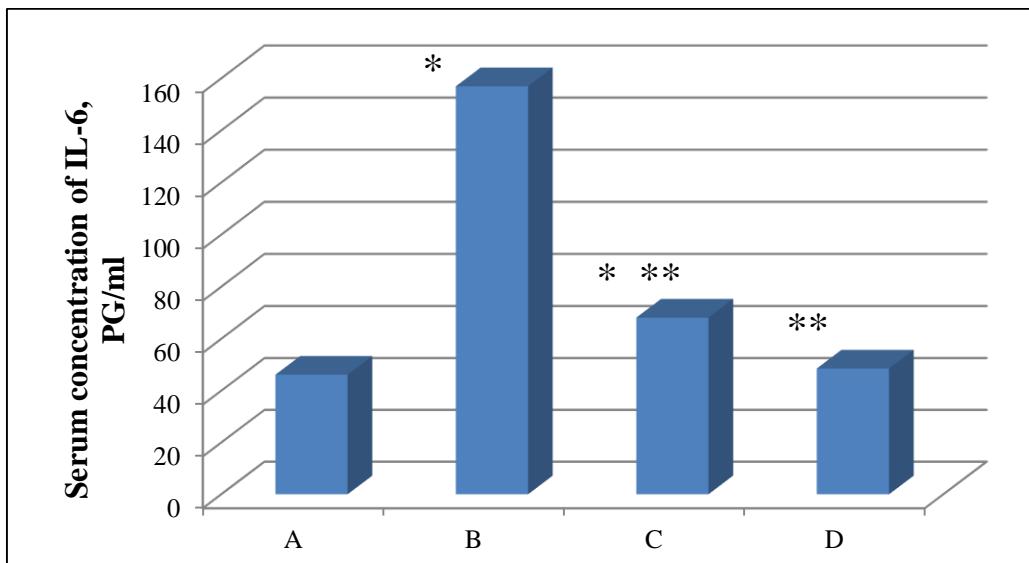


Fig. 2. The influence of the test compounds on the serum concentration of interleukin-6. * – $p < 0.05$ in comparison with the intact animals; ** – $p < 0.05$ in comparison with the control group

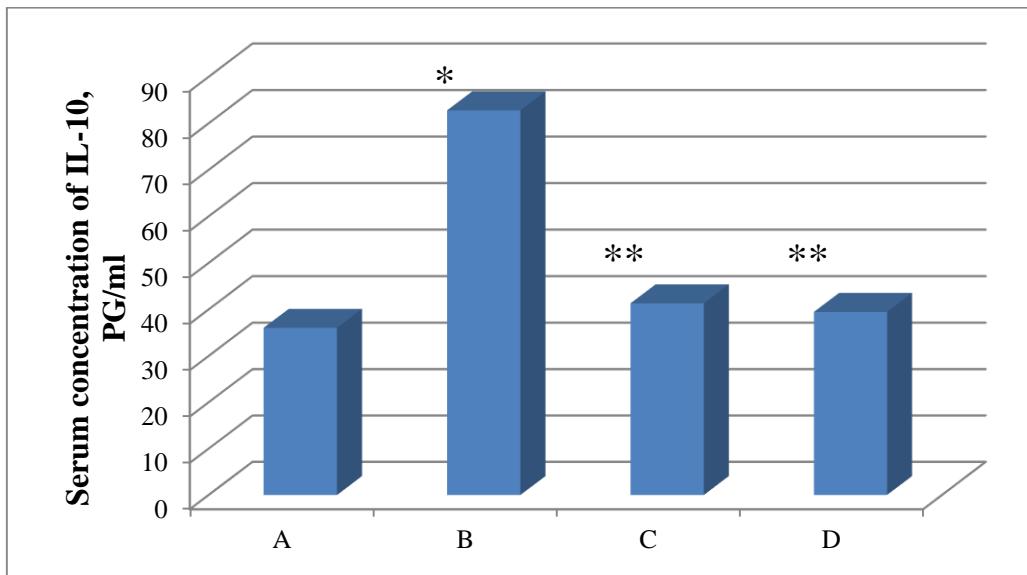


Fig. 3. The influence of the test compounds on the serum concentration of interleukin-10. * – p<0.05 in comparison with the intact animals; ** – p<0.05 in comparison with the control group.

IL-6 is a multifunctional cytokine that is involved in the pathogenesis of cardiovascular diseases. Increased secretion of IL-6 in response to inflammation, increases serum concentration of angiotensin II, exacerbates oxidative stress and vascular damage that is why IL-6 has become a marker of vascular inflammation, i.e. the increase in the level of circulating interleukin epidemiologically associated with a number of clinically significant diseases of the cardiovascular system [16, 17].

Increased level of circulating IL-6, leads to the following pathological changes, which plays an important role in the development of endothelium-associated pathology:

- activation and maintenance of the low-grade systemic inflammation (including a powerful increase in the synthesis of CRP in a liver);
- disruption of homeostatic functions (cellular protection from reactive oxygen species);
 - endothelial dysfunction,
 - activation of monocytes;
 - intimal proliferation, myocardial hypertrophy;
 - disturbance in metabolic control (insulin resistance).

These various effects indicate that IL-6 is not merely a passive biomarker, but actively modulate a course of cardiovascular diseases [18, 19].

In numerous clinical studies there was proved the role of interleukin-6 in the pathogenesis of atherosclerosis [20], ischemic heart disease [16], hypertension [21], chronic heart failure and myocardial hypertrophy [16]. At the same time, there is confirmed and discussed not only the role of the increased level of IL-6 in the development of cardiovascular disease, but adverse effects on the prognosis and outcomes of the disease.

In this regard, IL-6 began to be typified as a potential target for targeted therapy of such diseases as atherosclerosis, coronary heart disease, as well as with the aim of reducing cardiovascular risk with comorbidity.

The most studied pleiotropic effect of tocilizumab is the effect on the atherosclerosis progression. For example, SAMURAI and CHARISMA studies demonstrated the increase in the level of HDL cholesterol in 24% of patients on a background of tocilizumab administration, and in the CHARISMA study there was observed a dose-dependent effect of tocilizumab: the use of the drug in a dose of 8 mg contributed to the increase in the level of HDL cholesterol and reduced the atherogenic index [22, 23].

In experimental studies inhibition of IL-6 by tocilizumab led to improvement in endothelial function and reduced arterial stiffness [24].

Preliminary data obtained in the study of a single dose of tocilizumab in patients with myocardial infarction without ST-segment elevation showed a tendency to decrease in the

areas under the curve of concentrations of CRP and troponin T [25].

In another pilot study, there was investigated the effect of tocilizumab on endothelial dysfunction and arterial wall rigidity. According to the results, tocilizumab significantly improved the endothelium-dependent vasodilation from 3.3 ± 0.8 to 4.4 ± 1.2 (after 3 months of therapy) to $5.2 \pm 1.9\%$ (after 6 months of therapy) and decreased the vascular wall stiffness. Thus, the researchers concluded the beneficial effect of tocilizumab on endothelial function and the main pathogenetic links of cardiovascular diseases and complications [26].

The high prevalence of cardiovascular complications in rheumatoid arthritis has led to the need to study the effect of targeted therapy on cardiovascular risk in this group of patients. So, 52 weeks of tocilizumab therapy resulted in marked and significant increase in ejection fraction (+ 8.2%) and a significant decrease in left ventricular mass (-24.4%) [27].

Data review of clinical trials published in the Lancet in 2012, suggests that targeting inhibition of the IL-6 receptors may provide a new therapeutic approach to the prevention and treatment of ischemic heart disease [28].

Dysfunction of endothelium, as a rule, involves in the pathological process not only the cardiovascular system but also leads to the development of the so-called "cardiometabolic continuum". Therefore, it is necessary to consider the potential influence of the IL-6 inhibitors on carbohydrate metabolism and insulin resistance.

The first study of the influence of tocilizumab on carbohydrate metabolism was the study, during which patients with rheumatoid arthritis and coexisting diabetes mellitus type II was treated with tocilizumab at the dose of 8 mg/kg intravenously every 4 weeks [24]. In the subgroup of 10 patients, HbA1c decreased significantly after 1 month tocilizumab therapy (from 7.17 to 6.35%, $p < 0.01$), while the effect was maintained after 6 months (6.0%, $p < 0.001$).

In another study, children with systemic juvenile idiopathic arthritis in the presence of glucocorticoids dosage modification, only after 6 weeks of tocilizumab therapy HOMA-IR index reflecting insulin resistance, significantly decreased [29]. The obtained data were confirmed in another cohort study (n=11): after 3 months of

tocilizumab therapy HOMA-IR decreased significantly, indicating improving insulin sensitivity through inhibition of IL-6 signaling. To confirm the theory, LAR was designed as a new marker of insulin resistance. In keeping with HOMA-IR dynamics, LAR was constant during the first month of the study, but it was significantly lower than baseline after 3 months of therapy [30].

Thus, we have described the potential endothelium protective mechanisms of inhibition of signaling pathway triggered by overproduction of interleukin-6, which require further study, both in preclinical and clinical studies.

Conclusion

Thus, tocilizumab (at the doses of 4 mg/kg and 8 mg/kg) after a single dose has a pronounced endothelium protective activity in the model of endothelial dysfunction associated with the low-grade systemic inflammation and oxidative stress and prevents the imbalance of the cytokine profile.

Conflicts of interest

The authors have no conflict of interest to declare.

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**UDC 616.53 – 002+616.891: 615.03****DOI: 10.18413/2313-8971-2017-3-3-97-109****Samodai O.V.¹,
Reznikov K.M.²****COMPLEX CORRECTION OF PSYCHOEMOTIONAL
AND IMMUNOLOGICAL CHANGES IN PATIENTS WITH ACNE**¹NUZ «DKB at st. Voronezh-1» JSC« Russian Railways». 2, lane Zdorov'ya, Voronezh, 394024, Russia²GBOU VPO VSMU named by. N.N. Burdenko of the Ministry of Health of Russia. 10 Studencheskaya str., Voronezh, 394036, Russia.Corresponding author, ¹e-mail: samalyna@yandex.ru**Abstract.**

Introduction: The problem of complex therapy of acne attracts the attention of researchers because it accompanied with significant cosmetic defect and caused serious patient's experiences.

Objectives: The aim of this study was to find ways to increase the efficacy and safety of treating patients with acne by complex pharmacological correction of its manifestations.

Methods: The study included 110 people (32 men and 78 women) aged from 17 to 35 years, divided into 5 equal groups (according to the type of therapy) and observed for 6 months. All the patients underwent a comprehensive examination, including calculation of the dermatological index of acne (DIA), psychometric tests as well as an immunological study.

Results and discussion: Monotherapy with combined topical agents leads to a decrease in DIA (48.75%) and the length of remission for more than 6 months in 46.4% of observations. Phabomotisol decreased DIA count and improved psychometric parameters. Ionized liquid (IL) led to an increase in CD4 + lymphocytes (14.48%) and immunoregulatory index (40.88%), decreased in the number of CD8 + lymphocytes (17.92%). The most effective combination was phabomotisol and IL (negative redox potential): DIA decreased (74.36%), remission of more than 1 year was observed in 22.7% patients.

Conclusion: Effectiveness therapy with phabomotizol was shown. Effectiveness of IL in the complex treatment of acne in the medium-severe course of the disease is estimated for the first time. Presence of immunotropic properties of IL was established. Possibility of achieving long-term remission without the use of antibiotic therapy was demonstrated.

Keywords: acne, adolescence, adult, acne complex therapy, psycho-emotional state, pharmacological correction, phabomotizol, ionized liquor, redox potential.

Introduction

Acne is the most common skin disease. It occurs in about 85-95% of people aged 12-30 years and in 50% of patients – over 25 years [1, 2, 3]. The peak incidence corresponds to the period of social formation and psychological approval of a person [4, 5]. Acne is not a disabling pathology, but it can cause serious emotional disturbances. Patients with acne have a significant range of

psychological changes: anxiety disorders, dysmorphophobia, pathomimia, eating disorders, depression [6, 7, 8]. Severity of depressive symptoms is proportional to the degree of cosmetic defect in most cases [9, 10].

The pathogenetic basis of acne is hyperproduction of sebum, retentional hyperkeratosis of the sebaceous-hair follicle, proliferation of propionbacteria acne and

inflammation [1, 8, 9]. The presence of receptors in the sebaceous glands for neurotransmitters that are released in response to stimulation has also been established. It corresponds to the role of stress in enhancing of acne [9, 10, 11].

A variety of agents for the treatment of acne have been proposed. The main ones are systemic and topical antibacterial preparations of the group of tetracyclines, lincosamides, systemic and topical retinoids, which combined with oral contraceptives [1, 12, 13, 14, 15, 16, 17]. Systemic isotretinoin possesses indisputable efficacy in the cases of severe form of acne [18, 19]. However, many side effects and the absolute teratogenicity of this drug limits its wide application. Systemic antibacterial drugs (tetracycline, doxycycline, minocycline) act only on the ancestor propionbacteria without embracing other pathogenetic mechanisms [1, 2, 11, 16, 17, 20]. Yet they remain the drugs of choice for the treatment in case of moderately severe acne. The drugs are administered for a period of 7 days to several months, but in that case they have a significant negative impact on the microbiocenosis of the intestine and mucous membranes. Previous studies indicate the presence of changes in immunological parameters such as the increase in the content of CD8+ lymphocyte fraction, the decrease in the level of CD3+ and CD4+ populations of T-lymphocytes, the decrease in the immunoregulatory index ($IRI=CD4+/CD8+$) and the positive effect of immunomodulators in the complex treatment of acne [1, 16, 17, 18, 20]. Antibiotics and agents that affect immunity have several side effects. When they are combined, the pharmacological load and the spectrum of contraindications as well as the frequency of unwanted reactions increase. The greatest number of acne sufferers are teenagers with mild to moderate form [4, 9]. In this age group, it is better to prescribe drugs for topical use due to significant limitations and side effects of systemic drugs. However, the main topical anti-acne preparations (alcohol solutions of clindamycin, erythromycin, benzoyl peroxide, retinoids – adapalene compound) have side effects in the form of erythema, dryness and flaking of the skin. These reasons can cause premature self-canceling of the treatment by a patient.

Understanding the role of immunological, psychosomatic changes in acne as well as positive

assessment of the prospects for the use of appropriate drugs creates the prerequisites for the search of new effective and safe schemes of complex correction of this disease [21, 22]. The effectiveness of the use of psychotropic drugs in a number of patients with acne was proven [23, 24]. Many scientific researches suggested these methods of pharmacological correction for use in practical medicine about 10 years ago [1, 11, 12, 25]. However, there is no data on the use of phabomotisol in this disease. Ionized liquids with positive and negative oxidation-reduction potential that are used for the first time in the treatment of acne are currently prescribed to treat eczema and psoriasis in dermatology and to treat purulent wounds in surgery [26].

The purpose of this study is to find the way for the improvement of the effectiveness and safety of treatment in patients with moderate acne by using complex pharmacological correction of psychoemotional, immunological changes and cutaneous manifestations of acne.

Objectives

1. Investigate the possibilities of standard acne therapy.
2. Study the effectiveness of treatment of moderate acne with the use of topical monotherapy.
3. Establish the possibility of including in the complex therapy of acne anxiolytics such as phabomotisol and synthesized ionized liquids with different oxidation-reduction potential.
4. Conduct a comparative analysis of various programs for the treatment of acne according to clinical, psychoemotional and immunological indicators.
5. To substantiate the application of the most effective and complex ways for pharmacological correction of psychoemotional, immunological disorders and cutaneous manifestations of acne.

Scientific novelty of the study

The therapeutic efficacy of phabomotisol in the correction of psychoemotional changes in acne has been proved for the first time. It has been demonstrated that a combination of standard external therapy with taking phabomotisol in a dose of 5 mg 3 times a day during a month effectively corrects psychoemotional changes and cutaneous manifestations of acne.

A positive effect of innovative external therapy (anti-inflammatory lotion containing 3% alcohol solution of chloramphenicol once a day in

the morning 2 to 4 weeks, a peeling lotion containing benzoic acid 1.0, salicylic acid 2.0, camphor alcohol 5-15.0, boric acid 3.0, Streptocide 7.0 and whitening ointment containing zinc, boric acid at 1.5-2.5, 30% hydrogen peroxide 0.5 and zinc ointment 25.0 at night once a day on the skin of the face, excluding the paraorbital area, for 2-4 months) has been established.

The evidence of the immunomodulatory action of a liquid with negative AFP that is administered at a dose of 2 ml/kg of body weight was received by the basis of changes in the immunograms of patients with acne.

New data has been obtained on the positive effect of ionized fluids with positive and negative redox potential on acne eruptions and post-acne changes.

A positive pharmacodynamic interaction of phabomotisol and ionized liquid with a negative redox-potential was established, which is expressed in the greater effectiveness of their combination compared to the separate application with the standard external agents. This combination can be considered optimal for reducing the dermatological index of acne (DIA) and increasing the duration of remission. This therapeutic method allows to abandon the use of systemic antibiotic therapy. The incidence of side effects decreases from 81.2% to 4.5% in the case of using complex therapy as a combination of topical agents, phabomotisol and ionized fluids with different AFP.

The theoretical significance of the work lies in the development of a system that makes it possible to assess the possibility of changing the therapeutic activity of a pharmacological agent by changing the redox potential of body fluids. For practical medicine, the prospect of a comprehensive correction of acne with the use of phabomotisol and liquids with various oxidation-reduction potentials against the background of standard external pharmacotherapy has been proved. This allows to optimize treatment, withdraw from the systemic antibacterial drugs, minimize the frequency of side effects, increase the duration of remission, reduce the cost of treatment, which makes it reasonable to use the proposed scheme of dermatology and cosmetology. New data obtained in this study can supplement the relevant courses in dermatology,

cosmetology, pharmacology, clinical pharmacology.

All the studies were carried out in accordance with the requirements of the current guidelines: the ethical norms of the Helsinki Declaration of 1964, modified by the 41st World Assembly (Hong Kong, 1989) and the 52nd General Assembly of the WMA (Edinburgh, Scotland, UK, 2000), the Lisbon Declaration on the Rights of Patients, (Lisbon, Portugal, 1981), in section V of the Code of Medical Ethics, approved by the XVIII All-Russian Pirogov Congress of Physicians (07.06.1997). All patients signed informed consent, allowing at any time to stop the study.

Personal contribution to the study

Personal contribution consists in the direct participation of the author of the thesis in the study of many scientific sources of Russian and foreign literature, the selection of patients for participation in the study, filling out individual medical records, in systematizing and statistical processing of the data obtained and analyzing the results.

Methods

All patients with papulo-pustular form of acne were on outpatient treatment at the DKB at st. Voronezh-1 RZhD in the period from 2010 to 2014 inclusively. The work was carried out at the Stationary Unit No. 3 of DKB (the Chief Doctor – Doctor of Medical Sciences, Professor Novomlinsky V.V.), the Department of Pharmacology and the Department of Dermatovenereology of the VSMU named after N.N. Burdenko (Rector – Professor I.E. Esaulenko). The results of the examination and treatment of 110 patients of different sexes – 32 men (29.09%) and 78 women (70.9%) with acne of moderate severity were used for the thesis. Diagnosis – Acne vulgaris (L70.0) – was delivered in accordance with the X International Classification of Diseases on the basis of clinical and anamnestic data.

The majority of the subjects had an age of 16 to 25 years, which corresponds to literary data on the incidence of acne. Based on the clinical picture and by counting the elements of the rash, we evaluated the condition of the patients and included acne with a moderate degree of severity in the study. For the quantitative evaluation of each type of acne-elements, the dermatological index of acne (DIA) was calculated in points. The

duration of the disease varied from several months to 10 years. During this time, patients either did not seek help expecting spontaneous cure or visited cosmetic rooms. Some of them previously underwent outpatient treatment (with greater or less effectiveness), which did not allow achieving a stable remission. 73.6% (81 patients) of young men and women showed a background somatic pathology – diseases of ENT and gastrointestinal tract, allergic reactions. The criteria for inclusion in the study were: the presence of the disease – acne of moderate severity and post-acne changes (atrophic scars, post-inflammatory pigmentation, breach of skin microrelief), age from 17 to 35 years, written informed consent of the patient. The criteria for exclusion were: indication of the diseases in the acute stage, diabetes mellitus, pregnancy, lactation, individual intolerance of the proposed therapy, disagreement of the patient.

All patients were assigned standard laboratory tests – a general blood test, a biochemical blood test, a hormonal status. Subjects with the revealed endocrine pathology and changes in the content of sex hormones from laboratory data were sent to the appropriate specialists. In addition, the parameters of cellular and humoral immunity (number of T and B lymphocytes, immunoglobulins Ig M, Ig G, Ig A, immunoregulatory index (IRI=CD4⁺/CD8⁺) were estimated additionally. The value of the dermatological index of acne-DIA was calculated by the formula A + B + C + D, where A is the score by the number of comedones, B is the number of pills, and C is the number of pustules and D – nodes. The patient's psychometric data was assessed by determining the levels of anxiety and depression on the Tsung scale, State of health, activity, mood.

Regardless of age and sex, patients were randomly assigned to 5 groups (22 patients each) using a randomization table in accordance with the treatment regimen used. In the first group (n = 22) patients were prescribed standard therapy – doxycycline monohydrate tablets ("Unidox Solutab") 100 mg twice a day for 7-14 days, topicals – combination of adapalene with clindamycin (gel "Clenzite-C") 1 time per day for 2-4 weeks, then – adapalene (gel "Clenzite") 2-4 months. A cream with 20% azelaic acid (Skinoren) was applied 2 times a day 2-4 months after the main course of treatment to prevent

exacerbations and post-acne correction. To normalize the intestinal microflora, all patients within 14 days in addition received capsules containing Lactobacillus acidophilus, Bifidobacterium infantis, Enterococcus faecium ("Linex" preparation) 1 capsule 3 times a day. Sunscreen was prescribed if treatment took place in summer.

The second group (n = 22) consisted of patients who did not receive systemic drugs. For topical therapy, combined topical agents were used in the form of an anti-inflammatory lotion containing 3% alcohol solution of chloramphenicol once a day in the morning 2-4 weeks, a peeling lotion containing benzoic acid 1.0, salicylic acid 2.0, camphor alcohol 5-15.0, boric acid 3.0, Streptocide 7.0, and bleaching ointment containing zinc, boric acid at 1.5-2.5, 30% hydrogen peroxide 0.5 and zinc ointment 25.0, at night once a day on the skin of the face, excluding the paraorbital area, for 2-4 months. In addition, a moisturizing lipid-replenishing day cream was prepared from medicinal cosmetics for problem skin and sunscreen for treatment in the summer.

In the third group of patients (n = 22), in addition to the above-described topical combined external agents of standard therapy, phabomotisol was administered at 5 mg 3 times a day for a month.

The fourth group of patients (n = 22) did not receive any drugs internally. For external therapy, the same combination of topical anti-inflammatory agents in the form of lotion, lotion-peeling and whitening ointments were used. Ionized fluids with different AFP were appointed in accordance to the protocol of the study based on the patient's written consent and permission of the Ethics Committee. Anolit – ionized fluid with a positive redox potential (ORP = +600 +900, pH = 6 – 7) was used in the form of applications locally 1 – 2 times daily 2 – 4 weeks. Catholyte – ionized fluid with a negative redox potential (ORP = -400 – 500, Ph = 8 – 9) was administered internally in a dose of 2 mL/kg body weight 1 times a day for 14 days. Ionized fluid was obtained in an electrolytic cell «Karat-M» Tu 3468-001-51702726-2006, certificate of conformance № ROSS RU AJA60V21343, from bottled spring water.

The fifth group used complex therapy including the topical multicomponent agents

described above and containing chloramphenicol, streptocid, benzoic, boric and salicylic acids, camphor alcohol, zinc, and anolyte externally. Fabomotizol in the dose of 5 mg 3 times a day for a month and catholyte in the dose of 2 ml per kg of body weight once a day for 14 days were administered internally.

The results were evaluated before the treatment and after 1, 3 and 6 months of therapy on the basis of patient complaints, their subjective sensations, changes in the clinical picture, dynamics of DIA and psychometric tests, and also on the basis of changes in the parameters of cellular and humoral immunity. DIA was determined before and during treatment – after 1, 3 and 6 months. Psychometric tests were performed before and 3 months after the therapy. The immunogram was evaluated at the beginning of the study and within 20 days after the end of the catholyte intake. The data were recorded and statistically processed. During the statistical analysis, methods of descriptive variational statistics were used. Correlation analysis was used to identify the relationship among the features being investigated. Regression analysis was used to establish the nature of the dependencies of variables. The results were processed in IBM SPSS Statistics v.22 and Microsoft Office Excel 2003 SP3 in the Windows 7 operating system.

Various pharmacotherapeutic methods for the therapy of acne of moderate severity were used in the work.

Results and discussion

The first group (therapy included doxycycline monohydrate and bifidopreparation internally, a combination of adapalene with clindamycin, azelaic acid externally) included 22 patients (6 young people and 16 girls). The dermatological index of acne before treatment ranged from 6 to 10 and averaged 7.1 points. Duration of the disease was from 6 months to 5 years. Six months after the start of standard therapy, DIA on average decreased by 33.12% and corresponded to mild acne in 45.5% of patients (Figure 1).

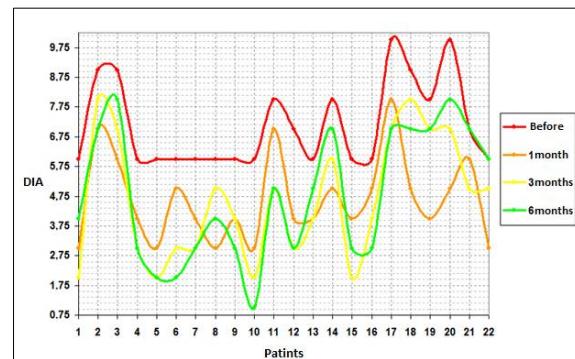


Fig. 1. Dynamics of changes in DIA (scores) in each patient with standard therapy

During the therapy, the following side effects were revealed: redness, dryness, burning, dyspeptic disorders, which were noted by 18 people (81.8%). Standard treatment promoted the onset of remission on average by 2.5 months (Figure 2).

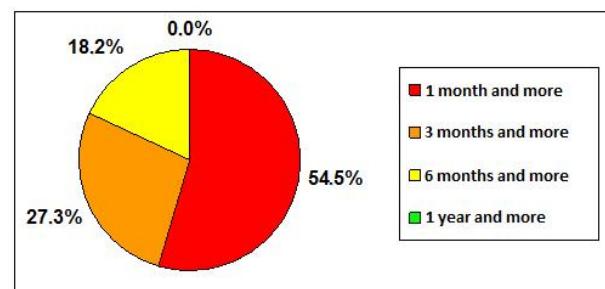


Fig. 2. Duration of remission in patients with acne (standard therapy, %)

When studying the parameters of the immunogram before and after the treatment, serious differences were not found in the patients of the first group, which leads to the conclusion that the standard complex therapy of acne does not significantly affect the parameters of the immune response.

After 3 months from the beginning of the main course of the treatment, positive changes in the indicators of the psychoemotional status of the patients of the first group were recorded. Their dynamics is presented in Table 1.

Table 1

Dynamics of psychometric indicators ($M \pm m$, score, %) standart treatment (n=22)

№	Index	Terms of observation	
		Before treatment	After treatment
1	Anxiety	30.91 ± 1.28	$28.91 \pm 1.07^*(93.53\%)$
2	Depression	39.00 ± 1.52	$35.50 \pm 1.21^*(91.03\%)$
3	Health	29.36 ± 0.97	$35.95 \pm 1.09^*(122.45\%)$
4	Activity	27.61 ± 0.74	$33.89 \pm 1.12^*(122.75\%)$
5	Mood	30.52 ± 0.86	$36.18 \pm 0.99^*(118.55\%)$

Note: * – $p < 0.05$ compared with the indicators before treatment

Thus, after 6 months from the beginning of the treatment, the DIA index on average decreased by 33.12%, anxiety of patients decreased by 6.47%, depression – by 8.97% and SAN test data improved by an average of 21.25%. Remission for more than half a year was observed in 18.2% of cases and averaged 2.5 months. Adverse effects of therapy were noted by 81.4% of patients ($p < 0.05$).

