Mechanisms of The Cardioprotective Effect of The Active Substance Based on Macromolecular Compounds of Humic Nature During Ischemia And Reperfusion of Isolated Heart of Rats

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Abstract

The article describes active substance of natural origin (ASO) based on high molecular compounds of humic origin according to developed parameters of their standardization, based on physicochemical analysis methods (titration, ultraviolet and infrared spectroscopy, elemental analysis). Cardioprotective and inotropic effects of gained ASO (0.1 mg/ml) have been researched during the experiments on global ischemia model (45 minutes) and reperfusion (30 minutes) of isolated perfused rat heart. It was found that preventive use of ASO before modeling of ischemia and reperfusion had decreased reperfusion contracture as well as contributed to decrease of necrotic death of cardiomyocytes and fuller recovery of contractility. Preliminary blocking of NO-synthase with a help of L-NAME (100 umol/L) had disabled cardioprotective effect of ASO. Supposition was made that NO-synthase plays an important role in development of ASO cardioprotective effect. Active substance of natural origin capability to inhibition of free-radical processes in test-system with use of DPPH was shown. It was surmised that improving cardiomyocytes resistance to ischemia / reperfusion after application of ASO is connected with its antiradical effect.

Key words: humic substance, peat, ischemia, reperfusion, cardioprotection, NO-synthase.

Key points

Active substance of natural origin (ASO) have cardioprotective effect in preischemia period;
Cardioprotective effect of ASO are results of increased synthesis of NO and, a consequence, cGMP in myocardium, and anti-oxidant action

1. Introduction

The protection of the heart from ischemic and reperfusion injuries remains an actual problem in cardiology. Currently, the cardioprotective properties of drugs of natural origin potentially having low toxicity are being intensively studied. The use of plant adaptogens slows the occurrence of irreversible damages of cardiomyocytes during ischemia-reperfusion, without provoking arrhythmia and without decreasing myocardial contractility (Lasukova, 2015; Maslov, 2009). At present time, cardioprotective features of herbal drugs with presumably low toxicity are being actively researched. Application of herbal adaptogens decelerates permanent damage of cardiomyocytes in case of ischemia-reperfusion without provoking of arrhythmia as well as without decreasing of myocardium contractility (Lasukova, 2015; Maslov, 2009). Biological effects of high molecular compounds of humic origin (humic acids) are also being actively researched (Belousov, 2014; Buzlama, 2010; Radomska-Lesniewska, 2016; Verticka, 2013). Structural features of the latter define their multi-purpose pharmacological effects: anti-inflammatory, immunostimulatory, hepatoprotective, antioxidant, antihypoxic and other (Belousov, 2014; Buzlama, 2010; Verticka, 2013). It is known that humic acids (HAs) can increase resistance of cerebrum's neurons and kidneys' nephrons to ischemia-reperfusion (Akbas, 2015; Sen, 2015; Rensburg, 2015), prevent damaging of cardiomyocytes (Rensburg, 2015), and possess some vas-

odilating features (Zykova, 2017). However there is a little data regarding implementation of HAs pharmacological effects. There are only few publications about HA-induced activation of NO-synthase (Hseu Y. W., 2002; Hseu, 2014), and enhancement of nitrogen oxide in case of cultivation of HAs with peritoneal mouse macrophage (Trofimova, 2016). These facts allow to make an assumption that the signal mechanism connected with NO-synthase is included in realization of myocardial effects. The purpose of the research is to investigate cardioprotective activity of standardized active substance of natural origin (ASO) based on high molecular compounds of HAs.

2.1. Extraction and standardization of ASO of HAs

Extraction and standardization of ASO based on HAs of lowland wood-grass type peat from «Tagan» deposit of Tomsk region was carried out according to methods described earlier (Zykova M. B., 2014; Zykova, 2017). Methods are based on the results of infrared spectroscopy (IR–Fourier – spectrometer – FSM 1201), on technology of back titration (content of acid functional groups), on elemental analysis (C,H,N – analyzer «Carlo Erba Strumentazione» 1106), and on spectroscopy in UV domain (spectrophotometer Unico 2800).