In 22 patients (5 young people and 17 girls aged 17 to 24 years) of the second group, the duration of the disease was from 1 to 6 years and DIA averaged 7.3 points. For acne therapy, only the above-described combined topical agents were used. One month after the start of the treatment, in 63.6% of patients, DIA decreased to mild acne and six months later decreased by 48.75% ($p < 0.05$). A graphic representation of the dynamics of the DIA is shown in Figure 3.

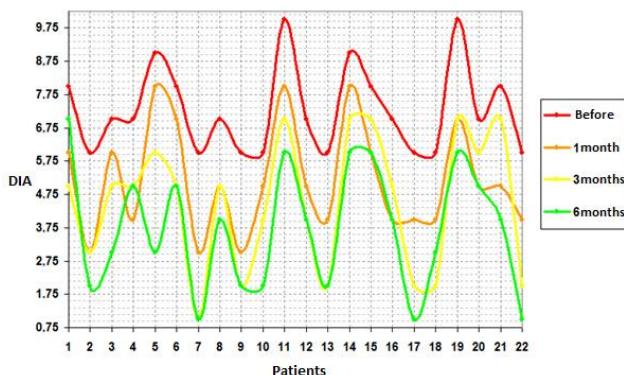


Fig. 3. Dynamics of changes in DIA (scores) in each patient in the appointment of combined topical therapy

The duration of clinical remission exceeded that for the administration of systemic antibiotic therapy and averaged 5.3 months (Figure 4).

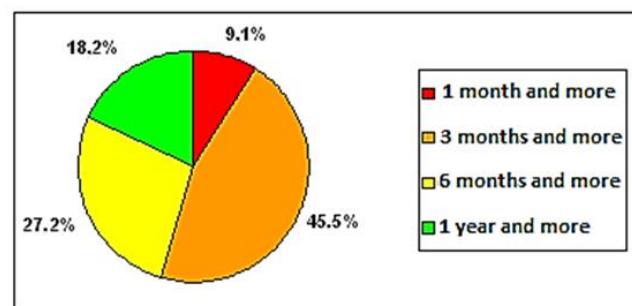


Fig. 4. The duration of remission in patients with acne in the appointment of combined topical therapy, %

Side effects of the therapy (flaking, erythema and dry skin sometimes) were noted by patients in 2 cases (9.1%).

When studying the parameters of the immunogram before and after the treatment, no significant differences were found in the patients of the second group. Thus, acne therapy with standard combined topical drugs did not affect the immune status indicators of the subjects.

After improvement of the clinical picture of the disease, according to the data of the anxiety, depression and SAN scales, positive changes were observed: in the majority of patients, anxiety and depression indicators decreased and SAN increased (Table 2).

Thus, as a result of therapy with combined topical agents, anxiety of patients decreased by 11.06%, depression by 12.71%, SAN test data improved by an average of 28.3%. The DIA indicator on average decreased by 48.75%. Remission of more than 6 months was observed in 46% of cases. The side effects of the therapy were sometimes noted by 9.1% of patients ($p < 0.05$).

Table 2

Dynamics of psychometric indicators ($M \pm m$, score, %) of patients with the appointment of combined topical therapy (n=22)

№	Index	Terms of observation	
		Before treatment	After treatment
1	Anxiety	31.64 ± 1.26	$28.14 \pm 0.98^*(88.94\%)$
2	Depression	37.55 ± 1.40	$32.77 \pm 1.83^*(87.29\%)$
3	Health	28.68 ± 0.95	$37.14 \pm 0.92^*(129.48\%)$
4	Activity	27.44 ± 0.92	$35.48 \pm 0.98^* (129.3\%)$
5	Mood	29.23 ± 1.04	$36.87 \pm 1.02^*(126.14\%)$

Note: * – $p < 0.05$ compared with the indicators before treatment.

Changes in the clinical picture and psychoemotional status of patients with acne when phabomositol was included in the program of the treatment. In the third group (n = 22), including 7 young people and 15 girls aged from 18 to 27 years, in addition to combined external therapy, phabomotisol was administered internally in a dose of 5 mg 3 times a day for a month. The duration of the disease varied from 6 months to 5 years. The average index of DIA was 7.1 points. After 6 months of therapy, the DIA index decreased by 56.41% ($p < 0.05$) and was equal to 1 in 36.4% of patients. The skin was almost pure. All patients noted the disappearance of fatty gloss on the face, which is associated with the normalization of sebum and the effect of lightening the skin. A graphic representation of the intensity of the rash in patients receiving external therapy in combination with phabomotisol is shown in Figure 5.

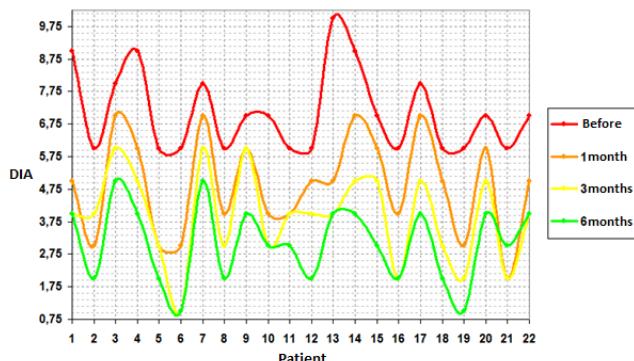


Fig. 5. Dynamics of changes in DIA (scores) in each patient treated with phabomotisol

A sufficient effectiveness of treatment is confirmed by a decrease in the frequency of side effects. Most of the subjects did not make any complaints. Redness and dryness of the skin were sometimes noted only by 2 patients treated in the autumn-winter period. The duration of remission also increased ($p < 0.05$) and averaged 5.2 months (Figure 6).

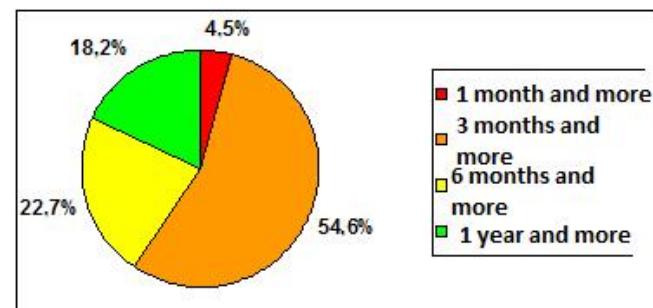


Fig. 6. Duration of remission in acne patients treated with phabomotisol, %

According to the received data, the main indices of the immunograms of the subjects were not changed significantly. Mathematical processing of the obtained results showed no significant effect of the inclusion in the therapy of phabomotisol on the immune status of patients of the third group.

The results of therapy indicate a decrease in anxiety rates by 20.55%, depression by 12.81% and an increase in SAN scores by an average of 32.03% ($p < 0.05$). These changes in the psychometric parameters of patients in the third group are presented in Table 3.

Table 3

Dynamics of psychometric indicators ($M \pm m$, score, %) of acne patients treated with fabomotisol (n=22)

№	Index	Terms of observation	
		Before reatment	After treatment
1	Anxiety	31.64 ± 1.39	$25.14 \pm 0.73^*(79.45\%)$
2	Depression	34.77 ± 1.49	$30.32 \pm 0.91^* (87.19\%)$
3	Health	27.59 ± 0.94	$36.69 \pm 0.92^* (132.98\%)$
4	Activity	28.91 ± 0.88	$37.55 \pm 0.82^* (129.87\%)$
5	Mood	26.98 ± 0.91	$35.95 \pm 0.88^* (133.24\%)$

Note: * – $p < 0.05$ compared with the indicators before treatment.

Mathematical processing of the obtained data indicates more significant changes in the clinical picture of this group than in the previous groups: DIA decreased by 56.41% ($p < 0.05$), remission of more than 1 year was observed in 18.2% of cases, the side effects of the therapy were sometimes noted by 9.1% of patients ($p < 0.05$). A significant decrease in anxiety scores was detected – by 20.55% ($p < 0.05$). Analyzing the presented results, we came to the conclusion that this effect can be explained by the inclusion of phabomotisol in the treatment program. The effectiveness of therapy for acne disease when included in the treatment of ionized fluids.

According to the design of the study, the number of patients in the fourth group corresponded to the previous ($n = 22$) – 6 young people and 16 girls aged 18 to 26 years. The duration of the disease varied from 1 to 7 years. The dermatological index of acne was consistent with the diagnosis and averaged 6.9 points. In addition to the combined topical drugs, according to the study protocol, patients were assigned ionized fluids: anolyte ($ORP = +600 + 900$, $pH = 6-7$) was used locally for inflammatory elements 1-2 times a day for 2-4 weeks and catholyte ($ORP = -400-500$, $pH = 8-9$) was administered internally in a dose of 2 ml / kg body weight once a day for 14 days.

After half a year of observation and treatment in 50% of cases, the skin was practically clean, the dermatological acne index was equal to 1-2 points. On average, the number of rashes decreased by 61.84% ($p < 0.05$). A graphic representation of the positive effect of treatment

on the dynamics of DIA of each patient of the fourth group is shown in Figure 7.

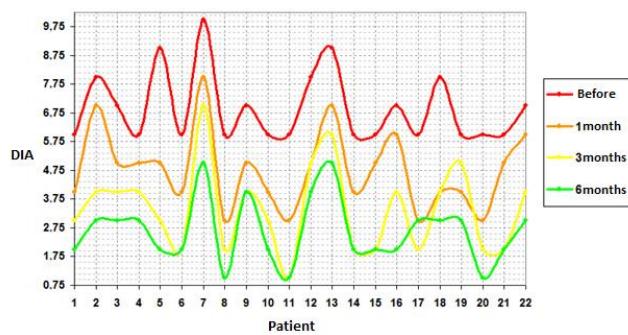


Fig. 7. Dynamics of changes in DIA (scores) in each patient patients when included in the treatment of ionized fluids

Only 1 person (4.5% of cases) during the treatment experienced redness and dryness of the skin. The remission of patients of the fourth group averaged 5.5 months (Figure 8).

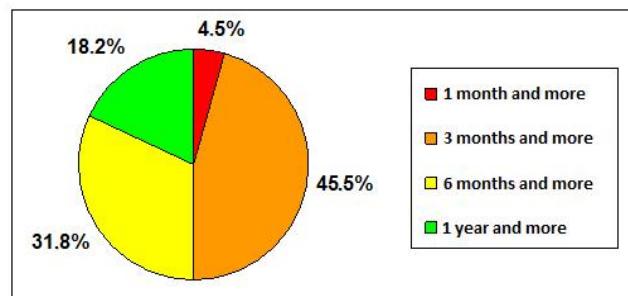


Fig. 8. Duration of remission of patients with acne when included in the treatment of ionized liquids, %

Analysis of the patients' immunograms revealed significant positive changes after the treatment: CD8 + lymphocyte count decreased by 16.9%, CD4 + lymphocyte count increased by 13.52%, immunoregulatory index (IRI) increased by 38.73% ($p < 0.05$).

After the therapy, anxiety and depression levels on the Tsung scale were significantly reduced. The dynamics of the investigated parameters is presented in Table 4.

Thus, as a result of the therapy, DIA

decreased by 61.84% ($p < 0.05$), the average duration of remission was 5.5 months. Side effects of the therapy were observed in 4.5% of cases. Mathematical processing of data made it possible to reveal more positive dynamics of the results of psychometric tests after the treatment in this group than in previous groups: anxiety of patients decreased by 22.53% ($p < 0.05$), depression – by 19.11% ($p < 0.05$), the indices for the SAN test improved by an average of 49.03% ($p < 0.05$).

Table 4

Dynamics of psychometric indicators ($M \pm m$, score, %) in patients with the inclusion of ionized fluids (n=22)

№	Index	Terms of observation	
		Before reatment	After treatment
1	Anxiety	31.27 ± 1.27	$24.23 \pm 0.70^*(77.47\%)$
2	Depression	36.86 ± 1.33	$29.82 \pm 0.77^*(80.89\%)$
3	Health	28.05 ± 0.78	$42.64 \pm 1.17^*(152.03\%)$
4	Activity	29.27 ± 0.81	$43.41 \pm 1.1^* (148.3\%)$
5	Mood	28.52 ± 0.9	$41.86 \pm 0.97^*(146.77\%)$

Note: * – $p < 0.05$ compared with the indicators before treatment.

The use of phabomotisol and ionized fluids with positive and negative AFP in complex acne therapy.

According to the protocol of the study, the number of patients in the fifth group who received complex therapy was 22 people – 8 young people and 14 girls aged 18 to 28 years. The duration of the disease varied from 1 to 10 years. Before the start of therapy in this group, the dermatological index of acne averaged 7.1 points. In addition to combined topical agents and anolyte externally, patients were prescribed phabomotizol in a dose of 5 mg 3 times a day for a month and catholyte in a dose of 2 ml / kg body weight once a day for 14 days internally.

A month after the start of the treatment in 68.2% of cases, DIA decreased to light acne by 30.77% ($p < 0.05$). After 3 months, 36.4% ($p < 0.05$) of the rash virtually disappeared DIA 1-2). On average, the number of rashes decreased by 56.41% ($p < 0.05$). After a half of a year of the observation and treatment, 81.2% of patients did not complain, because the skin was practically clean of the acne-elements. Only comedones were present in the seborrheic zones. The level of decline in DIA as a whole

was 74.36% ($p < 0.05$). In all, one patient (4.5%) was noted to have tightness of the skin (sometimes). A graphic depiction of the decrease in the intensity of acne eruptions in patients of the fifth group is shown in Figure 9.

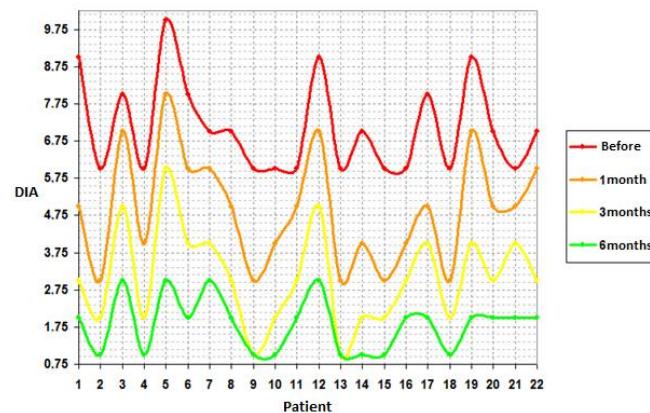


Fig. 9. Dynamics of changes in DIA (scores) in each patient treated with phabomotisol and ionized fluids

The results obtained also demonstrate an increase in the duration of remission, which averaged 6.1 months and 22.7% of patients continued for more than 1 year (Figure 10).

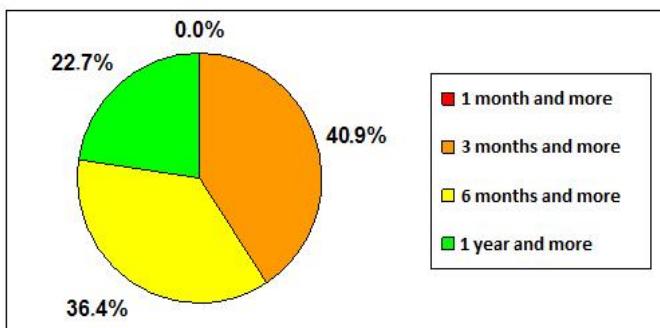


Fig. 10. The duration of remission when included in the treatment of acne fabomotisol and ionized fluids, %

After inclusion in the treatment regimen of acne phabomotisol and ionized fluids, the patient's immunograms changed significantly (table 5): the number of CD4 + T-cells increased by 14.48%, the immunoregulatory index increased by 40.88%, the number of cytotoxic CD8 + lymphocytes decreased by 17.92% ($p < 0.05$).

Table 5

Dynamics of changes in immunological indices ($M \pm m$, UD, %) of patients treated with phabomotisol and ionized fluids (n=22)

№	Indicators immunity	Terms of observation		Percent changes
		Before treatment	After treatment	
1	Leukocytes	6.26 ± 0.35	6.07 ± 0.17	96.7
2	Lymphocytes	25.41 ± 1.66	$28.55 \pm 1.46^*$	115.17
3	CD3+	71.93 ± 1.67	73.16 ± 1.16	101.91
4	CD19+	9.53 ± 0.91	10.90 ± 0.79	107.78
5	CD4+	36.31 ± 1.69	$41.05 \pm 1.71^*$	114.48
6	CD8+	27.32 ± 1.14	$22.50 \pm 0.85^*$	82.08
7	Immunoregulatory index	1.36 ± 0.10	$1.89 \pm 0.11^*$	140.88
8	NK	10.07 ± 1.06	10.01 ± 0.76	98.96
9	Phagocytic activity of neutrophils	59.55 ± 1.47	$63.68 \pm 1.35^*$	88.22
10	IgG	13.34 ± 0.67	$11.79 \pm 0.44^*$	86.84
11	IgM	1.87 ± 0.13	$1.63 \pm 0.08^*$	88.29
12	IgA	2.40 ± 0.18	2.10 ± 0.14	11.71

Note: * – $p < 0.05$ compared with the indicators before treatment.

According to the obtained data on the change in the psychoemotional state of patients (Table 6), as a result of treatment of patients in the fifth group, anxiety rates decreased by 25.68% ($p < 0.05$), depression by 15.17% ($p < 0.05$), The results of the SAN test improved by 52.75% ($p < 0.05$).

Analyzing the presented results, it can be concluded that a pronounced clinical effect (a decrease in DIA by 74.36%) and the longest remission (an average of 6.1 months) were observed with the inclusion of phabomotisol and

ionized fluids in the combination of topical acne therapy.

Comparative analysis of acne treatment results using different therapy programs.

One of the main indicators of the effectiveness of the treatment is the magnitude of DIA, which characterizes the degree of positive changes in skin condition. Analysis of the obtained data shows that in the fifth group the decrease in the intensity of the precipitation was maximal: after 6 months, decreasing by 74.36%.

Table 6

Dynamics of psychometric indicators ($M \pm m$, scores, %) in patients treated with phabomotisol and ionized fluids (n=22)

№	Index	Terms of observation	
		Before treatment	After treatment
1	Anxiety	31.68 ± 1.46	$23.55 \pm 0.58^*(74.32\%)$
2	Depression	34.45 ± 1.38	$29.23 \pm 0.90^*(84.83\%)$
3	Health	29.23 ± 1.32	$44.55 \pm 1.25^*(152.41\%)$
4	Activity	30.18 ± 1.05	$45.67 \pm 0.95^*(151.32\%)$
5	Mood	28.40 ± 1.16	$43.89 \pm 1.04^* (154.54\%)$

Note: * – p<0.05 compared with the indicators before treatment.

In the appointment of IL with negative AFP, in conjunction with phabomotisol, the maximum increase in the total SAN index was observed by 52.75% in patients of the fifth group and the decrease in anxiety scores by 25.68% (p <0.05), which is associated with a positive pharmacodynamic interaction of the drugs. In addition, the conducted correlation analysis demonstrated the dependence of the amount of dermatological index of acne and indices of the psychological profile, in particular – values of the scale of anxiety. Among patients who received phabomotisol and ionized fluids, an increase in the correlation coefficient was revealed (Table 7), which indicates an increase in the relationship between DIA indices and anxiety after the therapy.

Table 7
Spearman correlation coefficients for anxiety and DIA

Number	Before treatment	After treatment
Group 1	0.261	0.001
Group 2	0.198	0.357
Group 3	0.303	0.424*
Group 4	0.332	0.311
Group 5	0.315	0.398

Note: * – Correlation is significant at the level of 0.05 (two-sided).

In the results of the research, the effect on the immune processes of a combination of ionized liquid with negative AFP and a psychotropic drug was demonstrated. The absence of significant changes in the immune response, when standard

drugs and phabomotisol are included in the treatment program, allows us to state that IL with negative AFP has an immunotropic action.

One of the important indicators of the effectiveness of therapy is the duration of remission. The data of the study convincingly testify that remission for more than a year occurred in 22.7% of patients in the fifth group and in no one as a result of the application of a standard therapy regimen. As a result of the inclusion in the treatment program of acne phabomotisol and ionized fluids with different AFP on the background of combined topical drugs, the incidence of side effects decreased from 81.2% to 4.5% of cases.

Conclusion

The thesis presents the results of studying the effect of preparations of a complex pharmacotherapeutic regimen for the correction of immunological, psychoemotional changes and cutaneous manifestations of acne on the course of the examined dermatosis. In the course of the study, an effective way of standard topical combined agents not including retinoid-like substances on the pathological processes in the hair follicle and skin manifestations of acne was suggested. Given the role of stress in the implementation of the cascade of pathogenetic mechanisms of acne, including changes in the local and systemic immune response, new tools have been proposed to correct psychoemotional and immunological changes in patients with acne.

The effectiveness of the assignment of ionized liquids, which we use for the first time in

complex therapy of moderate acne, has been proved in the work. The physicochemical properties of the ionized liquid with negative AFP made it possible to assume the presence of its immunotropic properties, which was confirmed in the course of the study. In addition, it was proved that the therapeutic effect, expressed in the reduction of cutaneous manifestations of acne and the onset of a prolonged remission, was achieved without the use of systemic antibiotics with the minimum frequency of side effects.

Summing up, we can draw the following conclusions:

1. The use of standard therapy provides a reduction in the DIA index by 33.12% and does not lead to a stable remission. Side effects of therapy are recorded in 81.8% of cases.

2. When using monotherapy with combined topical agents, the DIA index decreases by an average of 48.75%, remission for more than a half of a year is achieved in 46.4% of cases.

3. The inclusion in the treatment of moderately severe acne of fabomotizol at a dose of 5 mg 3 times a day during the month positively affects the clinical picture of the disease and the psychometric parameters of patients (DIA after 6 months decreases by 56.41%) and does not affect the immune status. Undesirable effects of the therapy are recorded in 9.1% of patients.

4. The use of an ionized liquid with negative AFP at a dose of 2 ml / kg of body weight 1 time per day for two weeks in patients with acne leads to an increase in CD4+ lymphocytes by 14.48%, an immunoregulatory index by 40.88% and a decrease in the number of CD8+ cytotoxic lymphocytes by 17.92%.

5. The maximum decline in the DIA index (by 74.36%) and long-term clinical recovery (remission of more than a year in 22.7% of cases) with a minimum incidence of side effects (in 4, 5% of cases) was achieved by including in the treatment program phabomotisol and ionized liquids with different redox potential.

Conflicts of interest

The authors have no conflict of interest to declare.

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PHARMACOLOGICAL CORRECTION OF INTERCEPT HEMODYNAMICS IN ACUTE KIDNEY DAMAGE (PART 1)

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Abstract

Introduction: Development of vasoconstriction of kidney arterioles and reduction of renal blood flow is one of the main mechanism of acute kidney injury (AKI) formation. Methods for evaluation of intrarenal hemodynamics status are rather limited. Evident interest for the clinician is the possibility of rapid and non-invasive assessment of renal hemodynamics using the dopplerography method. The method makes it possible to visualize the kidney vessels and conduct a qualitative and quantitative evaluation of renal blood flow. Peculiarities of disturbed blood flow in the kidneys can determine the individuality of pharmacological correction and intensive care in patients with AKI.

Objectives: The aim of our study was to reveal the peculiarities of renal blood flow disorders, depending on the variant, stage and severity of AKI; to evaluate the opportunities of individual pharmacological correction and intensive care in studied AKI patients.

Methods: A prospective nonrandomized study. Inclusion criteria: patients with prerenal, renal and subrenal AKI in the stage of oligoanuria and restoration of diuresis; exclusion criteria: AKI patients after cardiac surgery and operations on the large vessels. 250 ICU patients with prerenal (130), renal (81) and subrenal (39) AKI were examined by ultrasound dopplerography.

Results and discussion: Comparative data of intrarenal blood flow dopplerographic examination in patients with various variants of AKI are presented. All patients initially, at admission in ICU revealed disorders of renal hemodynamics, the severity of which was different depending on the AKI module. During intensive care, as diuresis was restored, the parameters of renal blood flow improved. The speed and completeness of hemodynamics recovery was determined by both the modulus and severity of AKI. The heterogeneity of the prerenal module of AKI was determined due to the data of renal blood flow and the rate of restoration of diuresis. So, we divided prerenal module in 2 groups: 1) real (genuine) prerenal AKI and 2) AKI prerenal for reason. The expediency of such selection is substantiated. It was established that resistive index (RI) in the main trunk of the renal artery is an early criterion of severity of AKI (F), and its dynamics during intensive care makes it possible to diagnose the transformation of AKI in chronic renal failure (CRF). Strong direct correlation RI with the duration of oligoanuria ($r = 0.72$), which is the main retrospective marker of the severity of AKI, was revealed yet upon admission to the ICU. It was found that the peculiarities of renal hemodynamics disturbance are an important criterion for differential diagnosis of the AKI module: renal dopplerometry data significantly ($p < 0.05$) differ in prerenal, renal and subrenal AKI. It was found that the risk of death is statistically significant decreased ($p = 0.001$), OR =

0.009 (95% CI 0.001 – 0.050) when performing individual pharmacological correction (nephroprotection) in the ICU, based on the advanced diagnosis of the AKI module.

Conclusions: The revealed peculiarities of renal blood flow disorders in patients with different AKI modules point to the need for an individual pharmacological correction and intensive care in AKI patients.

Keywords: Acute kidney injury, renal blood flow disorders, dopplerography, pharmacological correction, intensive care.

Introduction

Treatment of AKI is an important social problem. In recent years, the incidence of AKI has increased, accounting for more than 35% of patients in intensive care units (ICU) [1, 2]. This severe complication is increasingly developing as a component of multiorgan failure in patients of the most able-bodied age [3, 4, 5]. Accession of AKI is an independent predictor of adverse outcome in critically ill patients and increases hospital mortality by 3-10 times [6, 7, 8]. With the advent of hemodialysis, the results of treatment of renal failure improved in comparison with the pre-dialysis era. However, at present, despite the high level of dialysis technologies, new classifications (RIFLE, AKIN, KDIGO), the results of treatment AKI patients, especially complicated by multiorgan failure, do not improve [9].

Mortality in this pathology remains high and exceeds 50% [10, 11]. Often the difficulty of conducting adequate intensive care is associated with the lack of timely diagnosis and the late determination of real cause of AKI [12, 13].

The development of vasoconstriction of kidney arterioles and reduction of renal blood flow is one of the main mechanism AKI formation. Spasm of renal arterioles causes the growth of peripheral vascular resistance and reduces the blood supply of the parenchyma. The expressed hypoperfusion leads to damage, first of all, cells of the proximal renal tubules. Tubulosclerosis and necrosis promotes the flow of ultrafiltrate into the interstitium. Interstitial edema, in turn, compresses the blood vessels, worsens blood circulation and aggravates the disorders of the basic processes that provide numerous kidney functions [14].

Methods for assessing the status of intrarenal hemodynamics are rather limited. The active use of traditional radioisotope, radiologic and radiopaque methods for evaluating the kidney in conditions of reduced renal function may be an

additional risk factor for AKI progression and is not always sufficiently informative [14, 15, 16]. Evident interest for the clinician is the possibility of rapid and non-invasive assessment of renal hemodynamics using the dopplerography method. The method allows to visualize the kidney vessels [15, 16]. Performing a complex ultrasound in the regime of pulsed wave dopplerography allows conducting a qualitative and quantitative analysis of arterial and venous blood flow in the kidneys and aorta [17, 18]. Most of the work is limited to the analysis of hemodynamics in patients with non-severe forms of AKI, in cases where persistent oliguria and severe impairment of renal functions have not yet developed [19, 20].

The possibility of dopplerography examination of renal blood flow only in the early stages (1 and 2 according to AKIN, KDIGO classification) is assessed. Some authors prefer resistive index (RI) in comparison with humoral factors, in particular, with an early marker of AKI – cystatin C [18, 21, 22]. A great number of publications are devoted to the possibility of differentiating the prerenal and renal form of AKI with tubular necrosis [19]. The analysis is limited to carrying out correlations between the RI and the possibility of forming the 3rd AKI stage. The main focus is on the resistive index (RI), without assessing the rates of arterial blood flow, on the basis of which RI is calculated [23, 24].

In ICU of Donetsk clinical territorial unit (DoCTU) work is performed to improve the pharmacological and non-pharmacologic correction of detected renal blood flow disorders in AKI patients to provide nephroprotection [25, 26].

Objectives. The aim of our study was to reveal the peculiarities of renal blood flow disorders, depending on the variant, stage and severity of AKI; to evaluate the opportunities of

individual pharmacological correction and intensive care in studied AKI patients.

Methods

250 patients were the main group (treated from 2009 to 2014 in DoCTU), 162 male (64.7%), 88 female (35.3%). The age was from 18 to 85 years, an average of 53 ± 8.2 . When enrolling in the ICU, an isolated AKI was only in 5%. All patients after diagnosis clarification were divided into modules: 1) patients with prerenal AKI module (130); 2) patients with renal module AKI (81); 3) patients with subrenal AKI module (39). The module was a relationship of etiology, pathogenetic mechanisms, risk factors, causes of AKI.

Renal replacement therapy was performed in 182 (72.8%) patients, in total 965 sessions, 5.3 sessions per patient on the AK-200, Innova (Hospal), Tina (Baxter), using dialyzers – Alwal GFE, GFS, Polyflux (Gambro), F-5 (Fresenius) and provided intermittent hemodialysis, hemodiafiltration, hemofiltration depending on the mode of operation and the dialysate membrane. The comparison group consisted of 107 patients with a prerenal (60), renal (32) and subrenal (15) variant of AKI treated at the DoCTU before 2009, aged 18 to 77 years, an average of 48 ± 7.9 . 74 male (69.2%) and 33 female (30.8%). Upon admission to the ICU, the isolated AKI was up to 25%. Dialysis treatment was performed in 79 patients (73.8%).

The groups were comparable by sex and age, although the average age group was higher in the main group. In addition, in the comparison group at admission, the percentage of isolated AKI was higher, without polyorganic complications. In the retrospective group, the treatment was performed without taking into account the peculiarities of the renal blood flow and the individual approach.

All patients underwent general clinical, biochemical studies, ultrasound of the kidneys, ECG, chest X-ray; Parts of them, according to the indications – echocardiography, computed tomography (CT) of the abdominal cavity and retroperitoneal space, magnetic resonance imaging (MRI).

In addition to the standard ultrasound, all patients underwent doppler scanning of the vessels of both kidneys (color Doppler mapping and pulsed dopplerography) on the HDI-5000 expert class device (Philips, Holland) in

dynamics. The velocity of blood flow was measured in cm / s. In automatic mode, maximum systolic (Vps), end diastolic (Ved) blood flow velocity was determined in the renal vessels. The resistive index (RI) was calculated. $RI = [(V_{ps} - V_{ed}) / V_{ps}]$.

The systolic-diastolic ratio was determined – Vps/Ved. Each patient was performed 2 complex ultrasound examinations in the stage of oligoanuria and recovery of diuresis.

Statistical processing of data. The analysis of the results was carried out in statistical packages MedStat v.4.1 (Lyakh Yu. E., Guryanov V.G., 2004-2013), MedCalc v. 13.2.2 (MedCalc SoftWare bvba, 1993-2014), Statistica Neural Networks v.4.0 B (StatSoft Inc., 1996-1999). The obtained results are presented in the text in the form of values of the arithmetic mean and its standard error. For the selection of adequate sample comparison procedures, a preliminary verification of the distribution for normality was carried out. The statistical significance of the differences in the mean values in the two samples was estimated using the Student's test (the normal distribution law) or the Wilcoxon test (the distribution law differs from the normal one).

When analyzing the dynamics of change in characteristics during the treatment, the methods of comparing the coupled samples were used. In all cases, the difference was considered reliable when $p < 0.05$. To identify the relationship between the signs used by the method of correlation analysis. The correlation coefficient was used to estimate the degree of linear connection between the two signs. In cases where the distribution was not different from normal, the standard Pearson correlation coefficient (r) was determined. If the distribution deviates from the normal the signs was measured in the rank scale, the Spearman rank correlation coefficient used. At $p < 0.05$, the hypothesis of the existence of a correlation link was accepted. Computer program Excel was used to create diagrams.

To assess the effect on the outcome of AKI in the main group, a number of factors were analyzed that could potentially have an impact on the results of treatment. During the analysis, the results of treatment of 106 patients were reviewed. The task was to identify the factor signs associated with the results of AKI patients

treatment. An estimation of the degree and direction of the influence of these factor signs on the obtained results was carried out.

For the analysis, methods of constructing multifactorial models of classification were used. The quality of the constructed models was estimated by their sensitivity and specificity, a 95% confidence interval (95% CI) was calculated [25]. To determine the factors most associated with the results of treatment, the "genetic algorithm" (GA) method was used. To assess the adequacy of multifactorial mathematical models and predictive efficacy trials, area under the ROC curve (Area Under Curve – AUC) of 95% CI was used. To evaluate the possibility of using the constructed models on new data, a method was used to verify their prognostic characteristics on the confirming variety (a random number generator was used for the selection). To assess the degree of influence of factor characteristics on the resulting characteristics, a method of constructing logistic regression models was used [27]. For the assessment, the odds ratio (OR), as well as their 95% CI, was calculated.

Results and discussion

The development of vasoconstriction of kidney arterioles and reduction of renal blood flow is the main mechanism for the formation of AKI. Normally, the microvascular system of the kidneys is low-resistant. We conducted a comparative analysis of changes in blood circulation in the kidneys with various modules of AKI in the stage of oligoanuria, as well as in the restoration of diuresis.

Visual assessment of renal blood flow using color Doppler duplex scanning in the oligoanuria stage revealed: 1) signs of its impoverishment in the cortical layer of the kidney parenchyma at the level of the arc and interlobar arteries, weak signals; 2) reduction of the diastolic flow, until its complete absence; 3) an increase in the rate of venous blood flow associated with shunting the

blood into the venous system of the pyramids and overflowing the venous vessels; 4) in 8 patients the presence of reversible blood flow to the diastole; 5) in 11 patients – with thrombosis of the main trunk of the renal artery – complete absence of blood flow.

In the oligoanuria stage, patients of all three modules had abnormalities of renal hemodynamics, which significantly ($p <0.001$) differed from those in the control group. The blood flow velocity in the main trunk of the renal artery in the oligoanuria stage was significantly lower than the corresponding parameters in the control group.

This involved both V_{ps} and V_{ed} blood flow velocities at the level of the main renal artery and its segments (arc, interlobar and segmental vessels). The ratio S / D was higher than the data in the control group, which can be explained by edema, lymphohistiocytic infiltration of the interstitial tissue of the kidney in AKI. RI in all cases was elevated and significantly ($p <0.001$) was different from the data in the control group.

In clinical practice, the severity of AKI (F) is determined retrospectively by the duration of the oligoanuria stage. A correlation between RI and the duration of the oligoanuria was performed, a strong ($r = 0.724$) direct correlation was found.

The following data were obtained: if at admission RI in the trunk of the main renal artery was > 0.79 , the duration of the oligoanuria stage was more than 18 days; At an RI in the trunk of the main renal artery from 0.71 to 0.79, the duration of the oligoanuria stage was 8 to 18 days; If RI <0.71 , the duration of the oligoanuria was less than 8 days. Thus, already at the admission in ICU it was possible to talk about the severity of AKI (F). In RI >0.79 , AKI was assessed as severe, in RI level of 0.70 to 0.79 – moderate , and in RI <0.71 light AKI (F) (Figure 1).

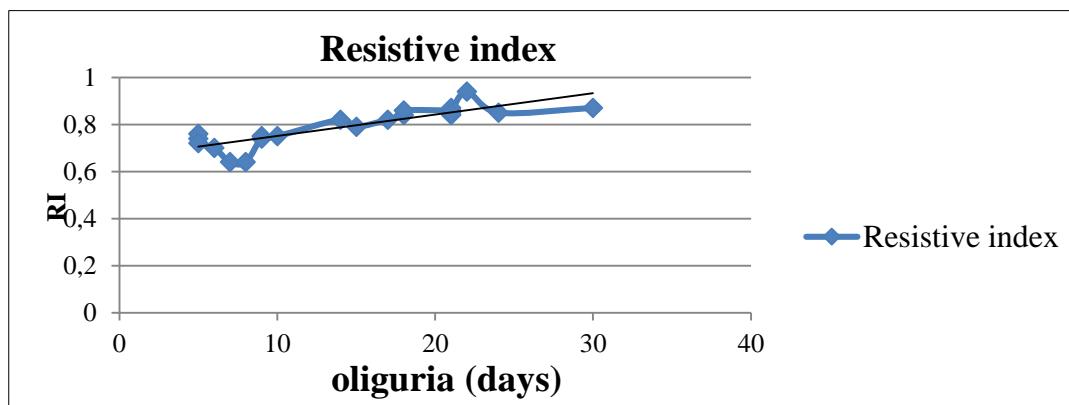


Fig. 1. RI and oliguria (in days) correlation link

Reduction of the rates of renal blood flow and an increase in RI was typical for all patients with AKI in the stage of oligoanuria. However, the studied data significantly ($p < 0.001$) differed in various modules of AKI.

Thus, the most significant decrease in the rate of renal blood flow was observed in renal AKI. In the main trunk of the renal artery V_{ps} was 42.4 ± 0.15 cm / sec; V_{ed} – 6.04 ± 0.03 cm / sec; S / D – 8.96 ± 0.27 ; RI was increased to 0.85 ± 0.01 and was the highest in comparison with RI in other AKI modules. The data of S / D in renal AKI variant were the highest. So, these data reflect, not only the decrease in the total blood flow, but also the edema of the kidney parenchyma, significant shunting of the blood into the venous network with depletion of cortical structures. The most significant edema of the renal parenchyma was revealed in the renal module of AKI.

In prerenal AKI module the following changes were observed according to the data of Doppler ultrasound: V_{ps} was reduced to 59.5 ± 0.2 cm / sec, V_{ed} was 15.2 ± 0.2 cm / sec; the S / D ratio was higher than the data in the control group and was 4.0 ± 0.2 ; RI increased to 0.75 ± 0.02 .

In subrenal AKI, V_{ps} was reduced to 64.01 ± 0.25 cm / sec; V_{ed} – to 20.3 ± 0.3 cm / sec; the S / D ratio was 3.12 ± 0.8 ; RI raised to 0.69 ± 0.02 (Figure 2, 3).

Further analysis of the Dopplerometry data in the oligoanuria stage revealed that groups of patients with prerenal and renal AKI were heterogeneous. And the hemodynamic data differed significantly in them.

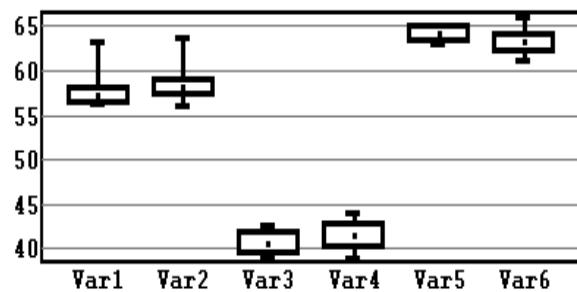


Fig. 2. Comparative characteristics of V_{ps} in the main trunk of the renal artery with prerenal (1, 2), renal (3, 4) and subrenal (5, 6) AKI in oliguria.

Note: Var 1, 3, 5 – right kidney; Var 2, 4, 6 – left kidney. V_{ps} right versus left kidneys $P_{1-2, 3-4, 5-6} \geq 0.05$

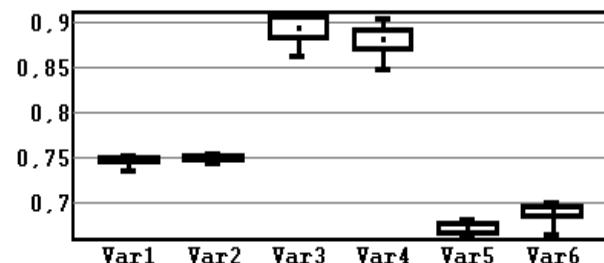


Fig. 3. Comparative characteristic of RI in the main trunk of the renal artery in prerenal (1,2), renal (3,4) and subrenal (5,6) AKI in oliguria.

Note: Var 1,3,5-right kidney; Var 2,4,6-left kidney. V_{ps} right versus left kidneys $P_{1-2, 3-4, 5-6} \geq 0.05$

In some patients (27) with prerenal AKI, V_{ps} and RI significantly ($p < 0.05$) differed from the majority of patients (53) in this group. Disturbances of renal hemodynamics in this subgroup were much less severe. Thus, V_{ps} was 67.4 ± 5.0 cm/sec, V_{ed} 18.9 ± 1.2 cm/sec, and RI was 0.715 ± 0.01 . While in the majority of patients V_{ps} was less (54.8 ± 6 cm/sec on average), V_{ed} – 13.38 ± 4.2 cm/sec, and RI was 0.75 ± 0.05 (Figure 4, 5).

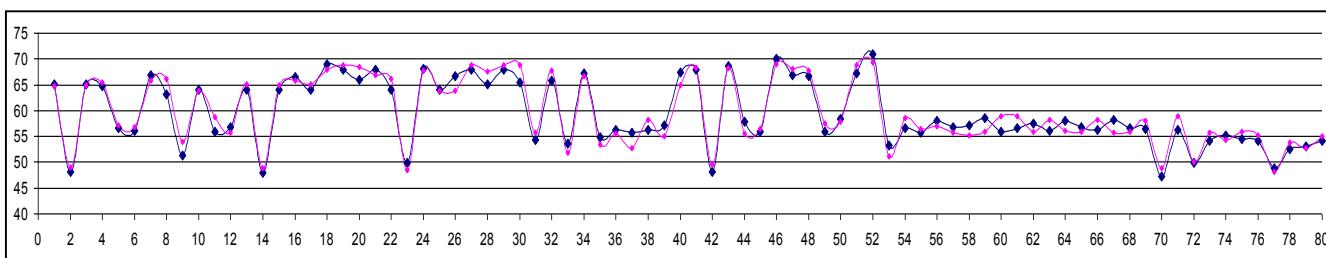


Fig. 4. Variation of Vps in the prerenal AKI group

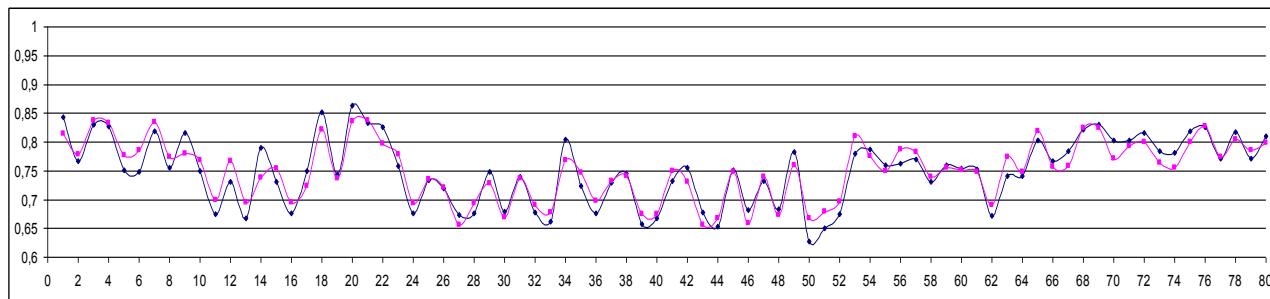


Fig. 5. Variation of RI in the prerenal AKI group

Subsequently it turned out that 27 patients of the prerenal group with less severe renal blood flow disorders responded well to pharmacological correction (nephroprotective measures) and diuresis was restored without hemodialysis treatment or 1-2 sessions were performed. So, we characterized this subgroup as a real prerenal AKI, which is not accompanied by severe tubular damage. In the remaining cases (53), AKI was evaluated as for the causal factor prerenal AKI.

In a group of patients with renal AKI, the significant variation of hemodynamic data was revealed too. Thus, the min value of Vps in the renal AKI group was 31.15 cm/sec , and max – 66.8 cm/sec . Accordingly, the min RI was 0.69, and max was 0.94 (Figure 6, 7).

In 17 patients of this group, very low Vps of $34.4 \pm 0.5 \text{ cm/sec}$ was detected and Ved – $8.7 \pm 0.3 \text{ cm/sec}$ that was less than in the other patients of this group. The reason of such peculiarities, on our opinion, was the prevalence of nephrosclerosis versus severe oedema of renal parenchyma in these patients. The RI in this subgroup was relatively lower in comparison with the rest of the patients in renal AKI group ($p < 0.05$) and was 0.74 ± 0.02 . Subsequently, in these 17 patients kidney function did not restore definitively. They were isolated as a group of AKI on CRF or – with transformation in CRF. More than 70% of these patients were transferred from the ICU to continue treatment with program hemodialysis or kidney transplantation.

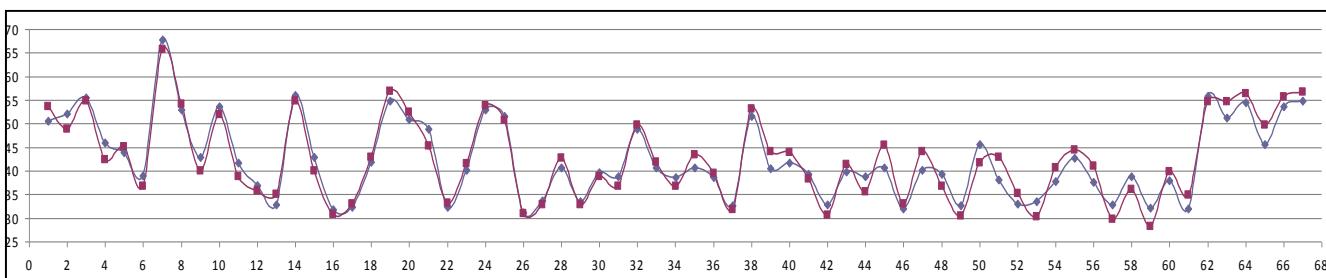


Fig. 6. Variation of Vps in the renal AKI group

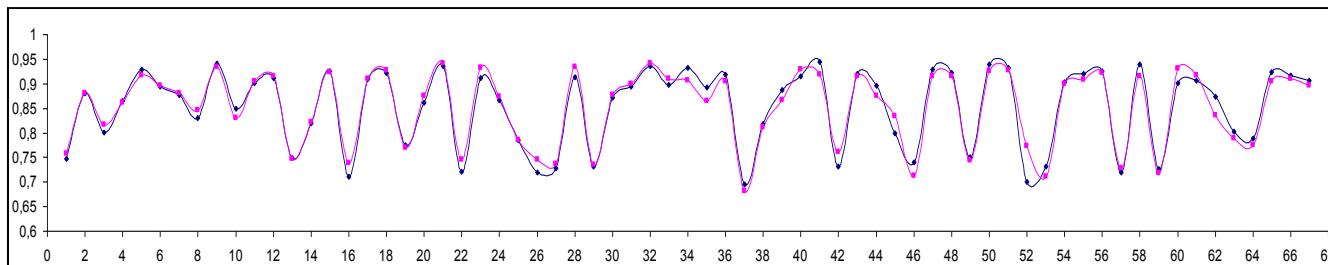


Fig. 7. Variation of RI indices in the renal AKI group

In polyuria phase of AKI, according to the data of complex ultrasound Dopplerography, restoration of arterial blood flow was observed in all groups, beginning with arched and interlobar arteries; normalization of the shape of the Doppler curve; reduction of echogenicity and thickness of the parenchyma, restoration of kidney size and cortico-medullary differentiation. Improvement of the clinical state, restoration of renal functions clearly correlated with improvement of renal hemodynamics and biometric parameters.

The absence of a reliable improvement in the parameters of renal blood flow, according to the data of Dopplerography, even in patients with restoration of diuresis, indicated loss of function and transformation in CRF.

As a model of the study, it is possible to present the diagnosis and treatment of patients with renal artery thrombosis of a single functioning kidney.

1. Diagnostics. The diagnostics based on: clinical manifestations – back pain, oliguria; The presence of risk factors for acute renal blood flow disorders: atherosclerosis, hypertension, permanent atrial fibrillation, history of myocardial infarction or transient cerebral circulatory disorders, malignant neoplasms, old age, wrinkling of one of the kidneys. The absence of expansion of the calyceal system in renal ultrasound examination was the reason for the Dopplerography;

2. Appointment of renal ultrasound in the Doppler mode allow fast, with minimal risk of complications and high accuracy to establish the diagnosis of renal artery thrombosis. Kidney ultrasound in the usual regime was uninformative in this pathology;

3. Carrying out x-ray endovascular intervention, including several steps: catheterization of the renal artery; tromboaspiration and balloon angioplasty of the vessel; selective thrombolysis (actilize100 mg for 1 hour). Endovascular intervention was advisable, regardless of the oliguria duration, as AKI was

prerenal. High levels of creatinine in this case were not a contraindication for the X-ray-contrast introduction.

In all 11 this group patients, diuresis recovered immediately after intervention and pharmacological correction, despite prolonged anuria (up to 7 days). Conducting hemodialysis was not required.

Figures 8-11 show the renograms of the patient P., 58 years (7 days of anuria), which demonstrate the restoration of blood flow after catheterization of the left renal artery and local thrombolysis.

After removal of the thrombus, intravenous heparin was administered at a dose of 600 to 800 U /h under the control of the coagulogram indices (the target level of APTT was 60-80 s). Heparin therapy was combined with clopidogrel (75 mg / day). Warfarin was prescribed at the stage of diuresis recovery. When target values of INR (2.5-3.5) were reached, heparin was canceled.

Such nephroprotective therapy was organ-preserving, prevented the wrinkling of the kidney and the need for program hemodialysis (in contrast to the comparison group). So, a good quality of life in extremely severe group of patients was provided.

It was principally important to provide the pharmacological correction during endovascular intervention (thrombolytics, anticoagulants), otherwise the effect was short-lived.

To assess the influence of various factors on the outcome in AKI patients, a multifactor analysis was carried out. The results of treatment in AKI patients were evaluated as: survived, died.

18 factors were analyzed, as the factor signs in mathematical models construction: age, sex, AKI module (prerenal -1, renal-2, subrenal-3), duration of the disease before admission to ICU, time of diagnosis, duration of oligoanuria in days before admission to ICU, the presence of multiorgan failure (MOF), the origin of multiorgan disorders (primarily, secondary), the development of AKI in the presence of CRF,

presence of risk factors (no = 0, is = 1), pre-dialysis intensive therapy (early pharmacological correction, nephroprotection) before admission to the ICU (0-none, 1-conducted), pre-dialysis and peridialysis intensive therapy (nephroprotection in ICU) (1- early, 0 late), the need for

hemodialysis (without HD = 0, HD = 1), the number of hemodialysis, the need for artificial ventilation, the level of urea and creatinine on admission to the ICU, the highest level of urea and creatinine.

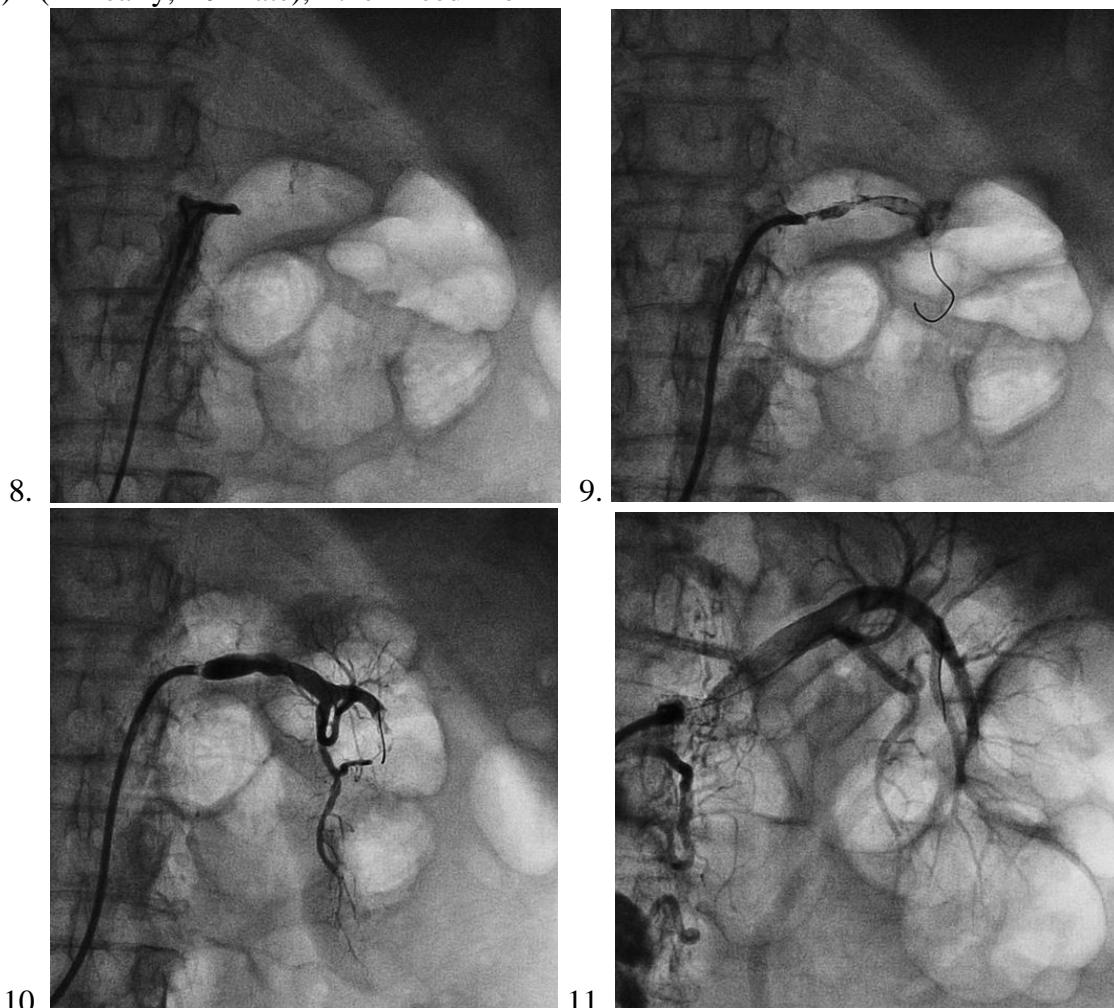


Fig. 8-11. Restoration of blood flow in the main trunk of the renal artery

To identify the factors most associated with the risk of death, a selection of significant signs was performed using the genetic algorithm (GA) method. As a result of the analysis, 2 factors were selected: the presence and origin of multiorgan disorders (primary MOF-1, secondary-2, absence-0) – (X1), nephroprotection in ICU (1 – early, 0 – late) (X2).

On the selected set of factor attributes, a linear neural network model for predicting the risk of death was constructed. The sensitivity of this model on the training set was 82.4% (95% CI 59.4% – 96.9%), specificity – 94.9% (95% CI 87.7% – 99.1%). On the confirming set, the

sensitivity of this model was 83.3% (95% CI 34.3% – 100%), specificity 100% (95% CI 92.3% – 100%).