2.2. Determination anti-radical activity of ASO of HAs

Anti-radical activity (ARA) of ASO was measured by reactions of interaction with a free stable radical diphenyl picrylhydrazyl (DPPH) on spectrophotometer Unico 2800 (λ=520 nm). Degree of optical density's decrease relative to initial point shows radical binding activity (%) – interaction intensity percent between DPPH and substance, which is characteristic of ARA (Nishizawa, 2005). Dihydquercetin was used as a drug for comparison (Psotova, 2002).

2.3. Research of myocardial effects of ASO of HAs

Research of myocardial effects of ASO was carried out on male Wistar rats that weight 250-300 g. The study was approved by the Ethical Committee of the Federal State Budgetary Scientific Institution, Research Institute for Cardiology, and it conformed to the European Union Directive 2010/63/EU. Retrograde heart perfusion was according to Langendorf method using Krebs-Henseleit solution, saturated with mixture of O_2 и CO_2 (95% and 5%, respectively). The temperature of solution was $37\pm0{,}5^{o}$C, pH =7.5. Rates of inotropic heart functions were measured in isovolumic mode with a help of pressure meter SS13 (Biopac System Inc., Goleta, California, USA). Pressure record in aortic ventricle was done with a device for electrophysiological research MP35 (Biopac System Inc., Goleta, USA). Effects of ASO that was standardized according to mentioned above method and dissolved in Krebs solution (0,1 mg/ml) on contractility and terminal diastolic pressure (TDP, mmHg). This concentration was selected as a result of preliminary screening research in vitro (Zykova, 2017). After 20-min stabilization period, the contractile parameters of isolated heart were recorded: the left-ventricular developed pressure (LVDP), which is the difference between the systolic and diastolic pressure, and the relative value of end-diastolic pressure (EDP). Global normothermic ischemia was modeled by arresting perfusion for 45 min; the duration of reperfusion was 30 min. In order to estimate the irreversible damage to cardiomyocytes, the definition of creatine phosphokinase in a perfusion solution flowing from the heart during reperfusion. The coronary effluent was collected during reperfusion to measure CK release. Creatine kinase activity was determined with a CK-NAc kit (Analyticon Biotechnologies, Lichtenfels, Germany) using a SmartSpec Plus Spectrophotometer (Bio-Rad, Hercules, CA, USA) at wavelength of 340 nmol/L and expressed as units per gram of heart weight for the 30-min collection period. Perfused hearts not subjected to I/R served as controls. Registration of contractility was done after 20 minutes adaptation to perfusion conditions then at the 10^{th} minute of perfusion made with Krebs solution, containing ASO, then at 5^{th}, 15^{th} and 30^{th} minute of reperfusion. Rat hearts perfused with similar method using Krebs solution without ASO were used as a control group. L-NAME blocker (Nω-nitro-l-arginine methyl ester hydrochloride) in end point

of $100x10^{-3}$ mM was used to study the role of NO-synthase in realization of myocardial effects of ASO (Andelova, 2005).

2.4. Statistical analyses

Statistical processing of data was performed with STATISTICA 6.0 software. Mann–Whitney U test was used to reveal intergroup differences. Results are expressed as mean ±SEM from indicated number of experiments. Statistically significant were the differences fitting $p<0.05$.

Influence of the characterized ASO on the contractility rate of isolated heart are shown in Table 1.

Table 1

Effect of ASO on the physiological parameters

Parameter	CONTROL (n=14)	ASO (n=14)	ASO + L-NAME (n=14)	L-NAME (n=14)
Stabilization period (20 min adaptation)				
LVDP, mm Hg	91±5,8	93±3,2	90,9±4	92±2,2
EDP, mm Hg	15,6 ±0,3	15,3±0,3	14±0,3	15,7±0,6
Perfusion (10 min)				
LVDP, mm Hg	90,9±6,1	70,8±4*	90,8±6,9	87±5,2
EDP, mm Hg	13,6±0,2	12,4±0,5*	13,4±0,9	14,7±0,8
Reperfusion (5 min)				
LVDP, mm Hg	10,3±2,7###	37,4±11*###	12,2±4,4###	10,2±3###
EDP, mm Hg	79,2±7,1###	65,8±1,4*###	77±3,6###	79±3,6###
Reperfusion (15 min)				
LVDP, mm Hg	18,5±2,4###	45,3±9*###	19,5±3,8###	16,5±2,4###
EDP, mm Hg	75,8±5,4###	60±7,1*###	74±6,3###	76±6,6###
Reperfusion (30 min)				
LVDP, mm Hg	25,7±3###	37,7±4,2*###	28,4±3,5###	20,7±3###
EDP, mm Hg	64±8,2###	59,8±4,5###	62,9±8###	65,9±5###

Note. # $p<0.05$, ## $p<0.01$, ### $p<0.001$ in comparison with the initial values. *$p<0.05$ in comparison with the control.