Sensitivity and specificity on the training and confirming sets are not statistically significant ($p = 0.57$ and $p = 0.63$, respectively, when compared by the criterion χ^2), which indicates the adequacy of the model constructed.

To evaluate the significance of the two isolated factors from the total of 16 factor and assess the adequacy of the constructed models for predicting the risk of death, a method was used to compare the ROC curves of the models (Figure 12).

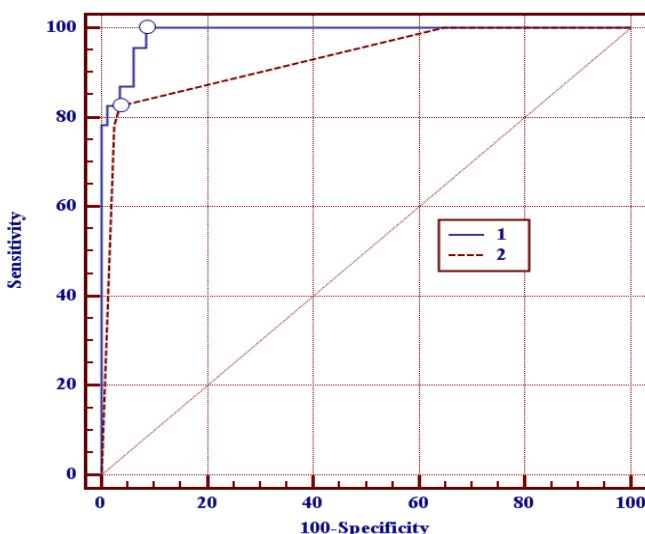


Fig. 12. ROC-curves of models for predicting the risk of death (0 – the optimal values of sensitivity and specificity of models are indicated, the curves are constructed on all 106 cases): 1 – linear neural network model built on all 16 factor attributes, 2 – linear neural network model built on 2marked factor characteristics

When analyzed, it was established that the area under the ROC curve for the linear neural network model constructed on all 16 factor signs was $AUC_1 = 0.99 \pm 0.01$, for the linear neural network model constructed on the 2 selected factor signs, $AUC_2 = 0.93 \pm 0.03$.

Thus, a decrease in the number of factor signs from 16 to 2 does not lead to a significant change in the predictive qualities of the model, which indicates the high significance of the identified

factors; such as presence and origin of MOF (primary -1, secondary-2, absence-0) (X1), nephroprotection in the ICU (1 – early, 0 – late) (X2) to predict the risk of death. To determine the strength and direction of the influence of the 2 selected factors, a logistic regression model was constructed, the model is adequate ($\chi^2 = 66.0$ with the number of degrees of freedom $k = 3$, $p < 0.001$). The results of analysis of the regression coefficients of the model are presented in table 1.

Table 1

Coefficients of the 3-factor model for predicting the risk of a lethal outcome (logistic regression model)

Factor	The prediction model coefficients Sign value, $b \pm m$	The significance of the coefficient difference from 0, p	odds ratio, OR (95% CI OR)
X1 1. vs 0	17,5±3000	0,995	–
X12vs 0	15,2±3000	0,996	–
X2	-4,8±0,9	<0,001*	0,009 (0,001–0,050)

Note: * – the difference from 0 is statistically significant, $p < 0.05$.

Analysis of logistic regression model coefficients suggests that when early nephroprotection with pharmacological correction is performed in ICU, the risk of death is statistically significant ($p = 0.001$) decreased, $OR = 0.009$ (95% CI 0.001 – 0.050). The sensitivity of this model was 83.3% (95% CI 34.3% – 100%), specificity 100% (95% CI 92.3% – 100%).

In the study group, out of 250 patients, 38 patients died, the mortality rate was $15.2 \pm 0.5\%$. In the comparison group (107 patients), 28 people died, the mortality was $26.2 \pm 3.7\%$. The absolute risk of death was statistically significant ($p = 0.027$) decreased by 11% (95% CI 2.1-20.8).

Conclusion

Disturbances of hemodynamics in AKI according to Dopplerography of the kidneys are most severe when patients enter ICU (in the stage of oligoanuria). In all groups of AKI, the changes in arterial kidney blood flow are the same and show: a decrease in Vps and Ved, as well as an increase in RI at all levels and a significant increase in systolic-diastolic ratio. At the same time, the renal blood circulation values significantly differ depending on the AKI module, which is convincingly presented when comparing in groups of prerenal, renal and subrenal AKI.

The RI is an indicator that reflects the severity of AKI in the early days (on the first days of oliguria) and reliably correlates with the duration of the oligoanuria stage, the main retrospective marker of the severity of tubulointerstitial kidney damage.

Thus, the implementation of complex ultrasound with Dopplerography of the kidney vessels in AKI patients allowed:

- 1) clarify the cause of AKI;
- 2) to identify patients with real prerenal AKI and AKI prerenal for causal factor.
- 3) in the renal AKI group, reveal patients with AKI on CRF, or transformation AKI into CRF.
- 4) to assess the severity of kidney damage upon admission and appoint an individual intensive care program with pharmacological correction (nephroprotection).

This confirms our hypothesis that evaluation of renal blood flow by the method of Dopplerography and the introduction of individual pharmacological correction in intensive therapy AKI patients in ICU improves the results of treatment of these patients.

Conflicts of interest

The authors have no conflict of interest to declare.

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ADAPTIVE DESIGN IN CLINICAL DEVELOPMENT OF NEXT-IN-CLASS DRUGS

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Abstract

Introduction: The next-in-class drugs are the original drugs that by chemical structure and mode of action similar to their predecessors of the same pharmacological group. The clinical development of the next-in-class drugs usually follows the same path as for innovative drugs including all phases. Since the effects of the next-in-class drugs can be predicted with certain accuracy, there is a potential for optimizing their clinical program in terms of duration and costs. Adaptive design represents the innovative approach that allows for efficiency and acceleration of drug development.

Objectives: The study objective was to assess the perspectives of the adaptive design methods in clinical development of the next-in-class drugs of different pharmacological groups including hypoglycemic agents, anticoagulants and anti-HIV drugs.

Methods: The adaptive designs were developed and implemented in phase II-III studies of three next-in-class drugs. The seamless two-stage design was used for sequential assessment of two dosing schemes of gosagliptin (DPP-4 inhibitor), as well as for the dose selection and its further efficacy and safety assessment in phase II/III studies of teixoban (factor Xa inhibitor) and elisulfavirine (NNRTI). The measures necessary to control a type I error and avoid biases were assumed at all stages.

Results and discussion: In three conducted trials the non-inferiority of the next-in-class drugs to the standards of care was demonstrated as well as comparative or improved safety profiles. The adaptive designs allowed for combining two trials/phases in one study providing efficient use of resources and expedited market access.

Conclusion: The adaptive design can be successfully implemented in clinical programs of next-in-class drugs.

Keywords: clinical trials, adaptive design, next-in-class drugs, non-inferiority, type 2 diabetes mellitus, prevention of venous thromboembolism, HIV, DPP-4 inhibitor, factor Xa inhibitor, NNRTI, gosagliptin, teixoban, elisulfavirine.

Introduction

The development of similar in pharmacotherapeutic effect or improved analogues of innovative drugs is one of the main focuses for the development of the Russian pharmaceutical industry in 2013-2020. The mechanisms of the government support of this

area include the Federal target program "Development of the pharmaceutical and medical industry of the Russian Federation for the period till 2020 and further perspective", including the rules for obtaining the subsidies for the next-in-class drugs development [1, 2].

The next-in-class drugs are original patented candidates that affect upon the known biological targets and in structure or mode of action similar to existing drugs. The development of a next-in-class drug can be considered a low-risk R&D strategy due to higher predictability of its effects in humans (i.e. similar to drugs of the same group), as well as possibly better efficacy and safety profiles due to “refining” of the original molecule.

The classic approach to the development of a next-in-class drug is the repetition of the clinical program of its innovative prototype that imposes similar expenses and timelines. Due to high volume of investments needed, the scientific and practical rationale for such drugs might be of question. Hence the pharmaceutical companies require effective planning of the clinical trials of the next-in-class drugs to ensure their reasonable price and expedited market access.

The adaptive design is one of the innovative approaches that allows for conducting clinical trials more efficiently (e.g. shorter duration, less patients, etc.) or with a higher probability of the demonstration of the drug's effects. The trials that use the adaptive design have a predefined possibility of modification of some of the design or hypothesis aspects based on the results of the interim analysis. The data analysis is performed according to the predefined plan at preliminary specified time points; it may be blinded or unblinded, with or without testing of the formal statistical hypothesis.

The first foreign publication describing the concept of the adaptive design appeared at the end of the 1980s (P. Bauer, K. Köhne, M. Posch, J. Wittes, E. Brittain, R.J. Simes) [3, 4, 5, 6, 7]. The enthusiasm towards the possibilities of implementation of the adaptive design came across the critical assessment of the complexity of the proposed statistical models. On the other hand, during the recent decades the regulatory requirements to safety and efficacy assessment have been reinforced that made the clinical programs more complicated, extended the time for the market access, and diminished every possibility for optimization of the clinical trial design.

Nevertheless in the 2000s the regulators of different countries indicated the slowdown of the innovative sector of the pharmaceutical industry that induced some stimulating initiatives such as the national US strategy for implementation of the

innovative approaches to drug development, assessment and manufacturing (FDA, 2004). The adaptive design was suggested among other methods for optimization and acceleration of drug development [8, 9].

Despite the obvious advantages of the adaptive design, this approach might increase the risk of biases and misinterpretation of the clinical trial results. The choice of statistical methods to ensure integrity and validity of study data poses a certain difficulty. These aspects are described in the EU and US guidelines for the adaptive design in clinical trials (EMEA, 2007; FDA, 2010) [10, 11, 12, 13].

In our country the use of the adaptive design is limited to the two-stage approach in bioequivalence studies, as well as few phase II-III trials that altogether are less than 1% of all clinical trials in Russia [14, 15, 16].

Thus the feasibility assessment of implementation of the adaptive design in clinical trials of the next-in-class drugs is important for the pharmaceutical science and industry.

Objectives

The objective of this study was to assess the perspectives of the adaptive design methods in clinical development of the next-in-class drugs of different pharmacological groups including hypoglycemic agents, anticoagulants and anti-HIV drugs.

Three clinical trial designs were developed in order to meet the objective: a phase III clinical trial of dipeptidyl peptidase-4 (DPP-4) inhibitor gosagliptin in patients with diabetes mellitus (DM) type 2, a phase II clinical trial of factor Xa inhibitor tearxaban in patients undergoing knee replacement surgery, and a phase II-III clinical trial of non-nucleoside reverse transcriptase inhibitor (NNRTI) elisulfavirine in patients with HIV-infection. Upon the trials completion, a comparative analysis and economic efficiency assessment of the adaptive designs was performed.

Methods

The clinical trials were conducted in 2012-2016 in the framework of the technologies transfer program financed by the Ministry of Industry and Trade of Russia. The study documents were reviewed and approved by the Ministry of Health of Russia: approval #136 of gosagliptin clinical trial dated March 01, 2013 (27

sites), approval #485 of tearxaban clinical trial dated August 01, 2013 (7 sites), and approval #219 of elsulfavirine clinical trial dated April 21, 2014 (12 sites).

The studies were conducted in compliance with the Guideline for Good Clinical Practice (International Conference for Harmonization, 1996), ethical principles outlined in the Declaration of Helsinki (World Medical Association, 2013), Russian legislation and applicable regulatory requirements (Federal law #61, 2010; National industry standard P 52379-2005) [17, 18, 19].

Study designs. The clinical trials were prospective multicenter randomized studies with active control and two-stage data analysis (the adaptive seamless design). In the gosogliptin study, the two-stage analysis was used to assess the efficacy and safety of the monotherapy at Period 1 and then the combination therapy with metformin at Period 2 in the same patients' population. In the tearxaban and elsulfavirine studies, the interim analysis was used to choose the optimal dose of the investigational product (Stage 1); then additional patients were enrolled in the study in order to assess the efficacy and safety of the selected dose (Stage 2) [20].

Original marketed drugs listed in the national standards of care and the Register of vital and pivotal drugs of Russia were used as comparators: DPP-4 inhibitor vildagliptin (Galvus®, Novartis Pharma Stein AG, Switzerland) was used in the gosogliptin study; low molecule weight heparin enoxaparin (Clexane®, Sanofi-Winthrop Industry, France) was used in the tearxaban study; NNRTI efavirenz (Stocrin®, Merck Sharp & Dohme B.V., the Netherlands) was used in the elsulfavirine study.

The primary efficacy endpoint (PE) in the gosogliptin study was the mean change of HbA1c at Week 12 (period of monotherapy) and at Week 36 (period of combination therapy); in the tearxaban study it was the composite of all venous thromboembolism (VTE) within 6 weeks of the knee replacement surgery; in the elsulfavirine study it was the rate of achievement of the viral load < 400 copies/mL at Week 12 (the surrogate endpoint for the interim analysis) and the undetectable HIV RNA level at Week 24 (the final analysis).

In the gosogliptin trial, the study treatment was not blinded (open study); in the tearxaban and elsulfavirine studies the doses of the investigational products were blinded at the first stage of the studies (partially-blinded study). The credibility of the PE assessment was ensured by its analysis in the central laboratory or by the independent central reviewer; the dose selection based on the interim data analysis in the tearxaban and elsulfavirine studies was performed based on the decision of the Data Monitoring Committee (DMC).

Patients' population and study procedures.

Treatment naïve patients with DM type 2 aged 18 to 78 with body mass index (BMI) of 22 to 40 kg/m², HbA1c from 7.5 to 11.0%, fasting plasma glucose (FPG) < 15 mmol/L, glomerular filtration rate (GFR) ≥ 60 mL/min/1.73m², with no severe acute or chronic complications of DM, significant and/or poorly controlled diseases, were enrolled in the gosogliptin study. A total of 299 patients were randomized to one of two treatment groups: gosogliptin or vildagliptin (at 1:1 ratio). The duration of the patients' participation in the study was about 42 weeks, including screening (1 week), diabetes education and run-in (1 week), randomization and the monotherapy period (12 weeks), the combination therapy period (24 weeks), and follow-up (4 weeks). The following parameters were controlled during the study: the state of systems and organs based on the physical examination, electrocardiography (ECG), and ultrasound; body weight, BMI, blood pressure (BP), heart rate (HR), respiratory rate (RR), and body temperature; HbA1c, FPG and safety laboratory parameters based on hematology, biochemistry and urinalysis in the central laboratory; pregnancy based on the urine pregnancy test; glycemia and hypoglycemic episodes based on glucometer and patients' diary data; adverse events (AE).

Patients aged 18 and older scheduled for the planned primary total knee replacement (TKR) surgery, with normal coagulation parameters, no history of thrombosis or coagulopathy, with no active bleeding, significant and/or poorly controlled diseases were enrolled in the tearxaban study. A total of 200 patients were randomized in one of four treatment groups: tearxaban 50 mg, 100 mg, 150 mg or enoxaparin (at 1:1:1:1 ratio at Stage 1 and at 1:1 ratio at Stage 2 after the dose

selection). The duration of the patients' participation in the study was about 8 weeks, including screening (2 weeks), randomization, surgery and study treatment (2 weeks: 12 ± 2 days) and follow-up (4 weeks). The following parameters were controlled during the study: the state of systems and organs based on the physical examination and ECG; wound assessment, VTE and bleeding symptoms; duplex ultrasound scanning, multislice computed tomography (MSCT); body weight, BP, HR, RR, and body temperature; safety laboratory parameters based on hematology, biochemistry, coagulation, and urinalysis in the central laboratory; pregnancy based on the urine pregnancy test; AE.

Patients aged 18 and older with serologically confirmed stable HIV-1 infection (Stage 1-2), HIV-1 RNA in plasma $\geq 5\,000$ copies/mL and CD4+ T-lymphocytes count > 200 cells/mm³, with no hepatitis B and C, tuberculosis, acute infection, significant, and poorly controlled diseases, who meet the criteria for starting the antiretroviral therapy (ART), were enrolled in the elsulfavirine study. A total of 150 patients were randomized in one of three treatment groups: elsulfavirine 20 mg, 40 mg or efavirenz (at 1:1:1 ratio at Stage 1 and at 1:1 ratio at Stage 2 after the dose selection). The duration of the patients' participation in the study was about 54 weeks, including screening (2 weeks), randomization and study treatment (48 weeks) and follow-up (4 weeks). During the study the following parameters were controlled: state of systems and organs based on the physical examination and ECG; body weight, BP, HR, RR, and body temperature; HIV-1 RNA load by polymerase chain reaction (PCR); CD4+ and CD8+ T-lymphocytes count, HIV resistance mutations by PCR, safety laboratory parameters based on hematology, biochemistry, coagulation, and urinalysis in the central laboratory; pregnancy based on the urine pregnancy test; AE including AE of special interest in central nervous system (CNS).

Statistical analysis. The sample size for each trial was calculated based on the non-inferiority hypothesis to compare two means taking into

account the standard deviation (the gosagliptin study) or to compare proportions taking into account the expected results in the study groups (the tearxaban and elsulfavirine studies); at the significance level of $\alpha = 2.5\%$ (one-sided), power of 80%, and predefined non-inferiority margin [21, 22, 23].

The number of patients required for the interim analysis was calculated based on the Simon's MiniMax statistical model in the tearxaban study and based on the non-inferiority hypothesis for the surrogate endpoint in the elsulfavirine study. None of the studies assumed any repeated testing of the same hypothesis. The two-stage statistical analysis didn't require a type I error correction since the null hypothesis should have been rejected at both stages of the studies. The power of the clinical trials was controlled by the algorithm of the patients' recruitment; the power correction was not performed.

The PE analysis was performed in the full analysis set of patients who received at least one dose of the study drug and who had at least one post-dosing assessment of the PE; in the two-stage design the patients from both stages were included in the analysis. Additional analysis was conducted in the per protocol population. The safety analysis was conducted in the population of patients who received at least one dose of the study drug (safety population).

The statistical analysis was performed in the SPSS Statistics and R programs. The 95% confidence interval (CI) was calculated for the PE and its lower bound was compared to the non-inferiority margin; based on this comparison it was concluded whether the null hypothesis should have been confirmed or rejected. The Simon's model was used for the interim analysis in the tearxaban study. The differences between the means were assessed with the Student's t-test and single factor ANOVA analysis. The comparison of proportions was performed with the Fisher's exact test. The Wilcoxon test was performed for non-parametric data. The significance of the parameter distribution between the treatment groups was assessed with the χ^2 criteria. AE were coded using the MedDRA dictionary [24, 25].

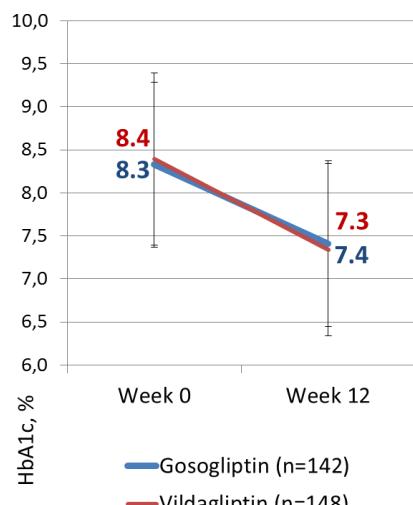
Results and discussion

1) Study results of DPP-4 inhibitor gosogliptin in patients with DM type 2

299 patients were randomized in the study including 149 patients in gosogliptin group and 150 patients in vildagliptin group. The treatment groups were similar in demographic parameters and main baseline characteristics ($p > 0.05$): sex (F 57.7% + M 42.3% vs. F 48.7% + M 51.3%), age (55.7 ± 10.0 years vs. 56.7 ± 9.7 years), race (Caucasian 98.7%), duration on DM type 2 (1.7 ± 2.6 years vs. 2.1 ± 3.8 years), baseline HbA1c ($8.3 \pm 1.0\%$ vs. $8.4 \pm 1.1\%$), FPG (9.5 ± 2.5 mmol/L vs. 9.5 ± 2.8 mmol/L), body weight (90.6 ± 14.8 kg and 91.7 ± 15.6 kg), and BMI (32.1 ± 4.3 kg/m² vs. 31.8 ± 4.3 kg/m²).

a) Period 1 – Assessment of efficacy and safety of monotherapy

The population for the efficacy assessment



a) HbA1c change during 12 weeks of monotherapy with DPP-4 inhibitors

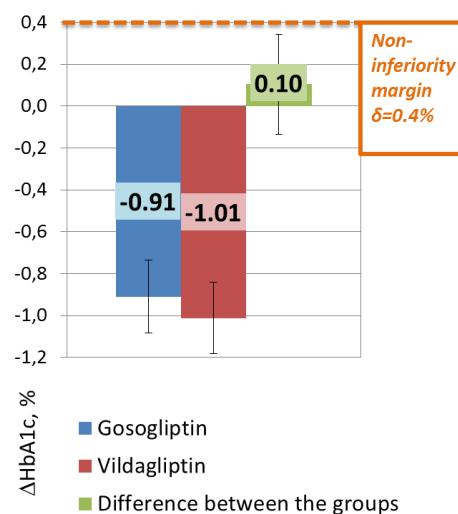
Fig. 1. HbA1c change at Week 12 for monotherapy

The analysis of the secondary endpoints after 12 weeks of monotherapy demonstrated that there were no statistically significant differences between the groups:

- the proportion of patients who achieved HbA1c $\leq 7.0\%$ was 41.0% on gosogliptin and 44.5% on vildagliptin ($p = 0.52$);
- the FPG decrease was -0.70 mmol/L on gosogliptin and -0.89 mmol/L on vildagliptin ($p = 0.44$);
- the mean change of post-prandial plasma glucose was -1.05 mmol/L on gosogliptin and -1.39 mmol/L on vildagliptin ($p = 0.09$);

of the monotherapy consisted of 144 patients on gosogliptin and 148 patients on vildagliptin; the per protocol population consisted of 134 and 140 patients; the safety population consisted of 149 and 150 patients, respectively.

Upon completion of the monotherapy period, the mean change of HbA1c at Week 12 was -0.93% in gosogliptin group (decrease from 8.3% to 7.4%) and -1.03% in vildagliptin group (decrease from 8.4% to 7.3%); the difference between the groups in mean change of HbA1c was 0.1% (fig. 1). The null hypothesis that gosogliptin was inferior to vildagliptin with respect to the PE was rejected and its non-inferiority was established since the 95% CI upper bound of 0.342% was below the non-inferiority margin ($< 0.4\%$). The result was confirmed in the per protocol population.



b) Difference between the groups in HbA1c change (upper bound of 95% CI $< 0.4\%$)

- the body weight change was -0.54 kg on gosogliptin and -0.78 kg on vildagliptin ($p = 0.44$).

During monotherapy, AE were reported in 37 (24.8%) patients on gosogliptin and in 25 (16.7%) patients on vildagliptin. All AE were mild or moderate; there were no severe AE. One serious adverse event (SAE) not related to the study treatment was registered in each group (i.e. carcinoma of the pancreas and furuncle, respectively).

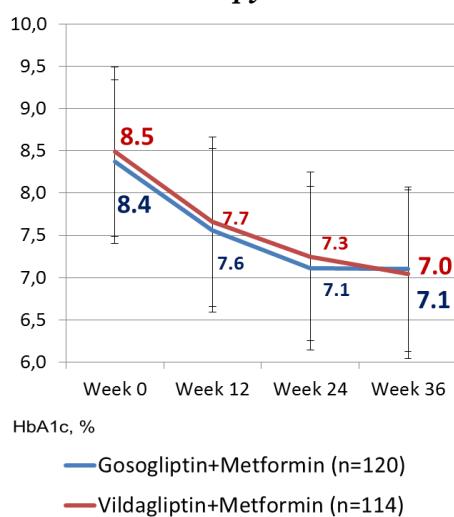
AE related to the study treatment were reported in 4 (2.7%) patients on gosogliptin (i.e.

constipation, allergic dermatitis, increase of transaminase levels, asthenia, and dizziness) and in 2 (1.3%) patients on vildagliptin (i.e. constipation, dizziness, headache).

During 12 weeks of monotherapy, hypoglycemic episodes were registered in 7 (4.7%) patients on gosogliptin and in 5 (3.3%) patients on vildagliptin; those included symptomatic episodes in 4 (2.7%) and 2 (1.3%) patients, respectively. There were no severe hypoglycemic episodes.

It was concluded that gosogliptin was non-inferior in efficacy and similar in safety profile to vildagliptin while prescribed as monotherapy during 12 weeks to patients with DM type 2. The patients in both groups who didn't achieve the target glucose parameters by Week 12 rolled-over to the combination therapy with metformin and continued the treatment for additional 24 weeks.

b) Period 2 – Assessment of efficacy and safety of combination therapy



a) HbA1c change during 36 weeks of treatment with DPP-4 inhibitors and metformin

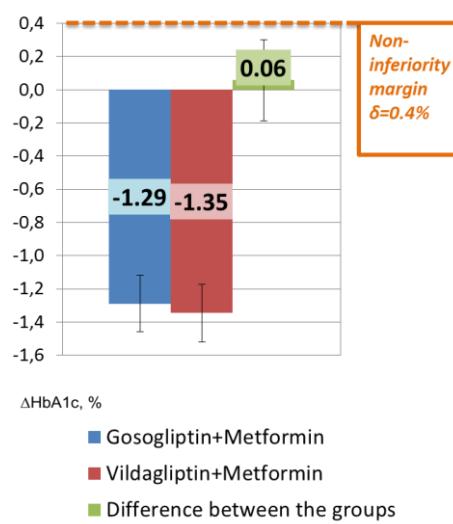
Fig. 2. HbA1c change at Week 36 for combination therapy

The analysis of the secondary endpoints at Week 36 demonstrated that there were no statistically significant differences between the groups:

- the proportion of patients who achieved HbA1c $\leq 7.0\%$ was 57.5% on gosogliptin and 54.4% on vildagliptin ($p = 0.74$);
- the FPG decrease was -1.62 mmol/L on gosogliptin and -1.64 mmol/L on vildagliptin ($p = 0.93$);
- the mean change of post-prandial plasma glucose was -2.30 mmol/L on gosogliptin and -2.51 mmol/L on vildagliptin ($p = 0.38$);

The population for the efficacy assessment of the combination therapy with metformin consisted of 120 patients on gosogliptin and 114 patients on vildagliptin; the per protocol population consisted of 104 and 105 patients; the safety population consisted of 122 and 114 patients, respectively.

Upon completion of the combination therapy with metformin, the mean change of HbA1c at Week 36 was -1.29% in gosogliptin group and -1.35% in vildagliptin group; the difference between the groups in mean change of HbA1c was 0.06% (fig. 2). The null hypothesis that gosogliptin was inferior to vildagliptin with respect to the PE was rejected and its non-inferiority was established since the 95% CI upper bound of 0.3% was below the non-inferiority margin (< 0.4%). The result was confirmed in the per protocol population.



b) Difference between the groups in HbA1c change (upper bound of 95% CI < 0.4%)

- the body weight change was -1.02 kg on gosogliptin and -1.35 kg on vildagliptin ($p = 0.48$).

During the combination therapy with metformin, AE were reported in 29 (23.8%) patients on gosogliptin and in 30 (26.3%) patients on vildagliptin. All AE were mild or moderate; there were no severe AE. SAE not related to the study treatment were registered in 1 (0.8%) patient on gosogliptin (i.e. stroke) and in 3 (2.6%) patients on vildagliptin (i.e. lumbar osteochondrosis, pneumonia, and diabetic neuropathy).

AE related to the study treatment were reported in 4 (3.3%) patients on gosagliptin (i.e. dyspepsia, diarrhea, polyposis of gall bladder, pancreatitis, cholecystitis, and steatohepatitis with increase of total bilirubin and aspartate aminotransferase) and in 2 (1.8%) patients on vildagliptin (i.e. ventricular extrasystole and increase of transaminase).

Hypoglycemic episodes were registered in 5 (4.1%) patients on gosagliptin and in 12 (10.5%) patients on vildagliptin ($p = 0.065$); those included symptomatic episodes in 3 (2.5%) and 7 (6.1%) patients, respectively. There were no severe hypoglycemic episodes.

It was concluded that gosagliptin was non-inferior in efficacy and similar in safety profile to vildagliptin while prescribed in combination with metformin during 24 weeks to patients with DM type 2 [26].

2) Study results of factor Xa inhibitor tearxaban in orthopedic surgery

92 patients were enrolled in the study at Stage 1 including 23 patients in tearxaban 50 mg group, 22 patients in 100 mg group, 23 patients in 150 mg group, and 24 patients in enoxaparin group. 108 patients were additionally enrolled at Stage 2 including 54 patients in tearxaban 100 mg group and 54 patients in enoxaparin group. Thus the total number of patients randomized in tearxaban 100 mg group was 76 and in enoxaparin group was 78 (both stages).

The treatment groups were similar in demographic parameters and main baseline

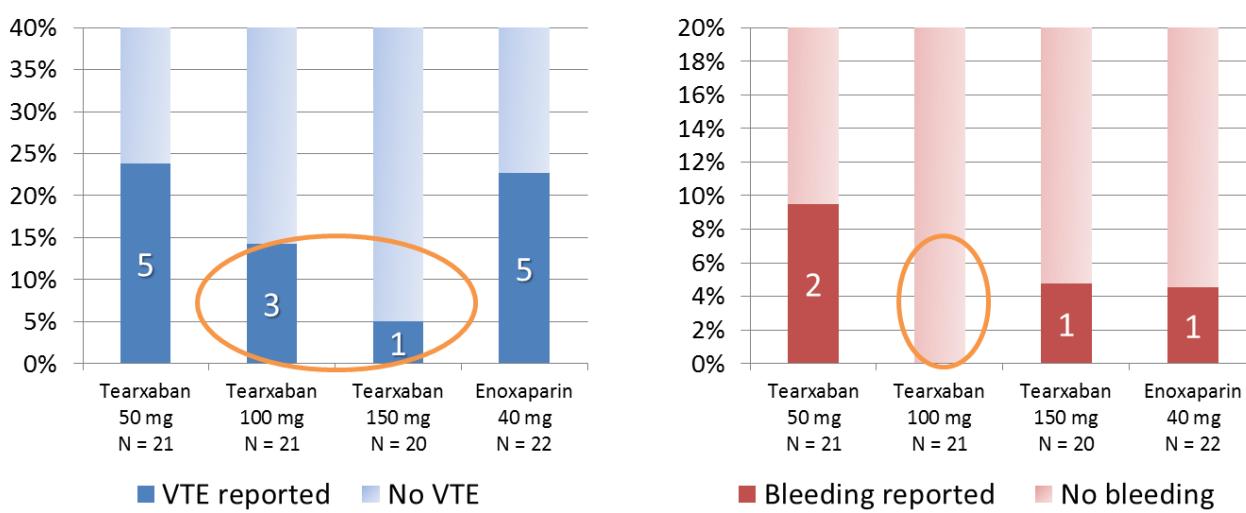
characteristics ($p > 0.05$): sex (F 95.2% + M 4.8%, F 83.6% + M 16.4%, F 90.0% + M 10.0% vs. F 81.6% + M 18.4%), age (67.0 ± 8.2 years, 65.9 ± 7.4 years, 64.3 ± 7.7 years vs. 63.9 ± 8.3 years), race (Caucasian 100.0%), body weight (85.1 ± 13.9 kg, 86.4 ± 13.7 kg, 87.5 ± 11.3 kg vs. 85.8 ± 13.4 kg) and BMI (32.8 ± 5.1 kg/m², 32.1 ± 4.5 kg/m², 32.5 ± 4.8 kg/m² vs. 31.8 ± 4.1 kg/m²).

a) Stage 1 – Dose selection

The population for the efficacy assessment at Stage 1 consisted of 21 patients on each tearxaban 50 mg and 100 mg, 20 patients on tearxaban 150 mg and 22 patients on enoxaparin; the safety population consisted of 21 patients in each group of tearxaban and 22 patients on enoxaparin, respectively.

The number of VTE on tearxaban 50 mg exceeded the predefined limit (i.e. > 4 VTE in 20 patients) and was similar to the number of VTE in the control group (23.8% and 22.7%, respectively). In tearxaban groups of 100 mg and 150 mg the number of VTE was within the predefined limit (14.3% and 5.0%, respectively), and the dose of 150 mg showed the best efficacy comparing to other doses of tearxaban and enoxaparin (fig. 3a).

Major and clinically relevant non-major bleeding within 6 weeks of surgery were reported in 2 (9.5%) patients on tearxaban 50 mg, 0 (0.0%) patients on tearxaban 100 mg, 1 (4.8%) patient on tearxaban 150 mg and in 1 (4.5%) patient on enoxaparin (fig. 3b).



a) Total VTE (target rate of VTE ≤ 4 in 20 patients)

Fig. 3. Risk/benefit analysis for tearxaban dose selection at Stage 1

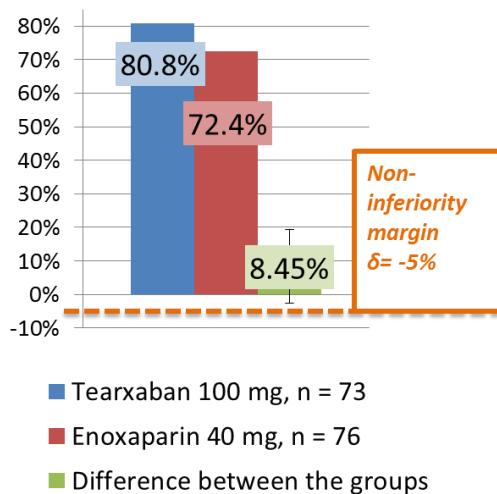
b) Major and clinically relevant non-major bleeding rate

It was concluded that Tearxaban 100 mg had the lowest risk of hemorrhage that on top of the efficacy results was the reason for choosing this dose for further study.

b) Stage 2 – Assessment of efficacy and safety

The population for the efficacy and safety assessment consisted of 73 patients on tearxaban 100 mg and 76 patients on enoxaparin; the per protocol population consisted of 63 and 64 patients, correspondingly.

The absence of VTE was confirmed in 59 (80.8%) patients on tearxaban 100 mg and 55 (72.4%) patients on enoxaparin; the difference between the groups was 8.45% (fig. 4a) The null hypothesis that tearxaban 100 mg was inferior to enoxaparin with respect to the PE was rejected and its non-inferiority was established since the 95% CI lower bound of -3.01% was above the non-inferiority margin ($>-5\%$). The result was confirmed in the per protocol population.



a) Efficacy – absence of VTE
(lower bound of 95% CI $>-5\%$)

Fig. 4. Efficacy and safety analysis of tearxaban 100 mg and enoxaparin at Stage 2

It was concluded that tearxaban 100 mg was non-inferior in efficacy and had lower risk of bleeding than enoxaparin when used for prevention of VTE in patients undergoing TKR surgery [27].

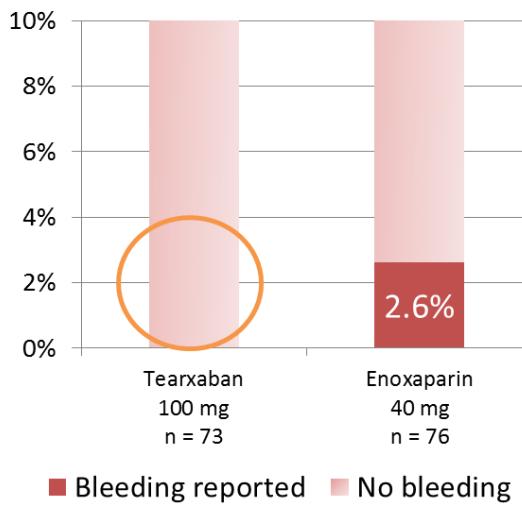
3) Study results of NNRTI elsulfavirine in patients with HIV-infection

90 treatment-naïve patients with HIV-infection were enrolled in the study at Stage 1 including 30 patients in each group of elsulfavirine 20 mg, 40 mg and efavirenz. 60 patients were additionally enrolled at Stage 2

There were no fatal events during the study. Symptomatic VTE were registered in 2 (2.6%) patients on enoxaparin, including 1 (1.3%) case of non-fatal pulmonary embolism (PE) that was confirmed by MSCT. No symptomatic or proximal VTE were registered on tearxaban.

AE were registered in 21 (28.8%) patients on tearxaban 100 mg and 33 (43.4%) patients on enoxaparin ($p = 0.0629$). All AE were mild and moderate, not related to the study drug. Cases of early discontinuation (ED) of the study treatment were related to the surgery complications. SAE not related to the study drug were registered in 2 (2.7%) patients on tearxaban 100 mg and 5 (6.6%) patients on enoxaparin.

Hemorrhage was reported in 1 (1.4%) patient on tearxaban 100 mg and 2 (2.6%) patients on enoxaparin. Whereas major and clinically relevant non-major bleedings were registered in 2 (2.6%) patients on enoxaparin and were absent in patients on tearxaban 100 mg (fig. 4b).



b) Major and clinically relevant non-major bleeding rate

Fig. 4. Efficacy and safety analysis of tearxaban 100 mg and enoxaparin at Stage 2

including 30 patients in elsulfavirine 20 mg group and 30 patients in efavirenz group. Thus the total number of patients randomized in each group of elsulfavirine 20 mg and efavirenz was 60 patients (both stages).

The treatment groups were similar in demographic parameters and main baseline characteristics ($p > 0.05$): sex (F 39.7% + M 60.3%, F 44.8% + M 55.2% vs. F 35.1% + M 64.9%), age (35.8 ± 8.7 years, 35.7 ± 9.7 years vs. 33.4 ± 8.3 years), race (Caucasian 98.3-100.0%), as well as duration of

HIV-infection from the time the diagnosis was established (2.6 ± 2.8 years, 2.4 ± 2.5 years vs. 2.1 ± 2.6 years), viral load at baseline (4.7 ± 0.6 log₁₀ copies/mL, 4.9 ± 0.6 log₁₀ copies/mL vs. 4.8 ± 0.8 log₁₀ copies/mL), body weight (72.1 ± 15.1 kg, 70.5 ± 14.0 kg vs. 71.2 ± 13.3 kg) and BMI (24.3 ± 4.1 kg/m², 23.7 ± 3.4 kg/m² vs. 23.7 ± 2.9 kg/m²).

a) Stage 1 – Dose selection

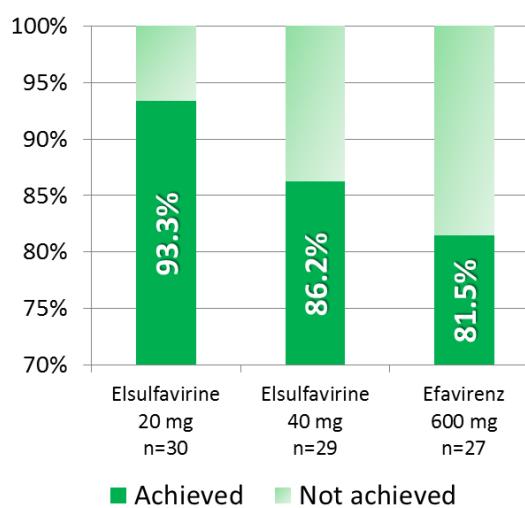
The population for the efficacy assessment at Stage 1 consisted of 30 patients in elsulfavirine 20 mg group, 29 patients in elsulfavirine 40 mg group and 27 patients in efavirenz group; the per protocol population consisted of 29, 28 and 24 patients; the safety population consisted of 30, 29 and 28 patients, respectively.

The HIV-1 RNA < 400 copies/mL at Week 12 was achieved by 28 (93.3%) patients on elsulfavirine 20 mg, 25 (86.2%) patients on elsulfavirine 40 mg and 22 (81.5%) patients on efavirenz (fig. 5a). The null hypothesis that elsulfavirine 20 mg and 40 mg were inferior to efavirenz with respect to the PE was rejected and

the non-inferiority thereof was established since the 95% CI lower bounds of -2.59% and -11.50% (respectively) were above the non-inferiority margin (> -15%). The result was confirmed in the per protocol population.

AE were reported in 21 (70.0%) patients on elsulfavirine 20 mg, 25 (86.2%) patients on elsulfavirine 40 mg, and 24 (85.7%) patients on efavirenz. AE related to the study treatment were reported in 8 (26.6%), 20 (69.0%) and 20 (71.4%) patients, respectively. The frequency of adverse reactions in patients on elsulfavirine 20 mg was 2.5 times lower than in patients on elsulfavirine 40 mg or efavirenz ($p < 0.005$). AE that caused early discontinuation of treatment were registered in 1 (3.4%) patient on elsulfavirine 40 mg (rash) and in 2 (7.1%) patients on efavirenz (rash and allergic reaction).

AE in CNS were registered in 8 (26.7%) patients on elsulfavirine 20 mg, 13 (44.8%) patients on elsulfavirine 40 mg and 16 (57.1%) patients on efavirenz (fig. 5b).



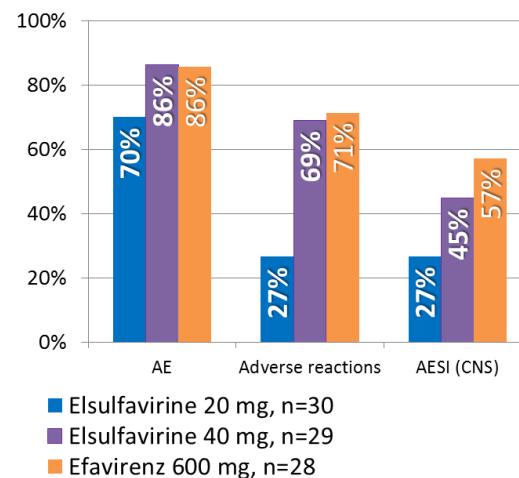
- a) Rate of achievement of viral load < 400 copies/mL at Week 12 (lower bound of 95% CI > -15)

Fig. 5. Risk/benefit analysis for elsulfavirine dose selection at Stage 1

It was concluded that elsulfavirine 20 mg had the lowest risk of adverse reactions that on top of the efficacy results was the reason for choosing this dose for further study.

b) Stage 2 – Assessment of efficacy and safety

The population for the efficacy assessment of the selected dose consisted of 58 patients on



- b) AE, adverse reactions and AE of special interest (CNS)

elsulfavirine 20 mg and 57 patients on efavirenz; the per protocol population consisted of 47 and 39 patients; the safety population consisted of 60 and 58 patients, respectively.

The undetectable level of HIV-1 RNA at Week 24 was achieved by 49 (84.5%) patients on elsulfavirine 20 mg and 38 (66.7%) patients on

efavirenz (fig. 6a); the difference between the groups was 17.8% ($p = 0.031$). The null hypothesis that elsulfavirine 20 mg was inferior to efavirenz with respect to the PE was rejected and its non-inferiority was established since the 95% CI lower bound of 2.4% was above the non-inferiority margin ($> -15\%$). The result was confirmed in the per protocol population.

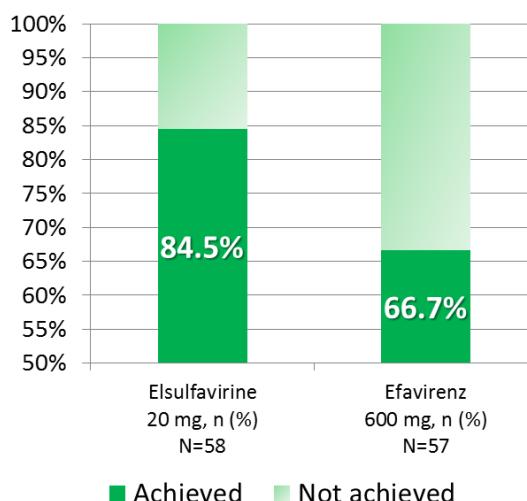
The analysis of the secondary efficacy endpoints demonstrated that there were some statistically significant differences between the groups:

- the decrease of the viral load (\log_{10} copies/mL) by Week 12 was -2.8 ± 0.7 on elsulfavirine 20 mg and -2.7 ± 1.0 on efavirenz; by Week 24 it was -3.3 ± 0.7 and -3.3 ± 0.8 ; by Week 48 it was -3.3 ± 0.7 and -3.4 ± 0.7 , respectively;

- the 10-fold decrease of the viral load by Week 4 was achieved by 56 (96.6%) patients on elsulfavirine 20 mg and 49 (86.0%) patients on efavirenz ($p = 0.053$);

- 48 weeks of the study treatment were successfully completed by 55 (91.7%) patients on elsulfavirine 20 mg and 47 (78.3%) patients on efavirenz ($p = 0.041$);

- the increase of CD4+ T-lymphocytes by Week 12 was 112.9 ± 127.6 on elsulfavirine 20 mg and 78.0 ± 135.8 on efavirenz; by Week 24 it was 145.0 ± 159.5 and 115.6 ± 168.0 ; by Week 48 it was 179.3 ± 156.3 and 182.6 ± 149.1 , respectively;



a) Rate of achievement of undetectable viral load at Week 24 (lower bound of 95% CI $> -15\%$; $p = 0.031$)

Fig. 6. Efficacy and safety of elsulfavirine 20 mg and efavirenz at Stage 2

It was concluded that elsulfavirine 20 mg was non-inferior in efficacy and had lower risk of

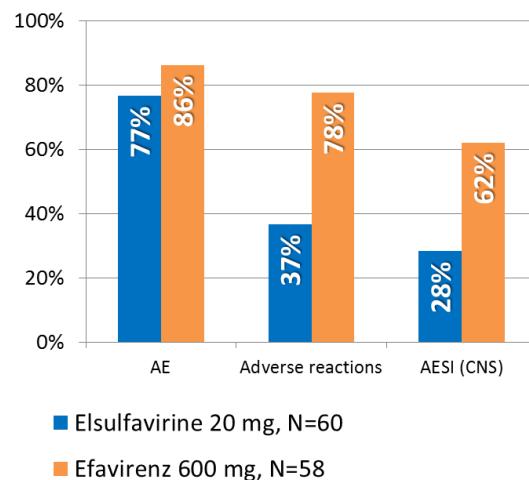
- the decrease of CD8+ T-lymphocytes by Week 12 was -60.5 ± 348.2 on elsulfavirine 20 mg and -143.5 ± 372.9 on efavirenz; by Week 24 it was -166.9 ± 346.3 and -175.3 ± 402.6 ; by Week 48 it was -214.2 ± 330.1 and -267.5 ± 401.4 , respectively;

- the HIV-1 drug resistance was not established during the trial.

AE were reported in 46 (76.7%) patients on elsulfavirine 20 mg and 50 (86.2%) patients on efavirenz. AE related to the study treatment were reported in 22 (36.7%) patients on elsulfavirine 20 mg and 45 (77.6%) patients on efavirenz (fig. 6b). The frequency of adverse reactions on elsulfavirine 20 mg was twice lower than on efavirenz ($p < 0.0001$). In elsulfavirine 20 mg group the following adverse reactions were reported in 5-15% patients: headache, increase of gamma-glutamyltransferase, mild proteinuria, sleep disorder, asthenia, dizziness, and nausea.

AE of grade 3 and 4 (severe and potentially life-threatening) were reported in 5 (8.3%) patients on elsulfavirine 20 mg and in 9 (15.5%) patients on efavirenz. SAE not related to the study drug were reported in 5 (8.3%) patients on elsulfavirine 20 mg; among patients on efavirenz, SAE were reported in 7 (12.1%) patients, including severe allergic reactions and cytotoxicity in 4 (6.9%) patients that were related to the study drug.

AE in CNS were reported in 17 (28.3%) patients on elsulfavirine 20 mg and in 36 (62.1%) patients on efavirenz ($p < 0.001$).



b) AE, adverse reactions and AE of special interest (CNS)

4) Comparative analysis and economic efficiency assessment of the adaptive designs

The adaptive seamless design was implemented in three clinical trials. In the gosogliptin study, the adaptive seamless design for two treatment regimens allowed assessing the

efficacy and safety of the monotherapy (Period 1) and then the combination therapy with metformin (Period 2) in the same patients' population (fig. 7). In a classic clinical trial model, this would require two independent studies with recruitment of more patients.

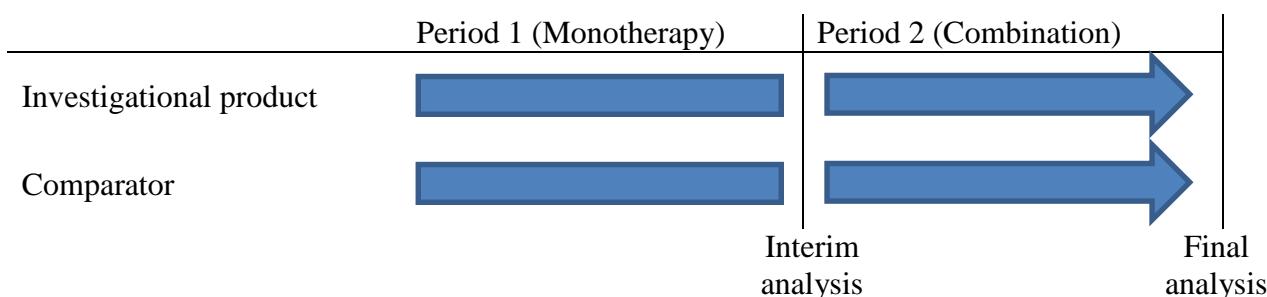


Fig. 7. Seamless design for two treatment regimens

The adaptive seamless design of phase II/III was used in the tearxaban and elsulfavirine studies (fig. 8). The optimal dose of the study drug was chosen based on the interim analysis (Stage 1); then additional patients were enrolled

in the study to assess the efficacy and safety of the selected dose in the overall patients' population (Stage 2). In a classic clinical trial model, this would take longer and require recruitment of more patients.

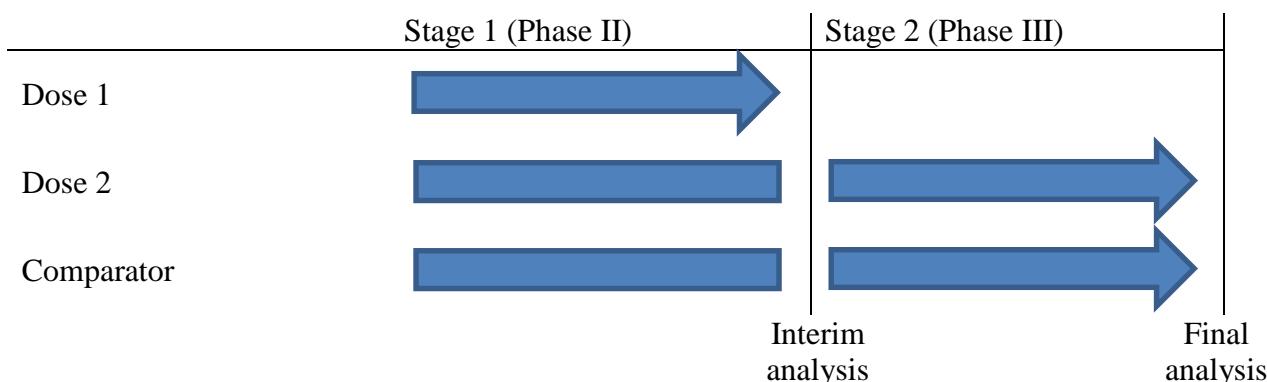


Fig. 8. Seamless phase II/III design

The comparative analysis of the clinical trial designs is presented in Table 1 [29].

The implementation of the adaptive design methods in the clinical trials of gosogliptin, tearxaban, and elsulfavirine provided the average decrease of the study budgets by 36% and the study duration by 27%

(i.e. 9 months) as opposed to the classic late-stage clinical development program.

As a result, gosogliptin and elsulfavirine have been registered in Russia: Saterex (registration certificate No. LP-003598 dated May 04, 2016) and Elpida® (registration certificate No. LP-004360 dated June 30, 2017).

Table 1

Comparative analysis of clinical trial designs

	Gosagliptin, DPP-inhibitor	Tearxaban, factor Xa inhibitor	Elsulfavirine, NNRTI			
Design	Multicenter open randomized, Phase III	Multicenter partially blinded randomized, Phase II	Multicenter partially blinded randomized, Phase II-III			
Adaptation type	Seamless design for 2 treatment regimens	Seamless phase II/III design	Seamless phase II/III design			
Period 1/ Stage 1	<ul style="list-style-type: none"> • Efficacy and safety of monotherapy • Gosagliptin / Vildagliptin • PE ΔHbA1c (W12-W0) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, SD = 1.1, $\delta = 0.4\%$ (95% CI), n = 238 (1:1) 	<ul style="list-style-type: none"> • Dose selection • Tearxaban 50 mg, 100 mg, and 150 mg / Enoxaparin 40 mg • PE % of VTE (W6) • $\alpha = 5\%$ (2-sided), power 80% • Simon's MiniMax, $p_0 = 60\%$, $p_1 = 85\%$, r $\leq 4/20$, n = 80 (1:1:1:1) • Unblinded • Central reviewer • Simon's model analysis • Dose selection based on DMC decision • No change of design or statistical assumptions • Efficacy and safety • Tearxaban (selected dose) / Enoxaparin • PE % of VTE (W6) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 60\%$, $p_1 = 85\%$, $\delta = 5\%$ (95% CI), n = 132 (1:1) 	<ul style="list-style-type: none"> • Dose selection • Elsulfavirine 20 mg, and 40 mg / Efavirenz 600 mg • PE % of < 400 copies/mL (W12) • $\alpha = 5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 80\%$, $p_1 = 90\%$, $\delta = 15\%$ (95% CI), n = 75 (1:1:1) • Unblinded • Central laboratory • Analysis of the surrogate endpoint • Dose selection based on DMC decision • No change of design or statistical assumptions • Efficacy and safety • Elsulfavirine (selected dose) / Efavirenz • PE % < 50 copies/mL (W24) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 67\%$, $p_1 = 77\%$, $\delta = 15\%$ (95% CI), n = 102 (1:1) 			
Interim analysis and adaptation	<ul style="list-style-type: none"> • Open • Central laboratory • Increased ED rate to control power • No change of design or statistical assumptions 	<ul style="list-style-type: none"> • Unblinded • Central reviewer • Simon's model analysis • Dose selection based on DMC decision • No change of design or statistical assumptions • Efficacy and safety • Tearxaban (selected dose) / Enoxaparin • PE % of VTE (W6) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 60\%$, $p_1 = 85\%$, $\delta = 5\%$ (95% CI), n = 132 (1:1) 	<ul style="list-style-type: none"> • Unblinded • Central laboratory • Analysis of the surrogate endpoint • Dose selection based on DMC decision • No change of design or statistical assumptions • Efficacy and safety • Elsulfavirine (selected dose) / Efavirenz • PE % < 50 copies/mL (W24) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 67\%$, $p_1 = 77\%$, $\delta = 15\%$ (95% CI), n = 102 (1:1) 			
Period 2/ Stage 2	<ul style="list-style-type: none"> • Efficacy and safety of combination therapy • Gosagliptin / Vildagliptin • PE ΔHbA1c (W36-W0) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, SD = 1.1, $\delta = 0.4\%$ (95% CI), n = 238 (1:1) 	<ul style="list-style-type: none"> • Efficacy and safety • Tearxaban (selected dose) / Enoxaparin • PE % of VTE (W6) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 60\%$, $p_1 = 85\%$, $\delta = 5\%$ (95% CI), n = 132 (1:1) 	<ul style="list-style-type: none"> • Efficacy and safety • Elsulfavirine (selected dose) / Efavirenz • PE % < 50 copies/mL (W24) • $\alpha = 2.5\%$ (1-sided), power 80% • non-inferiority, $p_0 = 67\%$, $p_1 = 77\%$, $\delta = 15\%$ (95% CI), n = 102 (1:1) 			
Estimated number of patients per trial	ED 20% (considering 2 periods) Period 1: 300 (150+150), <i>out of which</i> Period 2: ~264 (132+132) Total: 300 (150+150)	-46.4%	ED 15% (at each stage) Stage 1: 92 (23+23+23+23) Stage 2: 108 (54+54) Total: 200 (23+77+23+77)	-20.0%	ED 15% (at each stage) Stage 1: 90 (30+30+30) Stage 2: 60 (30+30) Total: 150 (60+30+60)	-27.9%
Study duration	Approval: 01.03.2013 Report Period 1: 02.07.2014 Report Period 2: 27.11.2014 1.7 years (21 months)	-0 months	Approval: 01.08.2013 Report Stage 1: 02.10.2014 Report Stage 2: 26.10.2015 2.2 years (27 months)	-14 months	Approval: 21.04.2014 Report Stage 1: 29.05.2015 Report Stage 2: 31.05.2016 2.1 years (25 months)	-13 months

Conclusion

The work demonstrated that the effects of the next-in-class drugs in development can be predicted with high accuracy due to known characteristics of the efficacy and safety of the drugs of the same pharmacological group. This allows minimizing the risks of using the adaptive design methods in clinical programs of the next-in-class drugs.

The adaptive design method for two treatment schemes can be used in assessment of the efficacy and safety of drugs that require dose titration and/or stepwise adding of other components of the combination therapy.

The preliminary assessment of efficacy using Simon's statistical model or surrogate endpoint prevents from testing the primary hypothesis during the interim analysis (for dose selection) and provides additional control of the type I error in clinical trials with the adaptive seamless phase II/III design.

The results of the study open a new perspective for further implementation of the adaptive design methods in clinical trials of next-in-class drugs that will allow for the optimization of the development programs and the accelerated market access for new drugs.

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Conflicts of interest

The authors have no conflict of interest to declare.

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**LIPID DISTRESS-SYNDROME AND PROSPECTS OF ITS
CORRECTION BY STATINES**

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Abstract

Endothelial dysfunction in peritonitis: The formed concept of lipid distress syndrome (LDS) allows us to develop a working hypothesis on the key role of endothelial dysfunction in the aggressive development of atherosclerosis.

The role of vascular endothelium in atherosclerosis: The process of NO production from L-arginine through eNOS involving tetrahydrobiopterin (BH4) is discussed. With the degradation of BH4 along the free radical path, an "eNOS uncoupling" (uncoupled eNOS).

The clinical role of statins: Statins manifest itself by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase). Many large, randomized clinical trials have shown that lipid-lowering strategies that include statins have an anti-atherogenic potential.

Pleiotropic effects of statins: Direct inhibition of small GTP-ase prenylation in vascular cells forms the essence of the hypothesis that explains the rapid pleiotropic effect of statins on the vascular wall, which does not depend on reducing lipid levels.

Endogenous antioxidant defense systems: Effects of statins. Statins have a beneficial effect on the vasculature not only by suppressing the prooxidant enzyme, but also by increasing the intensity and activity of endogenous antioxidant systems.

The effect of statins on redox-sensitive transcritic pathways: One of the most important pathways is the NF- κ B via the PI3K/Akt pathway.

Experimental evidence of endothelioprotective properties of statins in the modeling of endotoxin-induced pathology: The use of HMG-CoA reductase inhibitors in the background of the fashion modeling endotoxin-induced pathology, leads to the development of a dose-dependent endothelioprotective effect, which is expressed in the normalization of QED, as well as the normalization of biochemical markers of inflammation (C-reactive protein) and the level of pro-inflammatory drugs. At the same time, positive dynamics of the final products of NO and eNOS expression was detected.

Keywords: Statins, endothelial dysfunction, endotoxin-induced pathology, NF- κ B and PI3K/Akt pathways.

Endothelial dysfunction in peritonitis

In recent years, works of VS Savlyev, VA Petukhov and co-authors put forward the concept of lipid distress syndrome (LDS) [1, 2, 3]. The

concept was based on epidemiological studies, which indicate that in a remote period after an abdominal catastrophe in 10-20 years, patients develop atherosclerosis with catastrophic

consequences leading to the cardiovascular continuum [4]. The cause of arterial damage in persons who have undergone an abdominal catastrophe is not only endotoxin aggression, but also as a consequence of endothelial dysfunction with the further development of atherosclerosis and its complications [4, 5, 6, 7].

In the opinion of the authors, endotoxin aggression accompanies the human body from early childhood, but clinically significant pathology develops only when endotoxin-binding and endotoxin-limiting organism systems are damaged [8]. In case of abdominal catastrophe, excess amounts of endotoxins enter the bloodstream to disrupt the adaptive capacity of these systems and multi-organ failure [9]. It can be assumed that infectious diseases without adequate antibiotic therapy similarly deplete the antiendotoxin defense mechanisms, which primarily include high-density lipoproteins and lead to development endothelial dysfunction of atherosclerosis [10]. It seems that endotoxemia is a starting point in the development of organic disorders with LDS. Further long-term endotoxinemia against the background of the dysbiosis of the gastrointestinal tract forms the conditions for the progression of endothelial dysfunction [11].

Researches of the last years confirmed a role of endothelial dysfunction in a pathogenesis of many cardiovascular diseases. Clinical implication of endothelial dysfunction is the perversion of reaction to influence of physiological or pharmacological incentives when normal vessels react dilatation. Gravity of atherosclerotic process always correlates with endothelial dysfunction, and standard therapy of cardiovascular diseases doesn't eliminate endothelial dysfunction, and keeps risk of development of complication in several times [3, 6, 11].

It is possible to believe, that the trigger of endothelial dysfunction is the Gram – липополисахарид. At the same time realization has the prescription mechanism at the low concentrations, and at high – macrophagic CD 14 the effect is complemented with other mechanisms enlarging permeability of an endothelium [5, 12].

Developing in the abdominal catastrophe, endothelial dysfunction can not be treated in

isolation as soon as arteriolar vasculitis, an important role in the sequence of events plays the liver as the main target organ of LDS. Stimulated Kupfer cells reduce the synthesis of NO by 80-90%, causing local dysfunction of the hepatic vessels, followed by a powerful release of pro-inflammatory cytokines into the inflammatory bloodstream [13, 14]. At the same time, the concentration of TNF, C-reactive protein and other cytokines [15] and oxidized low-density lipoproteins significantly increase. The latter leads to a decrease in the synthesis of heparan and an increase in the size of the interendothelial gaps [16, 17, 18]. It is important to note that even a short-term increase in vascular permeability for macrophages with further endotoxin exposure significantly enhances endothelial dysfunction. At the same time, the protective role of high-density lipoproteins in limiting the activity of macrophages weakens [11, 19, 20]. At the same time, in addition to para-endothelial transport, the mechanism of transfer is accelerated by the co-endothelial mechanism and its activity is proportional to the level of endotoxemia [21].

The levels of P-selectin, interleukin 6 and others are the main markers of endothelial dysfunction [22, 23]. In clinical practice, a c-reactive protein is often used [24]. The quantitative determination of the latter correlates with Interleukin 1, Interleukin 6, TNF [25, 26].

C-reactive protein in its turn participates in the migration of monocytes to the atherosclerotic plaque and stimulates the formation of "foamy" cells by enhancing the capture of low-density lipoproteins by macrophages.

According to Savelyev V.S., Petukhov V. A., the information value S-reaktivnog about protein can SRB>; the relation of "XC/LPNP">; LDL>; XC>; гомоцистеин>; LP [9] is presented in the following form.

In this regard authors allocate several stages an endotoxin – the induced endothelial dysfunction. 1 stage – damage of a glycocalyx of an endothelium, 2 stage – permeability augmentation, 3 stage -damage, the last is fixed by emergence in a blood flow of the endotheliocytes circulating in a blood flow [12, 14, 18, 27, 28]

At early stages of atherosclerotic process, the formation of atherosclerotic and vasculitis is preceded by a thickening of the vascular wall.

This quantitative indicator received the name intimamedia thickness a complex of the "erotic media" of KIM [2, 7, 29] Essential value in recent years had an opportunity of an intravital not invasive research by means of an US, a doppler and td. UZVR technique (ultrasound of high resolution) the offered D.S. Celermajer et al. on the humeral artery, the greatest distribution [7, 8]

Pharmacological correction of LDS syndrome in abdominal catastrophe according to Saveliev VS and Petukhov VA [9] should be reduced to two components: 1 – endotoxin binding therapy (enterosorbent, plant hepatoprotectors, probiotics of metabolic action 2 – endothelioprotection (quercetin-glucuronide of red leaves of grapes)

Based on the above, one can conclude that the formed concept of LDS syndrome as the consequence of abdominal catastrophe allows us to develop a working hypothesis on the key role of endothelial dysfunction in the aggressive development of atherosclerosis and its complications that lead to the cardiovascular continuum. This hypothesis is confirmed by numerous data on the dynamics of biochemical markers of endothelial dysfunction (C-reactive protein, pro-inflammatory cytokines, circulating endotheliocytes), as well as the results of instrumental studies of ultrasonic tomography with evaluation of the intima-media complex and endothelium-dependent vasodilatation.

At the same time, the following questions arise:

Any endotoxemia can induce endothelial dysfunction or caused exclusively by lipopolysaccharides of Gram negative bacteria as for peritonitis ?;

What experimental models besides peritonitis can be used for the development of endotoxin-induced endothelial dysfunction ?;

Which of the biochemical markers of endothelial dysfunction or their combination with functional studies most adequately quantify the development of endotoxin-induced endothelial dysfunction ?;

Separate discussion requires a strategy of pharmacological correction of endotoxin-inducing endothelial dysfunction and the prevention of aggressive development of atherosclerosis. We basically agree with the first part of endotoxin-binding therapy, especially in conditions of peritonitis and other conditions,

accompanied by an infectious-toxic shock. However, the use of the term vegetable antioxidants as endothelial protectors from the standpoint of modern pharmacology is not sufficient. In this connection, in the following sections of the review, we propose to discuss the possibility of the pathogenetically justified use of HMG Co-A reductase inhibitors both in monotherapy and in combination with the donor NO L-arginine, nonselective and selective inhibitors of arginase and recombinant erythropoietin.

The role of vascular endothelium in atherosclerosis

As it known, endothelial dysfunction is currently considered as one of the key triggers in the development of the atherosclerotic process. Endothelial dysfunction is characterized by reduced availability of NO. This causes an increase in intracellular cyclic GMP, and induces vasodilation. In addition, NO also participates in the regulation of other processes, such as platelet aggregation and leukocyte adhesion, and plays an important role in vascular homeostasis [29]. The main enzymatic source of NO in the vascular wall is the endothelial synthetase of nitric oxide (eNOS), which is located mainly in endothelial cells. eNOS is a complex that uses L-arginine and molecular oxygen (O₂) as a substrate for the production of NO and L-citrulline.

This is achieved by electron transfer using NADPH as a donor from the flavin reductase domain from one monodomain to the other where the active gemm-iron site [30, 31] is located. The presence of calmodulin, which activates calcium binding, increases the rate of electron flow. In place of the heme, electrons are used to reduce and activate O₂, which in turn is used to produce NO through the two-step oxidation of L-arginine. This process requires the binding of the cofactor tetrahydrobiopterin (BH 4). The enzyme that limits the rate of biosynthesis of BH 4 is called cyclohydrolase guanosine triphosphate (GTPCH). When BH 4 is associated with eNOS, the enzyme is considered "linked" [3]. The adhesion of eNOS is important for the physiological function of the vascular endothelium. Degradation of BH 4 on the free radical path, especially with peroxynitrite (ONOO⁻), leads to a condition known as "uncoupling eNOS" of the enzyme. In the

absence of BH 4, the electron flux in eNOS is disrupted; this leads to the dissociation of a divalent-molecular oxygen complex, resulting in eNOS for converting O₂ to superoxide (O₂ -) radicals instead of producing NO [32]. O₂ - reacts with NO to form ONOO -, which can additionally oxidize to BH 4, creating a vicious circle of eNOS disconnection. As a result, the drop in NO production inhibits endothelium-dependent vasodilation, disrupts vascular homeostasis and leads to endothelial dysfunction [31]. In addition, ONOO - has a fixed role in the oxidation of low density lipoprotein (LDL), and is also responsible for the nitration of various cellular components. The accumulation of nitrated proteins is a potent marker of oxidative nitroergic cell damage. ONOO - and can induce cell apoptosis and necrosis even at high concentrations, further exacerbating endothelial dysfunction [33].

Endothelial dysfunction was established as an important predictor of future cardiovascular events, regardless of other risk factors. A number of studies have shown a link between impaired endothelium-dependent vasodilatation of the brachial artery and an increased risk of cardiovascular complications in patients undergoing vascular surgery [34], including in the absence of obstructive arteriosclerosis [35]. Recently, using meta-analysis, it was concluded that an increase in the diameter of the brachial artery during the cuff test is inversely associated with the risk of the cardiovascular continuum. At the same time, the risk is more pronounced in populations at high risk in the presence of traditional predisposing factors, and an increase in vasodilatation of the brachial artery by 1% leads to a 9% decrease in cardiovascular risk [36].

Oxidative stress and atherosclerosis. Modern understanding of the pathogenesis of cardiovascular diseases confirms the important role of free radicals (ROS) in atherogenesis [37]. Classical risk factors for atherosclerosis, such as diabetes mellitus, hypertension and smoking, are associated with increased production of ROS in the vascular wall [38]. One of the main factors in the development of the atherosclerotic process is the oxidation of lipoprotein molecules. First of all, the reaction of LDL + ROS leads to the formation of oxidized low density lipoproteins (oxLDL). This occurs primarily in the

subendothelial space, rather than in the plasma, in which there are many antioxidant defense mechanisms [39, 40]. ROS-mediated dissociation of eNOS causes dysfunction of the endothelium layer, through the mechanisms presented earlier. The binding of oxLDL to a lectin-like receptor induces activation of redox, proinflammatory transcription pathways (eg, nuclear factor kappa B [NF- κ B]) in endothelial cells. This induces the release of inflammatory cytokines (such as interleukin-6 (IL-6) and increases the expression of cell adhesion molecules (eg, vascular adhesion molecule 1 (VCAM-1).) As a result, circulating monocytes are attracted to the vascular endothelium, where they adhere to the increased expression of adhesion molecules and penetrate the subendothelial space. There they absorb (phagocytate) oxLDL molecules, acquire macrophage-like characteristics, and eventually turn into foamy cells. This further exacerbates the inflammatory processes triggered in the vascular wall and creates a vicious circle where more monocytes / macrophages are attracted to the subendothelial space. In addition, ROS inflammatory cytokines cause the migration of smooth muscle vascular cells in the intima. The accumulation of saturated lipid membranes of foam cells and the parallel migration / spread of smooth muscle cells ultimately lead to the formation of atherosclerotic plaques.

Despite the obvious role of oxidative stress in the development of atherosclerosis, the actual causal relationship in the human body has not been conclusively proven. There is a question: – is the accumulation of ROS the cause of atherosclerosis or free radicals appear during its development [40]. Prove a causal relationship is difficult. Oxidative stress is widely studied in experimental models. Clinical studies have yielded results that are largely consistent with fundamental research. Thus, oxidative stress is now universally recognized as one of the key components of cardiovascular disease [39, 40].

Taking into account the participation of ROS in the pathogenesis of atherosclerosis, a therapeutic approach is designed logically to introduce biologically active antioxidant supplements such as ascorbic acid (vitamin C), alpha-tocopherol (vitamin E), and others. These compounds act as exogenous traps of free radicals and their use was considered a rational therapeutic

strategy for restoring the vascular oxidation-reduction balance and preventing cardiovascular diseases. Indeed, since 1994, more than 50 large randomized studies that used antioxidant vitamins in primary and secondary prevention of cardiovascular disease have been published, with disappointing results. The latest meta-analysis, based on the total population of nearly 300,000 participants, scattered across 50 randomized trials, concludes that there is no evidence to support the use of antioxidants for the prevention of cardiovascular disease [41].

The disappointing results of the use of antioxidant additives, in our opinion, should in no case be interpreted as invalidating the hypothesis of oxidative stress. For example, Patrignani et al. [42, 43, 44, 45] found that supplements with doses of vitamin E to 1200 mg / day did not affect the urinary concentration of 8-iso-PGF2a (a noninvasive marker of oxidative stress *in vivo*) in healthy smokers. In contrast, comparable doses of vitamin E significantly affect the level of 8-iso-PGF2a in clinical settings in patients with risk factors for cardiovascular disease. It is interesting that there is a linear correlation between the basal rate of excretion of 8-iso-PGF2a and changes in this index of lipid peroxidation depending on changes in the level of vitamin E in plasma. These results are consistent with the hypothesis that the basal rate of lipid peroxidation is the main factor determining response to the exogenous introduction of vitamin E.

The clinical role of statins: an effective lipid-lowering strategy

Statins are organic molecules, originally derived from fungi. They exhibit their effect by inhibiting the enzyme 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA reductase) by competitive inhibition of substrate binding to the active site of the enzyme, due to their structural similarity to HMG-CoA [46, 47]. Inhibitors of HMG-CoA reductase is a rate-limiting enzyme in the biosynthesis of cholesterol. This enzyme converts HMG-CoA to mevalonate, which is then phosphorylated and converted to isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). These molecules serve as isoprenoid precursors farnesyl pyrophosphate (FPP), which is converted to geranyl-geranium pyrophosphate (GGPP) and squalene. In hepatic cells, squalene forms the

basis of ergosterol and subsequently the synthesis of cholesterol. Since a significant percentage of total cholesterol in the human body is endogenously produced, inhibition of HMG-CoA by statins leads to a subsequent decrease in cholesterol and, in particular, low-density lipoprotein [48].

Many large randomized clinical trials have shown that lipid-lowering strategies that include statins have an anti-atherogenic potential and reduce the risk of developing myocardial infarction and stroke. A 31% decrease in the number of coronary events in hypercholesterolemic patients after treatment with pravastatin (40 mg / day) [49, 50]. A recent review of the Cochrane database leads to the conclusion that statins reduce vascular complications and mortality from all causes in patients with no history of coronary artery disease [50]. Similarly, prolonged use of pravastatin in patients with ischemic disease demonstrates a significant reduction in coronary death from all causes in patients receiving pravastatin (40 mg / day [51]. In addition, in patients with CABG, aggressive lovadipidemic therapy of lovastatin + cholestyramine was more effective in compared with more conservative treatment [52]. As a result of these and other large-scale studies, statins are integral part of the drug therapy that recommended after major cardiovascular event. The latest WHO recommendations and the Russian guidelines recommend the initiation of statin therapy as a secondary prophylaxis in the presence of ischemic heart disease or other atherosclerotic vascular disease (class IA), with target LDL levels below 100 mg / dl, directed at least by reduction 30% of previous levels [53].

The pleiotropic effects of statins

Most of the early clinical trials of statins have been focused on correcting dyslipidemia in patients with CVD with high LDL cholesterol. However, an analysis of further studies reveals the beneficial effect of statin therapy in patients with CVD on newly diagnosed coronary heart disease or in patients who need revascularization, regardless of their LDL level [54, 55, 56, 57]. Similarly, post-factum analysis of the POST-CABG study shows a positive effect of intensive statin therapy on cardiovascular disease regardless of the decrease in lipid levels [55, 56, 57]. The concept, that statins can potentially have

effects beyond hypolipidemic capabilities is further enhanced by the verdict of the JUPITER trial in which non-lipidemic healthy subjects with high C-reactive protein levels (CRP) were randomized to receive rosuvastatin 20 mg / day or placebo [58]. The results of this study show that treatment with statins reduces the level of CRP and the frequency of major cardiovascular events. The effects of statins do not depend on LDL. This decrease in the level of C-reactive protein was termed "pleiotropic" and was the focus of extensive research in the last decade.

Direct pleiotropic effects of statins on vascular endothelial function in humans have been demonstrated by comparing the effects of various lipid-lowering strategies. In a randomized trial of patients with chronic heart failure for 4 weeks with simvastatin or ezetimibe (10 mg / day), statin showed a marked improvement in the results of the cuff test on the brachial artery compared with ezetimibe, despite a similar reduction in LDL levels [59, 60]. Similarly, in patients with dyslipidemia without cardiovascular disease, 40 mg / day of simvastatin improved cuff test results and the level of vascular Rho kinase was significantly higher than simvastatin 10 mg / day plus ezetimibe 10 mg / day [61]. Another recent meta-analysis, which examined the effects of statin therapy on cuff test results in diabetic patients, concluded that statins improve endothelial function, but only in patients who have no signs of severe endothelial dysfunction [62]. The latter highlights the role of prevention and modification of risk factors that can be realized with the use of statin therapy. Interesting, theat sudden cessation of treatment with simvastatin in patients with coronary heart disease leads to a significant reduction in the cuffing in the cuff sample in the first week after cessation of treatment, the effect does not correlate with an increase in rebound levels of LDL [63]. Conversely, in healthy people, the abrupt discontinuation of statin treatment also leads to a reduction in vasodilation in the cuff test on day 1 after discontinuation, which is restored to baseline within 1 week [63]. These results emphasize the potential of endothelium-protective pleiotropic effects of statins, as well as the need for an appropriate treatment regimen.

As mentioned earlier, isoprenoids FPP and GGPP are intermediate products of cholesterol

biosynthesis, which is inhibited by statins. Protein prenylation is an important post-translational modification. The family of small GTP-as (Ras, Rho, Rac, etc.) is an important cellular target for prenylation. The addition of FPP or GGPP residues in proteins is crucial for lipid anchoring and activation of Rho and Rac [64]. Geralulerization from Rho activates Rho-associated protein kinase (ROCK); Po / ROCK pathway is involved in the control of various cellular functions, including proliferation, cell migration, oxidation-reduction signaling and apoptosis [65, 66]. In addition, Rac is involved in the activation of the NADPH oxidase complex and thus plays an important role in regulating the generation of reactive oxygen species, as will be discussed in detail later.

Cholesterol biosynthesis is active not only in hepatocytes, where it was first described, but it is also important for the cellular homeostasis of vascular cells. Lipophilic statins (for example, atorvastatin and simvastatin) can passively diffuse through the lipid bilayer of the cell membrane and, therefore, can be assimilated by a large number of cells, and not only by hepatocytes (which also have such active transport mechanisms) [67]. Direct inhibition of small GTP-ase prenylation in vascular cells forms the essence of the hypothesis that explains the rapid pleiotropic effect of statins on the vascular wall, which does not depend on lowering lipid levels [67, 68].

Sources of free radicals as therapeutic targets of statins in the vascular wall. Cell redox imbalance, characterized by increased production and reduced utilization of ROS, is involved in many pathophysiological processes, including the development of cardiovascular diseases. Statins are probably the most effective and currently available pathway that inhibits prooxidant enzyme systems present in the vascular system and enhances antioxidant defense mechanisms. The effect of statins on the redox systems of the cell has been extensively studied in cell cultures *in vitro*. Nevertheless, the results obtained in experiments performed in such "model systems" do not necessarily reflect the processes *in vivo* and can not be applied to human diseases. A large number of experiments have been performed in human umbilical vein endothelial cells, which have significant functional differences compared

to arterial endothelial cells, which are directly related to the development of atherosclerosis.

The effect of statins on eNOS. The effect of statins on eNOS is well documented in the literature. It has been proven that pleiotropic effects of statins in the vasculature are at least partially mediated by changes in eNOS expression.

It was shown that geranylgearylation from GTPase Rho leads to a decrease in eNOS in the endothelial cell. Statin therapy is inhibited by GGPP formation and therefore leads to increased expression of eNOS, which was revised in the case of co-incubation with GGPP [69, 70]. Increased endogenous levels of LDLs also adversely affect the expression of mRNA in eNOS [71]. The mechanism by which statins increase the stability of eNOS mRNA is an increase in the eNOS mRNA of polyadenylation, due to changes in the cytoskeleton, after Rho inhibition [71]. In addition, the exposure of the H₂O₂ -induced, aging, smooth muscle cells to statins activates the phosphatidylinositide of the 3-kinase (PI3K) / Akt pathway and enhances the expression of eNOS [72]. More recently, eNOS mRNA has been identified as a target of micro-RNA-MIR-155, which is activated by inflammatory stimuli, such as tumor necrosis factor alpha (TNF). It is important to note, that this effect was weakened by simvastatin. The beneficial effect of statin was found when combined with megalonate or GGPP. Interestingly, the authors mimicked the effect by inhibiting RhoA, suggesting that the mevalonate / GGPP / RhoA pathway underlies the observed effects of simvastatin at the microRNA-155 level [73]. In animal experiments with diets induced by endothelial dysfunction, increased expression of eNOS mediated by statins leads to an improvement in endothelial function [74].

In addition to increasing the expression of eNOS at the transcriptional and post-transcriptional levels, statins cause an increase in eNOS activity at the post-translational level. eNOS has several sites of phosphorylation activation. The effect of fluvastatin on smooth-muscle cells of the human umbilical cord showed an increase in the phosphorylation of eNOS upon activation of Ser-633 and Ser-1177 genes via protein kinase A (PKA) – and PI3K / Akt-mediated pathway, respectively [75]. The statin-

induced induction of eNOS phosphorylation by Ser-1177 is realized through the heat shock protein-90 [76]. On endothelial cell culture, it was shown that atorvastatin to increase eNOS phosphorylation by Ser-633 in adenosine monophosphate-activated protein kinase (AMPA) -mediated pathway [77]. In addition, in experiments on the mesenteric artery of rats incubated with simvastatin, it was found that the drug causes rapid AMPK-mediated phosphorylation of eNOS on Ser-1177 [78]. This leads to an improvement in endothelium-dependent vasodilation. The effect is removed both by inhibiting eNOS L-NAME and by joint incubation with mevalonate. In addition, statins also increase eNOS activity at the post-translational level by inducing its dissociation from caveolin-1. Caveolae are invaginations of the cytoplasmic membrane, formed mainly by the protein caveolin-1, which has the ability to bind eNOS and inhibit its enzymatic activity. Atorvastatin reduces the content of caveolin-1 and activates eNOS in endothelial cells, regardless of the presence or absence of LDL cholesterol [76]. This mechanism was also demonstrated in experiments on apolipoprotein E deficient mice, where rosuvastatin reduced caveolin-1 and improved cardiac function and blood pressure variability [79, 80].

Activation of eNOS does not necessarily result in an improvement in NO production. If there is significant dissociation of eNOS, then its phosphorylation causes induced activation to increase production of O₂ – instead of NO [81]. Nevertheless, cerivastatin or fluvastatin to prevent the dissociation of eNOS increase the expression and bioavailability of BH 4 in human umbilical cord endothelial cell culture [75, 82, 83]. In the experimental model of diabetes in laboratory animals, the administration of atorvastatin prevents the separation of NOS through the same mechanism [84]. Recently, in a randomized, double-blind, placebo-controlled clinical trial, it was demonstrated that on the 3rd day of treatment with atorvastatin (40 mg / d) in patients after coronary artery bypass grafting, the level of BH 4 and eNOS of the internal thoracic artery increased compared to the placebo group [85]. This is accompanied by an improvement in the results of the cuff test on the brachial artery. It is important that these effects are rapid and

independent of the lipid-lowering effect of atorvastatin. In experiments on isolated segments of the human thoracic artery, atorvastatin causes an increase in the expression of the GCH1 gene, encoding guanidine triphosphate cyclohydrolase followed by an increase in the level of total tetrahydrobiopterin. The effect was reversed by joint incubation with mevalonate [85].

There is another mediated mechanism by which statins can enhance eNOS activity, improve its communication and enhance metabolism or prevent ADMA effects. Endothelial cells exposed to ADMA exhibit an increased inflammatory response, which is markedly reduced by the action of simvastatin indirectly through the extracellular pathway of the kinase receptor [86]. Treatment of rats with spontaneous hypertension with rosuvastatin revealed a decrease in the level of circulating ADMA and a decrease in vascular oxidative stress, regardless of the decrease in cholesterol [87]. Similarly, in the rat model of pulmonary hypertension, rosuvastatin increases DDAH expression, and then decreases serum ADMA levels, while increasing eNOS phosphorylation through the PI3K / Akt pathway [88]. Nevertheless, the results of clinical studies were contradictory. Some randomized studies have shown that short-term statin therapy reduces the level of circulating ADMA in humans [89, 90, 91], while other studies have not been able to find such an effect [92, 93, 94]. These conflicting results might be related to differences in the characteristics of the patients examined. It is interesting to note that in a small randomized trial, simvastatin failed to improve endothelial function in patients with high ADMA levels, but managed to do this in combination with oral administration of L-arginine in patients with low ADMA [95].

The effect of statins on NADPH oxidases. NADPH oxidase is the most important source of ROS in the vasculature, both in isolated segments of the arteries, and in culture of human umbilical vein endotheliocytes. It catalyzes the conversion of O₂ into O₂• – radicals, using NADPH as an electron donor. This membrane-bound enzyme complex; components that depend on the corresponding homologue of the membrane subunits: Nox1-5 and Duox1 or 2. Endothelial cells possess Nox2, nox4 and Nox5, while Nox1, nox4, and Nox5 were found in the umbilical cord

endotheliocytes. Nox2 is a form of the enzyme present in phagocytes. It consists of two membrane-associated subunits, p22 phox and gp91 phox-Nox2 (which make up the complex with cytochrome b558), as well as four cytosolic subunits, p40 phox, p47 phox, p67 phox and RAC1 or 2, which upon stimulation are transformed with flavocytochrome b558, which can lead to the assembly and activation of the enzyme complex. The activity of Nox1 and Nox2 can be stimulated with angiotensin II, growth hormone and pro-inflammatory cytokines, via p47phox phosphorylation [96]. At the same time, Nox5 is in a state without a Ca-dependent stimulation [97]. Conversely, nox4 does not require ROS for activation [96]. It has been found that increased expression and activity of NADPH oxidase isoforms in the vasculature, together with increased production of ROS, contribute to the onset and maintenance of the atherosclerotic process. Nevertheless, it should be noted that recent studies have found a potentially useful role for the ROS-independent nox4 isoform in the vascular wall, by increasing vasodilation in the H₂O₂-mediated pathway [98].

A significant number of publications are devoted to the role of statins in inhibiting the activity of NADPH oxidase. As mentioned earlier, activation of ROS and its localization in the membrane is necessary for the activation of Nox1 and 2-complexes, which are widely distributed in vascular cells and are involved in the pathophysiology of atherosclerosis. The most important step in the activation of ROS is its geranylgerinaliticim on geranyl-geranyl triphosphate through biosynthesis of cholesterol. Given that statins suppress both the formation of isoprenoids and geranyl-geranyl triphosphate, a large number of studies have attempted to determine whether inhibition of HMG-CoA reductase can lead to a decrease in the activity of NADPH oxidase.

In experiments on culture of human umbilical cord endotheliocytes, statin therapy reduced p22 phox mRNA and p47 phox protein levels [99]. Endotheliocytes of human umbilical arteries under the influence of atorvastatin reduce the expression of Nox1 in the membrane localization of Rac1, which leads to a decrease in the production of ROS [100]. Statins can also inhibit the generation of reactive oxygen species in

isolated segments of coronary arteries of pigs, reducing p22 phox levels of mRNA [101]. Thus, cerivastatin in human cord tissue culture of endotheliocytes induces the activation of LPO in the Rac1 region, followed by activation of NADPH oxidase and an increase O₂ – [102]. The mechanism by which NADPH oxidase exerts its effect is the colocalization of the acid ceramic of sphingomyelinase with the formation of membrane adhesions in the cell membrane. Statin treatment prevents the formation of these membrane adhesions induced by low-density, oxidized lipids, and then reduces the generation of O₂ – [103].

The effects of statins on NADPH oxidase have also been studied in animal models simulating cardiovascular pathology. Thus, the administration of statins in rats suppressed the activity of NADPH oxidase by means of mevalonate-dependent activation of ROS. This resulted in improved vascular NO production and further improvement in endothelial function [104]. In experiments with the modeling of atherosclerosis in rabbits, fluvastatin prevented an increase in p22 phox and gp91 phox induced by a high fat content and improved endothelial function and reduced plaque size [105]. Similarly, simvastatin suppressed generation of reactive oxygen species and restored endothelial function in rats with modeling of diabetes mellitus [106]. In the normocholesterolemic model of hypertension in rats, statins reduced the expression of the p22 phox and Nox1 [100] gene, as well as the levels of the p22 phox protein of the angiotensin I receptors, and thereby reduced the production of oxygen reactants and improved endothelial function [107]. In the experimental model of ischemic stroke in rats, atorvastatin prevented an increase in generation and NADPH oxidase activity of the ROS in, by reducing the gp91 phox levels of mRNA and P47 phox localized in the membrane [108]. On the other hand, statins prevent the development of endothelial dysfunction in mice through Rac1-mediated activation of NADPH oxidase [109].

The cultivation of the cell lines obtained from patients demonstrated the relevance of the above observations to human physiology. Thus, treatment with statins reduced the level of gp91 phox in cultures of endotheliocytes of the internal thoracic arteries of a person obtained from

patients undergoing aorto-coronary bypass grafting [110]. In a cross-over study, it was found that treatment with statins with hyperlipidemia leads to a decrease in the level of circulating gp91 phox, as well as markers of systemic oxidative stress [111]. The blockade of AT1 receptors in a randomized group of 49 patients with cholesterol-dependent oxidative stress after aorto-coronary bypass surgery compared with the placebo group showed that pravastatin (40 mg / day), irbesartan (150 mg / day), or both for 4- x weeks showed a significant positive effect [112]. Thus, statin therapy as well as combination therapy significantly increased eNOS expression and suppressed gp91 phox in the brachial artery during the cuff test, which indicates a synergistic effect on endothelial function, regardless of LDL-lowering effects [112]. Recently, it has been shown that short-term treatment with atorvastatin rapidly suppresses O₂ – the formation and activity of NADPH oxidase in patients undergoing aortocoronary bypass irrespective of LDL-lowering effects [113]. This is also accompanied by a decrease in the plasma levels of malonic dialdehyde as a marker of systemic oxidative stress. In vitro incubation of human endotheliocytes with atorvastatin results in a decrease in p67 phox and ROS localization in the membrane. In this case, the effects were removed in the presence of mevalonate [113]. A similar mechanism of NADPH oxidase was found in human mycardiocytes [114].

Effect of statins on endothelial cells. As is known, endothelial cells synthesize prostacyclin (PGI 2) with the help of the enzyme PGI 2 – synthase. Its molecule, the precursor of prostaglandin H₂, is itself synthesized from arachidonic acid by oxidation of fatty acids with cyclooxygenase. PGI 2 is an important vasodilator and an antithrombotic molecule that balances the effects of thromboxane A 2. Recently, it has been found that vascular PGI 2 can participate in the regulation of eNOS expression [115]. Di Francesco et al. [116] showed that inhibition of cyclooxygenase-2 induced by shift of heme oxygenase 1, results in the cessation of the effect of tumor necrosis factor in human endothelial cells [116]. Given that fluvastatin causes an increase in the expression of PGI 2 synthase and increases PGI 2 in human endothelial cells [117], this pathway can also

participate in the realization of vasoprotective effects of statins.

A statin-induced change in the activity of pro-oxidant enzymes may also partially explain the useful antithrombotic effects of statins, as evidenced by the reduced release of thromboxane A2 from the isolated rat aorta in the cyclooxygenase-2-mediated pathway [118]. Thus, the yield of 8-isoprostanate is elevated in rat-resistant hypertensive rats. This is eliminated by the appointment of atorvastatin and improves endothelial function and restores the redox potential of the vascular wall [119]. Similarly, atorvastatin-induced modification of 5-lipoxygenase leads to a reduction in the vulnerability of plaques in rabbits [120].

Endogenous antioxidant defense systems: effects of statins. Due to the constant impact of ROS, almost all living cells possess several enzyme antioxidant defense systems that control the final availability of ROS. Superoxide dismutase (SOD) is an enzyme that catalyzes the conversion of O₂ – to H₂O₂. Catalase is an enzyme located in peroxisomes that decomposes H₂O₂ in water and O₂. Glutathione (GSH) is a tripeptide that acts as an important antioxidant of the limiting effect of H₂O₂; this is achieved by reducing the sulfhydryl groups in the cysteine residues of other proteins, thereby protecting these proteins from oxidative damage. It is catalyzed by glutathione-S-transferase, as well as glutathione peroxidase (GSH-Px), which leads to the formation of glutathione disulfide (GSSG) from two GSH molecules; GSH can be replenished through the effects of glutathione reductase. The GSH / GSSG ratio is widely used as a cellular marker of oxidation-reduction processes. Paraoxonase is an enzyme involved in protecting against oxidation of the LDL molecule. Other antioxidant enzyme systems are also present in cells such as thioredoxins, peroxiredoxins, and so on.

It has been shown that statins have a beneficial effect on the vasculature not only by suppressing the prooxidant enzyme, but also by increasing the intensity and activity of endogenous antioxidant systems, in experimental models and clinical studies. Simvastatin can partially restore the renal levels of all three major cellular antioxidant systems protection (SOD, GSH-Px and catalase) and reduce levels of

biomarkers of oxidative damage in diabetic animals [121, 122]. In addition, an improvement in endothelial function was found by increasing the SOD level in rats chronically treated with the eNOS L-NFME inhibitor [123]. The beneficial effect of atorvastatin on reducing the size of plaques in animals on an atherogenic diet was observed against the background of an elevated level of circulating paraoxonase [124].

The effects of statins on catalase activity levels have been demonstrated in a number of studies. For example, endotheliocytes of aorta of rats and umbilical artery of a person show an increase in the levels of expression of genes and catalase proteins after treatment with atorvastatin [100]. The effect is mediated by PI3K / Akt by [125]. In addition, in animal models of hypertension and diabetes mellitus, statins increase the expression of aortic catalase [100, 125]. An increase in statin-induced catalase levels has also been demonstrated with an aneurysm of the human abdominal aorta [126].

The effect of statins on redox-sensitive transcritic pathways

It has been established that the main mechanism by which ROS production contributes to the development of atherosclerosis is the initiation of oxidation-reduction sensitive transcriptional pathways in the vascular endothelium. These pathways regulate the production of pro-inflammatory, pro-atherogenic cytokines and cellular components that enhance oxLDL and macrophage infiltration, as well as the proliferation and migration of smooth muscle cells to the intima. One of the most important oxidation-reduction pathways is the NF-κB pathway. NF-κB is a transcription factor that controls the expression of a large number of proinflammatory genes. In its non-activated form, NF-κB is in the cytosol bound to its inhibitor (IκBα). H₂O₂ was originally proposed as a candidate for direct activation of NF-κB [127]. Currently, it is understood that ROS have the ability to indirectly modulate the function of NF-κB [128]. Proinflammatory mediators, such as TNF; activate IκB kinases (IKK). This leads to its degradation, thereby freeing NF-κB molecules. Therefore, NF-κB is translocated to the nucleus and binds to its response elements, initiating the transcription of pro-atherogenic mediators, such as IL-6, TNFα, , adhesion

molecule VCAM-1 and intercellular adhesion molecule (ICAM) -1, and others. Another pro-inflammatory transcription factor that can be induced by ROS is a protein-1 activator (AP-1). Like NF- κ B, AP-1 regulates the expression of genes involved in inflammatory processes, cell proliferation and apoptosis, and plays a role in the atherosclerotic process.

The role of statins as potential inhibitors of NF- κ B has been investigated in cell cultures where NF- κ B activity was induced by various factors. Atorvastatin prevented the induced TNF α and angiotensin II-induced activation of NF- κ B and the subsequent release of inflammatory mediators. Effects were prevented by farnesyl pyrophosphate (FPP) and geranyl-geranyl pyrophosphate (GGPP) [129]. Fluvastatin also attenuated CRP-induced activation of NF- κ B in endothelial cells [130] of smooth muscle cells of the umbilical vessels [131]. Simvastatin and atorvastatin reduced oxLDL-induced activation of NF- κ B in the human coronary artery [132]. These statin-mediated effects have also been demonstrated in experimental models of cardiovascular disease. Thus, simvastatin reduced NF- κ B activity in atherosclerotic plaques and circulating mononuclears in animals with an atherosclerotic diet, regardless of hypolipidemic effects [133]. Similarly, the administration of cerivastatin significantly reduced the activity of NF- κ B in the hearts induced by angiotensin II and did not affect the level of cholesterol in the blood [134]. LDL-independent anti-inflammatory effects of statins have also been extensively studied in cellular models both at the forefront of atherosclerosis and inflammation induced by pathogenic microorganisms. In the culture of human umbilical cord endothelial cells, the inflammation induced by Chlamydia pneumoniae was significantly lowered under the influence of cerivastatin. Similarly, the release of inflammatory mediators was reduced by suppressing the activation of NF- κ B [135, 136]. Also, in the culture of portal vein endotheliocytes infected with cytomegalovirus, the effect of fluvastatin slowed the activation of NF- κ B, which reduced viral replication [137].

The impact of statins on the pathway of NF- κ B can play a decisive role in preventing monocyte infiltration, the formation of foam cells

and the proliferation of smooth muscle cells of the vessels. Simvastatin dose-dependently inhibits the TNF-induced increase in ICAM and VCAM-1 in endotheliocyte culture of the portal vein due to decreased activation of NF- κ B; this leads to a decrease in the interaction of monocytes with endothelial cells [138]. Importantly, in vitro pravastatin prevents the effect of human monocytes on LDL-induced activation of NF- κ B and the subsequent expression of inflammatory mediators [139]. In addition, atorvastatin inhibits interleukin-18 induced migration of smooth muscle aortic cells and the inactivation of NF- κ B [140].

Various hypotheses have been proposed to explain the ability of statins to reduce NF- κ B activity. It has been shown that statins cause an increase in the expression of I κ B α genes in endothelial cells, and also reduce the expression of NF- κ B and decrease binding to smooth muscle cell proteins, which leads to a general decrease in NF- κ B activity in vascular cells [141]. In addition, statins decrease ROS-mediated activation of NF- κ B in monocytes by decreasing the activity of I κ B kinase [142]. Phosphatidylisothiazidyl 3-kinase-independent pathway, which involves inhibition of the I κ B kinase / Akt signaling pathway in human endotheliocytes [143].

The ability of statins to inhibit NF- κ B signaling has also been found in clinical studies. In a small nonrandomized study, the appointment of pravastatin (40 mg / day) 3 months before the planned carotid artery prosthetics led to stabilization of the plaque in the carotid artery, as evidenced by a decrease in NF- κ B activation lipids [144]. In an aneurysm of the abdominal aorta simvastatin suppresses the generation of active oxygen species and the activity of NF- κ B [145]. In a randomized trial, treatment with atorvastatin (80 mg / day) for 1 month prior to surgery resulted in a decrease in NF- κ B activation in circulating mononuclears in combination with a decrease in inflammatory gene expression and cellular plaque infiltration [146].

One of the main ways that is crucial for the cellular antioxidant response is the nuclear factor (erythroid origin 2 (Nrf2). In its non-activated form, Nrf2 is in the cytoplasm, binds to proteins callin 3 and endothelium-associated protein 1 (Keap1). cysteine residues that are sensitive to

changes in ROS because of their sulphydryl group (-SH). The oxidation of these -SH groups dissociates the callin3 / Keap1 complex from Nrf2, which translocates into the nucleus and initiates the transcription of genes encoding endogenous antioxidant defense proteins. It has been shown that simvastatin is able to activate Nrf2 via the PI3K / Akt pathway and then suppress the generation of reactive oxygen species in primary embryonic fibroblasts of the mouse [147]. A similarly, fluvastatin significantly increased the Nrf2 nuclear translocation in smooth muscle cells of the coronary artery through the same intracellular pathway. This leads to increased activity of antioxidant enzymes and reduced production of ROS [148].

Thus, the use of statins is currently a key strategy for reducing cardiovascular risk, both in primary and secondary prevention. In addition to their hypolipidemic properties, statins also have a number of direct, or pleiotropic, effects on vascular function, suppressing atherogenesis, which in some cases may even lead to regression of atherosclerotic plaques. For the past decade, the vascular endothelium has been identified as one of the main targets for antiatherogenic / pleiotropic effects of statins. Statins have the ability to restore the physiological balance between NO and ROS in the vascular endothelium, through a series of LDL-dependent and -independent effects. They increase expression, and the enzymatic activity of eNOS, the main source of O₂• – radicals in the human vascular endothelium, which leads to an increase in NO biosynthesis. At the same time, they suppress the activity of prooxidant enzymes (such as NADPH oxidases, unbound Enos, and others) and increase the effectiveness of endogenous antioxidant systems in the vascular endothelium, which leads to a net reduction in ROS. By restoring the balance between NO and ROS in the vascular endothelium, statins also control the activation of the inflammatory processes of the vessels, and prevent the proliferation / migration of smooth muscle cells and lead to suppression of atherogenesis.

At the same time, there are several unresolved issues that need to be understood regarding the biological role of statins.

1. *Their indirect influence requires study. For example, by changing the biology of*

perivascular fat tissue, which has a paracrine effect on the vascular wall.

2. *The role of statin therapy in the pathogenesis of vascular disease and especially in atherosclerosis is clear. Statins are now involved in all therapeutic strategies in primary and secondary prevention of the cardiovascular system. They stabilize atherosclerotic plaques and improve survival (3). However, it is difficult to imagine that these effects are mediated primarily because of their direct or indirect "antioxidant capacity." Further studies are needed to study the efficacy of statins in other cardiovascular diseases with a large involvement of ROS in their pathogenesis. The last in full priority refers to endotoxin-induced pathologies. This would further expand their broad clinical application.*

3. Finally, the ability of statins to exert a biological effect on the vascular endothelium, regardless of their primary pharmacological action, can serve as a "pharmacological model" for the development of new antioxidant strategies that aim to control the intracellular balance between production / elimination. These smart strategies should aim to imitate the pleiotropic effects of statins on the vascular endothelium.

Experimental evidence of endothelioprotective properties of statins in the modeling of endotoxin-induced pathology

The use of simvastatin HMG-CoA reductase inhibitors (2.2, 4.3 and 8.5 mg / kg), atorvastatin (1.1, 2.2 and 4, 3 mg / kg), rosuvastatin (2.2, 4.3 and 8.5 mg / kg) and nanoparticulated rosuvastatin (3, 6.3 and 11.6 mg / kg) against the background of endotoxin-induced pathology modeling leads to the development of a dose-dependent endothelioprotective effect, expressed in the normalization of QED, the prevention of increased adrenoreactivity and exhaustion myocardial reserve, as well as the normalization of biochemical markers of inflammation (C-reactive protein) and the level of pro-inflammatory cytokines. At the same time, positive dynamics of the final products of NO and eNOS expression was detected. It is noteworthy that the most effective was the nanoparticulated form of rosuvastatin, which supports the hypothesis of a change in the volume of distribution of the drug. Parallel to this, there was a decrease in hypertrophy of mycardiocytes and

a normalization of the morphological picture of the endothelium of small vessels of the kidneys.

Use of monotherapy with donor NO L-arginine (70 and 200 mg / kg), non-selective inhibitor of arginase BEC (5 and 10 mg / kg), selective inhibitor of arginase 2 Arginazine (1 and 3 mg / kg) and recombinant darbepoetin (50 and 500 µg / kg) in the modeling of endothelial dysfunction of the pathology revealed their high activity, expressed in preventing the increase in QED, adrenoreactivity, preservation of myocardial reserve and normalization of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF). In this case, the drugs had a dose-dependent effect and were approximately equally effective.

Use of combined use of L-arginine (200 mg / kg) with inhibitors of HMG-CoA reductase by simvastatin (8.5 mg / kg), atorvastatin (4.3 mg / kg), rosuvastatin (8.5 mg / kg) and nanoparticulated rosuvastatin (11.6 mg / kg) against the background of endotoxin-induced pathology modeling proved to be so effective that the values of QED, adrenoreactivity, conservation of myocardial reserve and biochemical markers (Total NO, eNOS expression, C-reactive protein, IL -6, TNF) did not differ from the indices of intact animals. The combined use of L-arginine and statins in the modeling of L-NAME-induced pathology also revealed a pronounced additive endothelial and cardioprotective effect, manifested in a decrease in QED, prevention of a decrease in NO_x concentration, and an improvement in myocardial contractility in performing adrenoreactivity and load tests resistance. reduction of hypertrophy of myocardiocytes and normalization of the morphological picture of the endothelium of small vessels of the kidneys.

Combined use of non-selective inhibitor of arginase BEC (10 mg/kg) with simvastatin (8.5 mg/kg), atorvastatin (4.3 mg/kg), rosuvastatin (8.5 mg/kg) and nanoparticulated rosuvastatin (11, 6 mg/kg) did not show a positive pharmacodynamic interaction both in the modeling of endotoxin-induced and L-NAME-induced pathologies.

Combined use of the selective inhibitor Arginase 2 Argazine (3 mg/kg) with simvastatin (8.5 mg / kg), atorvastatin (4.3 mg / kg), rosuvastatin (8.5 mg/kg) and nanoparticulated rosuvastatin (11, 6 mg/kg) showed ectothelial and

cardioprotective effect, which is expressed in preventing the increase in QED, adrenoreactivity, preservation of myocardial reserve and normalization of biochemical markers values (Total NO, eNOS expression, C-reactive protein, IL-6, TNF). At the same time, combined therapy revealed the additive effect of drugs. A similar dynamics was observed with L-NAME-induced pathology.

Use of concomitant use of recombinant darbepoetin (500 mcg / kg) with simvastatin (8.5 mg / kg), atorvastatin (4.3 mg / kg), rosuvastatin (8.5 mg / kg) and nanoparticulated rosuvastatin (11.6 mg / kg) on the background of modeling endotoxin-induced pathology exhibits ectothelial and cardioprotective effects. At the same time, combined therapy revealed the additive effect of drugs. Similar dynamics was observed in L-NAME-induced pathology, which was reflected in a decrease in the endothelial dysfunction coefficient, prevention of NO_x concentration decrease, and improvement of myocardial contractility in performing functional tests and reducing myocardiocyte hypertrophy.

Vector analysis of the additive effects of the combined use of simvastatin, atorvastatin and rosuvastatin inhibitors and nanoparticulated rosuvastatin with L-arginine, arginase inhibitors-BEC and Arginazine and darbepoietin showed that, with endotoxin-induced pathology, the highest probabilistic percent of additions turned out to be in combinations of rosuvastatin with "Arginazine" (3 mg / kg) and dabropoietin (500 µg / kg), respectively, 31.9 ± 2.8 and 30.2 ± 2.9%.

Thus, the foregoing indicates that the problem of pharmacological correction of endotoxin-induced pathology and its components of endothelial dysfunction and multiple organ failure does not have a pathogenetically grounded pharmacological correction strategy. One of the possible ways is the use of drugs in the mechanism of action of which the principles of pharmacological pre- and post-conditioning, restriction of the activation cascades of LPO, NF- κ B and the release of pro-inflammatory cytokines, as well as an increase in the level of ADMA and TNF-induced increase in ICAM are laid. It seems that for the role of "drugs of choice" in this situation the most meaningfully claim statins, including nanoparticulated dosage forms. At the same time, the potential of L-arginine endothelioprotective

agents, nonselective and selective inhibitors of arginase 2 and darbopoetin is not fully disclosed. In this case, the approaches to experimental research in this direction should be formed taking into account the possibilities of a complex comparative evaluation of the dose-dependent anti-inflammatory effects of HMG-CoA reductase inhibitors, their cardioprotective effects in coronary-occlusive infarction, endothelioprotective effects in endotoxin-induced endothelial dysfunction in both monotherapy and in combination with drugs possessing endothelioprotective effects of various mechanisms of action. As a control of endothelioprotective activity, a well-developed model of L-NAME-induced deficiency of nitric oxide can be used in our laboratory. At the same time, taking into account the special role of the processes of "low gradation inflammation" in the development of endotoxin-induced endothelial dysfunction, it is necessary to control the level of inflammatory markers and the dynamics of morphological changes in the "target organs" of the myocardium and microvessels of the kidneys.

Conflicts of interest

The authors have no conflict of interest to declare.

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Semin A.A.

**STATE SUPPORT FOR RESEARCH IN PHARMACOLOGY:
AN ANALYSIS OF FOREIGN AND DOMESTIC EXPERIENCE**

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Abstract

Introduction: The goal of research was to analyze the management of innovative activities for innovative medicines market launch in Russia and abroad.

Materials and methods: In the study there were used methods of cognition, including methods of empirical (observation, comparison) and theoretical studies (analysis, synthesis, aggregation), scientific assessment, SWOT-analysis.

Results and discussion: The analysis of domestic and foreign experience in the planning and management of research in pharmacology was carried out. Despite the fact that at the present time in the Russian Federation a powerful ramified state apparatus for regulating scientific research and development in the field of medicine has been formed, the pharmacological science remains divided. This leads to a reduction of efficiency of innovative drugs development studies. At the same time, in developed countries, interdepartmental coordination and advisory bodies (councils, commissions) are active, often at the highest level. This testifies to the high attention given in these countries to interdepartmental interaction and intersectoral projects in the field of research and development.

Conclusion: Foreign R & D support systems are characterized by a number of differences from domestic mechanisms. A number of recommendations based on the experience of foreign colleagues for future measures to modernize existing and introduce new mechanisms for state support of research in the field of pharmacology in Russia were proposed.

Keywords: pharmacology, research and development, forecasting, strategic planning document, innovative territorial cluster, small and medium-sized enterprise.

Introduction

Innovative transformation of the Russian economy is a system tool by which the government can provide in the long term national competitiveness, successfully to solve the accumulated problems in the socio-economic domain.

Pharmaceuticals is the most high-tech and knowledge-based industry in the world economy with research and development rate in total sales amount of more than 14%, that in monetary terms in 2015 amounted to 150 billion dollars, and in

2022 it is expected that they will rise to 182 billion dollars [1].

The effectiveness of investment in research and development is confirmed the information voiced by the Minister of industry and trade of the Russian Federation Manturov D. V., at the meeting of the Government Commission for import substitution 8 July 2016: "...last year the sales volumes of 17 drugs, created with the participation of the state, were in 28 times higher than their budgetary development cost. Russian

drugs also allow to save money and ordinary consumers, and the state" [2].

However, at the present time in Russia there is no continuous value chain of an innovative product at all stages of its implementation (from the research and innovation order to their manufacturing application and creation of innovative companies).

As a result, the manufacturing application of R & D and commercialization of innovative ideas are the exception rather than the rule in the long-established administration system of innovation [3].

The President of the Russian Federation V. V. Putin in his address to the Federal Assembly in 2017, said: "...We need to transform research projects into successful commercial products; by the way, we have always suffered from it, from development to implementation the huge time goes by... It is true not only of our time, and not even the Soviet, and even in the Russian Empire everything was the same. We need to reverse this trend – we can do it..." [3].

In this regard, the stated goal of the study the management of innovation in bringing to market innovative drugs in Russia and abroad is relevant and will help to formulate recommendations on future actions for the modernization of existing and introduction of the new mechanisms of state support of scientific research in the field of pharmaceuticals in the Russian Federation.

Materials and methods

In the study there were used the methods of cognition, including methods of empirical (observation, comparison) and theoretical studies (analysis, synthesis, aggregation), scientific assessment, SWOT-analysis. Each of these methods is applied adequately on the functionality. Targeted application of these methods ensured the reliability of the estimates and conclusions obtained in the study.

The study subject was the state policy in the field of strategic planning in the Russian Federation, and in economically developed countries. Matrix regulation of state strategic planning in the Russian Federation in the field of pharmaceuticals unifying key concepts: forecasting, goal setting and planning is presented in figure 1.

From the matrix it follows that the President of the Russian Federation and the government of

the Russian Federation determine the goals, objectives and priorities strategic planning and the Federal Executive authorities carry out a direct development of the strategic planning documents, monitoring and control of their implementation. However, a promotion of coherence, balance and agreement of the strategic planning documents and determination of sequence of their development are a function of the Government of the Russian Federation.

With the aim of developing recommendations and new mechanisms for more effective management in the field of pharmaceutical science it was necessary to study the experience of foreign countries.

Results and discussion

The analysis of activity of Federal Executive authorities of the Russian Federation for the planning and management of research in the field of pharmaceuticals

It is obvious that research and development in pharmaceuticals are becoming more difficult to separate from other fields of science, and as a result, inter-industry and/or interdisciplinary projects take on greater and greater economic and social importance. A number of sectoral strategies (the Strategy for medications supply to the population of the Russian Federation for the period until 2025, the development Strategy of the pharmaceutical industry of the Russian Federation for the period until 2020) approved at the departmental level, which cannot be conducive to effective cooperation between the interested public authorities [5, 6].

At the same time, it is necessary to create a unified system of priorities and research planning, which will allow to eliminate duplication of scientific topics, to concentrate available financial and other resources in the most "critical" points, not to break the innovation chain and to coordinate all its stages from research to implementation.

Strategic planning documents have repeatedly emphasized the importance of organization priority lines of development of science and technology.

President of the Russian Federation

Shall define the directions, objectives and priorities of socio-economic policy
 Determines the goals of socio-economic development and national security
 Determines the areas of achieving the strategic goals and overarching objectives to be addressed

Forecasting

Strategic forecast of the Russian Federation

Goal setting

Presidential Address to the Federal Assembly

Planning

Priority Development Fields of science, technology and engineering in Russia

National security strategy

National technology initiative

Socio-economic development strategy

Science and technology development strategy

Government of the Russian Federation

Defines the goals objectives and performance measures of the Federal Executive authorities
 Ensures the coherence and balance of the strategic planning documents
 Determines the order of formation of system of target indicators on the basis of priorities of socio-economic development

The forecast of socio-economic development of the Russian Federation

Innovative development strategy of the Russian Federation

The main activities of the Government of the Russian Federation

The forecast of science and technology development of the Russian Federation

Medical science development strategy in the Russian Federation

The state program of the Russian Federation

Budget projection of the Russian Federation

The list of strategically important drugs

Federal Executive authorities

Develop strategic planning documents at the Federal level
 Involve in the coordination and methodical support for strategic planning in individual sectors of public administration
 Monitor and control the implementation of strategic planning documents at the Federal level

The sectoral forecasts

Strategy of development of pharmaceutical industry of the Russian Federation

The plans of the Federal authorities

The list of biotargets for the development of innovative medicines

Strategy for medications supply to the population of the Russian Federation

A comprehensive program of biotechnology development in Russia

Scientific platform of medical science

The development and production road map of modern immunobiological medicines

Fig. 1. The matrix of strategic planning in the Russian Federation in the field of pharmaceuticals

In accordance with the decree of the President of the Russian Federation of 07.07.2011 No. 899 "Life sciences" related to priority lines of development of science, technology and engineering in Russia. The list of critical technologies of the Russian Federation included biomedical technology, genomics, proteome, post-genomic, cellular technologies, technologies of loss enhancement from socially significant diseases, which are direct products of scientific research and development in pharmaceutics.

However, the question arises, how narrow should be the line and to what extent should specify the priorities of the state in the field of science.

The decree of the President of the Russian Federation of 01.12.2016 No. 642 adopted the Strategy for scientific and technological development of the Russian Federation, which envisages the transformation of science and technology in a key factor of the development of Russia and ensure the country's ability to respond effectively to big challenges¹.

One of the most important from the point of view of scientific and technological development of the Russian Federation the great challenge is "...rising threat of global pandemics, increasing the risk of new and return of extinct infections."

In the Strategy of scientific and technological development of the Russian Federation there are stated that, in the next 10 to 15 years, the priorities of scientific and technological development of the Russian Federation should be considered to be those areas that will allow to obtain scientific and technical results and to create technologies which are the basis for innovative development of the domestic market of products and services, a stable position of Russia on the foreign markets, and provide, including "the transition to personalized medicine, high-tech health care, and technology of health care, including through the rational administration of drugs (especially antibacterial)". Financial support for the implementation of that Strategy effected on account of federal budget allocations, including those allocated for realization of state programs of the Russian Federation.

In the approved state programs of the Russian Federation concerning the development of pharmaceutical science ("Health Development",

"Development of pharmaceutical and medical industry" for 2013-2020, "Development of science and technologies" for 2013-2020) there is a pronounced emphasis on the technological aspect of production, market development, improving the competitiveness of domestic products. In addition, a lot of attention from the state is paid to the effective implementation of the results of scientific research and development in industry, their commercialization and the development of mechanisms of state-private partnership [7, 8, 9].

In addition, when comparing the activities of these state programs, there is a high proportion of inter-sectoral activities requiring effective interagency cooperation.

Analysis of indicators of efficiency of realization of the state programs of the Russian Federation allows to highlight 4 of the most used groups of indicators:

indicators of publication activity;

indicators of scientific and innovative activity;

the indicators characterizing financial and economic efficiency of implemented actions;

indicators of changes of scientific and innovative infrastructure.

However, at the present stage of development of the span of control of scientific research and development, it seems advisable to move to more complex relative indicators that will characterize the efficiency performance of R & D and their practical significance.

For example, to the present day the demographic indicators and the indicators characterizing the health status of the population are not used as indicators of the effectiveness of the implementation of measures for development of pharmaceutical science. Also, such indicators could serve indexes of the effectiveness of individual methods of diagnosis and treatment.

Thus, there is a flagrant necessity for a substantial modification and improvement of the system of indicators of efficiency of realization of measures on development of scientific researches and developments area in the field of pharmaceuticals. The reason for this is not only a need to gather more informative performance indicators, but also the insufficiency of basic data

for planning of concrete lines of development of the pharmaceutical science.

Reviewing strategic documents stem from a common understanding of the key systemic problems prevailing in many knowledge-intensive industries of Russia's economy. These issues include:

technological backwardness and product obsolescence caused by a development gap of industries, infrastructure and markets;

dissociation of participants of innovative activity caused by the lack of development of the environment within the country;

fragile integration in international markets, caused by low competitiveness.

These problems mutually cause each other and have their own peculiarities in every industry, but most of the objectives formulated in the strategies aimed at overcoming these problems.

For general characteristics of the system of strategic documents it should be noted that their developers among the possible approaches to the regulation of innovative activity chose a combined strategy based on coordination of efforts of the state, large corporations, research organizations and market insiders, a high value set on attracting international participants.

In the development scenarios of industries there is also commonality. The initial stages of the strategies implementation give pride of place to creating a stimulating innovation, information and analytical infrastructure, reforming the system of scientific organizations. Further stages of integration are meant to be integrative when systemic effect from the provided resources and infrastructure, organized and stimulated interactions between process actors begins to appear. In other words, the initial stages can be characterized as an investment, and subsequent stages are as innovative.

All documents separately consider a problem of staffing system of training, retraining and retention of personnel. Given problem is accentuated by the gap between generations of professionals. The decision of the personnel problem lies in the interaction of the most competitive educational institutions and scientific organizations and enterprises, as well as through invitation of high-level specialists from abroad.

An obligatory element of these strategies is the use of a model of scientific-educational-

industrial clusters as groups of enterprises, suppliers of equipment and components, specialized production and services, research and educational organizations, linked by relations of territorial proximity and functional dependence in the production and sale of goods and services. It provides for the formation and promoting the activities of a few dozens of these associations in different regions of the Russian Federation.

So, now the following innovative territorial clusters in the field of pharmaceuticals created and developed:

Biotechnological innovation territorial cluster Pushchino;

Kaluga pharmaceutical cluster;

Cluster "Phystech XXI" (Dolgoprudny);

The cluster of medical and ecological instrument engineering and biotechnologies of St. Petersburg;

Altai biopharmaceutical cluster "ALTAIBIO";

Pharmaceutics, medical equipment and information technology of Tomsk region;

Innovative cluster of information and biopharmaceutical technologies of Novosibirsk region.

Similar approaches are used in management strategies. To coordinate the efforts of the implementing strategies there are created interagency councils, funds of an intellectual property, expert board.

Based on the above information and documents we can conclude that in the Russian Federation an extensive system of state regulation of the scientific researches and developments area in the field of pharmaceuticals has formed. The most influential actors in this process are the Ministry of education and science of the Russian Federation, the Ministry of health of the Russian Federation and the Ministry of industry and trade of the Russian Federation.

However, other Federal Executive authorities also appropriate significant budget funds for research and development for further application of their results in the field of health, including those ministries and agencies that do not have real powers in the development of pharmaceutical science.

Thus, despite the fact that currently in the Russian Federation an extensive state apparatus for the regulation of scientific research and

development in medicine has formed, pharmaceutical science remains fragmented. One-level activities and work can be carried out in institutions of the Ministry of health of Russia (including the Federal Medical and Biological Agency of Russia and Federal Service for Supervision in Healthcare), and Ministry of education and science, Ministry of industry and trade, Federal Agency for Scientific Organizations of Russia, whose activities in the field of pharmaceutical research is not coordinated. It leads to a reduction of the efficiency of research works in the field of pharmaceuticals.

The creation of a supra-departmental agency for the most effective regulation of scientific research and development in the field of pharmaceuticals and ensure the necessary level of coordination is obvious.

It emerges full blown in connection with a simultaneous increase in the Russian Federation the number and significance of intersectoral and/or interdisciplinary research and development projects in the field of pharmaceuticals.

The analysis of the foreign experience of planning and managing scientific research in the field of medicine

Even with the recognition of the high importance of development of science and innovation for social and economic welfare of the country and national security, no state may conduct scientific research and development at a modern level in all lines. In this regard, it is necessary to select priority lines to focus the main efforts of the government and where funds should be invested primarily.

Thus, the most important task of science policy is to develop tools for identifying scientific and technological priorities, and mechanisms for their implementation.

In the course of analysis of experience in application a systematic approach in forecasting research activities in the field of medicine in foreign countries at the state level there was found:

relevant agencies determine, in the first place, the priority lines of development of health in general, in some cases, the list of the priority lines of development of medical science was compiled,

but the principles and methodology of formation of the specified lists in the official resources are not given;

the circle of "critical technologies" for medical science identified through a comprehensive study on formation of the list of critical technologies at the state level;

the most progressive at the moment forecasting method "foresight" is also used to determine the priority lines of development of medical science in integrated studies.

The choice as a priority line the pharmaceutical science, in one degree or another, reflected in scientific and technical policy of such countries as the USA, the UK, France, Japan, Germany and Finland.

In these foreign countries (except the USA), in the structure of Federal Executive authorities there is defined the agency responsible for the integrated development of science and innovation in the country. And not always the relevant agency plays a significant role in the regulation of scientific research and development in the field of medicine. Fundamental researches is usually planned and financed by the agency responsible for scientific and technical state policy implementation.

The tasks of ensuring the interaction of science and industry, successful commercialization of scientific development and the coordination of industrial R & D in general, as a rule, are also provided by the agency responsible for scientific and technical state policy implementation, or organizations, subordinated to it. The presence in developed countries, special institutions (agencies) responsible for commercialization of scientific developments and technology transfer reflects the recognition of the special importance of these tasks for the interests of the state.

In all listed above countries the interagency coordination and consultative organizations (councils, commissions) actively operate, often at the highest level. It testifies to the high attention in developed countries, interagency cooperation and joint industry projects in the field of scientific research and development.

The allocation of the funds on priority lines of the science and technology development, as a rule, is through special state funds.

The foreign R & D support systems are characterized by a number of differences from the domestic mechanisms:

the implementation of the personal grant support of specialists in various fields to support the research of young people from college to heads of laboratories;

a presence in many countries of Europe, BRICS, the USA, in addition to the general natural-science funds and support programs, specialized funds for specific fields of science (e.g., biological, biomedical researches, etc.);

wider involvement of "corporate" science to the solution of public problems and coordination of the efforts with the University and Federal science.

A possible solution of a number of the scientific tasks in the field of pharmaceuticals, could be the introduction in Russia of the financing instruments of the international scientific consortia.

So, in the USA there is a few years of successfully operating a support program for internal research teams from the EU countries. The EU, in turn, through the Horizon 2020 program actively involves in-house development of participants from around the world.

All European funding for Russian scientists since 2014 amounted to 1.38 million euros (in this case, funds are mainly personal grants, for example, as part of the MSCA (Marie-Sklodowska-Curie Action)).

It should be noted that the analysis of instruments of international cooperation, Western partners have allocated considerable funds for the promotion of the private science, research and policy. For example, Partnership Instrument 2015 Annual Action Programme covered by the 2014-2017 Multi-annual Indicative Programme intend specifically related to "public diplomacy".

Planned within the program activities fit into the strategy of the public diplomacy of the EU, which covers four areas: work with the scientific community; joint policy research and discussions having a mutual interest; cooperation with representatives of civil society on issues of common interest, using culture as a vector for the public diplomacy. Grants as a part of one of the program sections (the "EU Policy and explanatory partnership" – "Cultural diplomacy") apply to Russia, Japan, China, the USA, Central America,

South America, the Asia-Pacific region with a budget of 15.5 million euros.

However, one of the major goals of state regulation in the area of scientific research and development, including in the field of the medicine, is to encourage the continuous transfer of knowledge and technology from science to the economy, because the market independently, without special incentives, is unable to provide the necessary economic development level of funding for science.

In developed countries, there was accumulated a vast experience in the integration of research results into practice industries. In this aspect, the involvement of the private sector plays an important role, as the volume of the budget of any state is limited. The analysis of foreign experience revealed common mechanisms for attracting commercial organizations for financing of scientific activities, namely:

1) Economical motivation for research cooperation in the private sector and research cooperation of the private sector with the state and university sectors, including tax incentives of the cooperation, concessional lending of the common projects, cost sharing major science and technology projects by the state.

It should be noted that if before the tax benefits acted only in respect of companies conducting research and development on their own, currently in most countries these benefits apply to companies conducting research and development by outsourcing.

Of course, tax benefits are not considered by governments of developed countries as the only effective method of stimulation of the research and innovation, but they have some advantages, for example, in comparison with government programs providing subsidies or grants.

In particular, tax incentives do not reduce the autonomy of companies in relation to the state, in contrast to programs providing subsidies or grants, realizing which the state retains significant control functions. Therefore, when choosing of research areas, the companies in this case follow by real market needs and not demand from the state. In addition, tax incentives require less paperwork, that facilitates the operation and the companies themselves, and government bodies [10].

In the USA there are over hundreds of benefits which are not an advance but as an incentive for real activity. The main difference between the Western system benefits from the Russian is that they are provided not scientific organizations, but investors. Thus regular review of benefits allows the government to purposefully stimulate innovation in priority sectors, to influence the strength and structure of scientific and innovation organizations [11].

However, direct financing of research and development from the state budget remains the main economic instrument of the state scientific and technical policy. In developed countries the share of public funding can reach 40% of national research expenditures. In addition, for basic research, it is much higher: from 50 to 70% in different countries [10].

2) Donation or grant on preferential terms in the permanent or temporary use of state property for conducting research and development to private sector companies.

One of the main forms of transfer of Federal property for temporary use to private companies in the US is the agreements on joint research and development. The company conducts research on the subject, given by the Federal Agencies.

Also in developed countries there is used the organization of common use centers (CUC), which allows companies to use equipment to conduct research (on a paid basis or free of charge). This mechanism is an effective incentive for companies with high research potential, but lack of funds to purchase expensive equipment. When using the equipment of the CUC, the organization determines its own theme independently, the problem is only that whether the CUC with the necessary equipment.

3) Creation of demand from the state to the results of research and development through the mechanisms of a state order.

In fact, government procurement of the high-tech products is the creation of a new market for domestic manufacturers.

Example of practical implementation of this mechanism is launched in 2004 in the USA, the project "Biological shield" under which the state guarantees the demand for vaccines and medicines of new generation, able to protect Americans from threats of bioterrorism. The project assumed that within 10 years, the

Department of homeland security will spend of \$5.6 billion for the purchase of anti-anthrax, smallpox and other biological and chemical poisons [12].

However, without in-depth development of the issue the public procurement can easily become inefficient spending of the budget funds. It is true both for the procurement mechanisms and mechanisms for the selection of objects for purchase. It is obvious that even the most prosperous state is not able to procure the entire range of the high-tech products (even very promising), produced in the country. First of all, an attention should be paid to the departments, products purchase of that simultaneously contributes to solving other national problems (in particular, enhancing the country's defense, improve the health of the nation, etc.).

4) The development of public-private partnership.

In many countries of the Organization for Economic Co-operation and Development (OECD), the share of state budget allocated through public-private partnership (PPP) is constantly growing [13]. For example, in Germany the PPP programme in the research field began to be implemented back in the 1970-ies, so the share of the public funding of R & D has currently dropped from 70 to 30% [14].

It should be noted that the instrument of PPP can be seen not only as a way of dividing the costs of implementation of any initiatives in research and innovation area, but also as a method of regulating relations between subjects of scientific and innovative activities. In most developed countries, PPPs are used as co-financing, and regulation. As a tool of co-financing PPP prevails in most the EU countries, but as an instrument of regulation it is in the United States and Japan [15].

The most common forms of PPP are co-financing of research projects on pre-competitive stage (and then the incentive for industry participation is the transfer of rights to the results of research and development for their further commercialization); co-financing of the early stages of commercialization ("seed", venture financing); organization of joint research centers in the areas that are traditionally in the state area of responsibility (including health service); acceleration of the development of technical standards necessary to regulate research and

innovation activities, the development of clusters and innovation infrastructure, support of small innovative enterprises and stimulate the creation of new small firms.

5) The creation and support of technology platforms

Under the "Seventh framework programme for the development of scientific research and technological development" (FP7), which served as the main instrument of the EU to support scientific research and development and to attract private financing in the period from 2007 to 2013, there were established the European Technology Platform (ETP).

Through the work of the ETP, the representatives of European industry, universities and research institutions affect the policy of the European Commission in the field of science and technology. In particular, the topics of the research unit of Cooperation in the last FP7

program were formed on the basis of programs of strategic researches of the technological platforms. It ensures a relevance for the industry of sponsored research topics.

6) The use of potential of small firms as sources of innovation

Initiatives in relation to small and medium innovative entrepreneurship are realized in most developed countries. Sufficiently developed forms of such support are in the USA, the UK, France, Germany, Finland.

Various schemes and programmes of state support for entrepreneurship, especially innovative, are formed taking into account dynamics of development of the respective projects and the level of their private investments. The typical model of development of innovative project and its financial support is presented in figure 2.

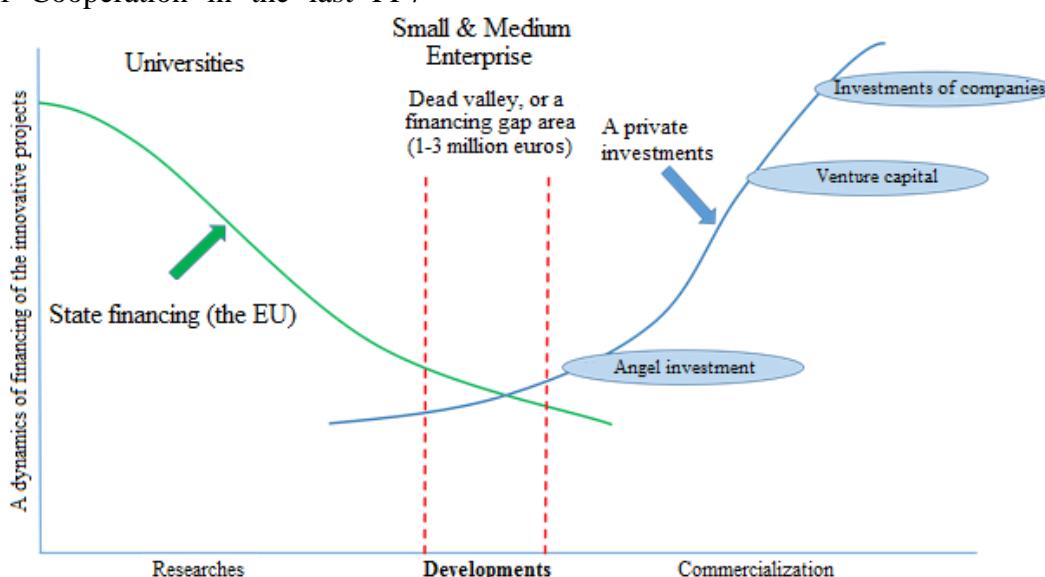


Fig. 2. Scheme of financial support for innovative projects by the EU and individual EU members

Herewith they use various mechanisms, including financial support in various forms, activities in the field of training and retraining of specialists, access to information, technology and markets, provision of tax preferences, such as the benefits for small firms or tax credit for investors, funding a small business [16].

In the United States to encourage the cooperation between state organizations and the private sector there was enacted a law on cooperative research and development between Federal research institutions and commercial companies [17]. The law provided access to all

interested USA companies to the scientific and technical resources of the Federal laboratories at the account of restrictions related to national security. In this case we are talking about research and development, customer of that is the Federal Executive authority and that was reckoned on an achievement of results that have a commercial value. The Federal laboratories are permitted to transfer the rights of ownership of the results of scientific and technological activities to the private enterprise. Small and medium-sized innovative firms are granted advantages in the form of exclusive licenses.

Another example is the support program for cooperative research centers operated by the National Science Foundation of the United States. The cooperative research centers are the pools, concentrated around the universities and involving at least six partners from industry. They are to promote the results of research conducted in the universities or the government laboratories, to industry.

Also the most well-known, having more than 30 years of history, and therefore well-studied the US programs: Small Business Innovation Research and Small Business Technology Transfer can be the example of a mechanism to provide financial assistance for the development of small innovative enterprises [18].

Innovative research program in the small enterprise has become a kind of model for other countries, which also began to support small innovative entrepreneurship as an important component of the national innovation system. It is meant to be start-up capital for the small enterprise and help them to participate in R & D funded by the government.

In the EU within the program Horizon 2020 for the first time there is being implemented the initiative (program) aimed at creating a special system of state co-financing and other forms of support for the innovative micro, small and medium enterprises. This program is called Horizon's 2020 SME Instrument – SMEI [19]. The SMEI program has a solid budget, which is about 3 billion euros for the period 2014-2020, that is slightly less than 30% of the amount allocated within the Horizon 2020 program on the research and development of the small and medium-sized enterprises [20].

SMEI largely copies successfully used in the USA Small Business Innovation Research and Small Business Technology Transfer programs.

SMEI unlike other programs allows for the possibility to be a member of one private small and medium enterprise (SME), while other similar programs require that the innovative

project was implemented by a consortium of two, three or more partners.

Also, from January 2015 within the Horizon 2020 program a new SME support program "Fast Track to Innovation pilot – FTI" has started in a pilot mode [21]. In the EU appeared first program aimed at the direct financial support of the innovative projects in advanced stages that precedes the entry of innovations into market, i.e. their commercialization. This period corresponds to technology readiness levels TRL 6 – TRL 9.

Also the experience of support for small firms in France by the National Agency for improving the innovative attractiveness of scientific research (ANVAR) is of some interest. The Agency has the industrial and commercial status and operates as an independent concern, but its mission is defined by the government, and it also provides the essential tools [22].

The Agency may provide interest-free loans for up to 5-6 years, covering up to 50% of the total costs associated with the implementation of the innovative project or a project on technology transfer which are repayable in case of successful completion of the project. In addition, grant-making is possible for the preparation and completion of innovative programs, floating innovative companies, increasing the technological level of small and medium-sized enterprises (through the involvement of researchers, formulation and use of scientific and technical information, etc.), as well as encouraging greater participation by small and medium-sized enterprises in the European projects of a technological cooperation.

Figure 3 shows the three sources for financial support to SMEs in the EU, which operate through financial intermediaries, using a wide range of instruments: loans; guarantee for loans; counter-guarantees; mezzanine financing, or, as it is called bridge financing; venture capital investments; joint-stock investment etc.

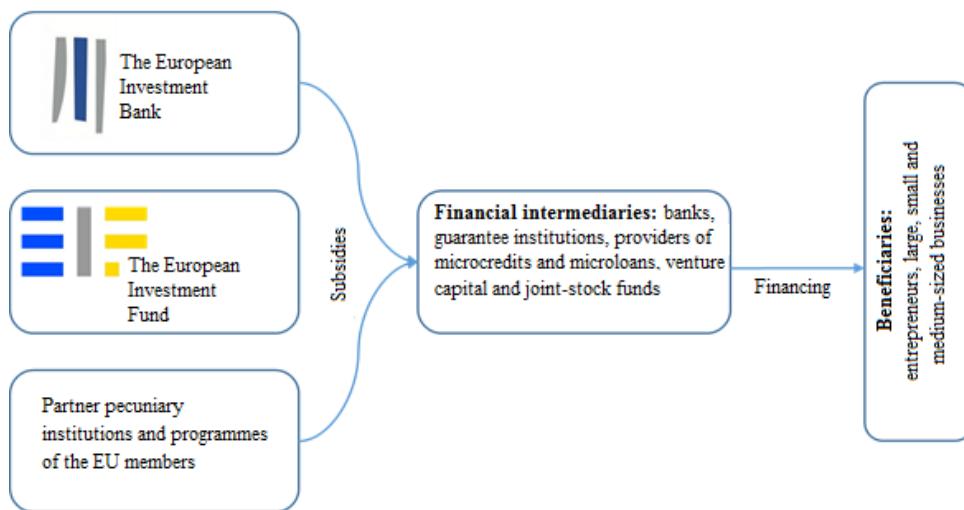


Fig. 3. Scheme of financial support for SMEs and larger companies by the EU and individual EU members

The EU supports entrepreneurship through numerous financial institutions on the ground. Such institutions throughout the EU there are more than 1 thousand Annually, support is provided to over 200 thousand enterprises of different size and degree of development, since the starting and ending expanding enterprises [23]. It is important that the EU aims to make financial support to SMEs the most convenient and quickly. In particular, there was created a specialized portal that makes it easy to find the appropriate financial institution in any EU country.

Thus, based on the experience of foreign colleagues, we can outline a number of recommendations regarding future measures on modernization of existing and introduction of new mechanisms of state support of scientific research in the field of pharmaceutics:

the development of narrow strategic development plans of specific areas of the science;

support of certain direction projects included in the development strategy of the priority industries;

creation of the specialized fund of the projects focused support in the field of pharmaceuticals.

orientation of activities aimed at international cooperation in the field of pharmaceuticals, on the advanced countries for specific scientific tasks (creation of new lines) and neighboring countries

(developing and maintaining a well-established cooperatives);

development and adoption of measures aimed at popularization of the Russian science (support popular science publications, conferences and exhibitions for pupils and students, etc.);

individual support of young scientists within the science organizations, designed to stimulate the development of domestic competencies.

Conflicts of interest

The authors have no conflict of interest to declare.

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Best regards,
The Editorial Board