The decrease of contractility force, as well as decrease of end diastolic heart pressure in preischemia period took place in view of perfusion with solution, containing ASO (Table 1). At the same time, preventive application of ASO promoted more effective recovery of heart's inotropic function in reperfusion period (Table 1) under simultaneous decrease of end diastolic heart pressure by an average of 30% (Table 1). Reliable decrease of reperfusion emission of creatine phosphokinase after application of ASO was proved during the experiments that shows a cardioprotective effect of the studied compounds (Figures 1).

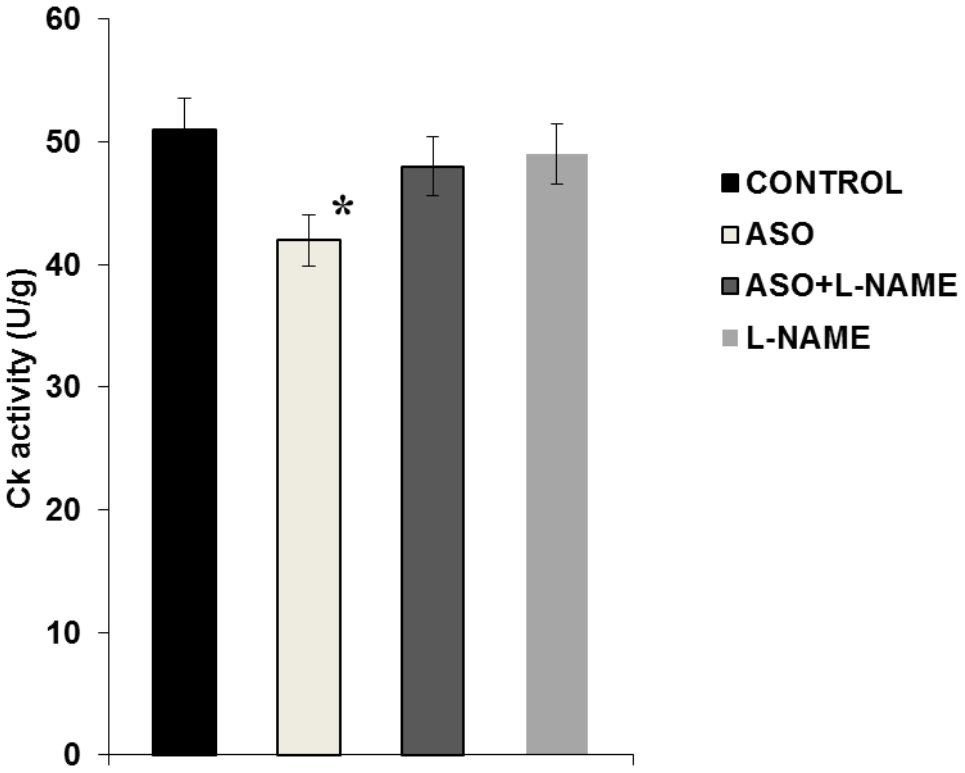

Figure 1 Effects of active substance of natural origin (ASO) on creatine kinase (CK) activity in the coronary effluent from isolated hearts rats

Experiments with L-NAME blocker of NO-synthase were conducted. Data are presented in Table 1 and Figures 1. It is evident that in case of NO-synthase blocking cardioprotective effect of HAs is not revealed (Figures 1). In the same series change of pressure in the left ventricle of heart as well as change of end diastolic pressure were not reliably differ from corresponding figures during the all stages of experiments (Table 1). Application of NO-synthase blocker did not affect the dynamics of all the studied indicators (Table 1, Figures 1).

Antiradical activity of the ASO investigated (Figures 2).

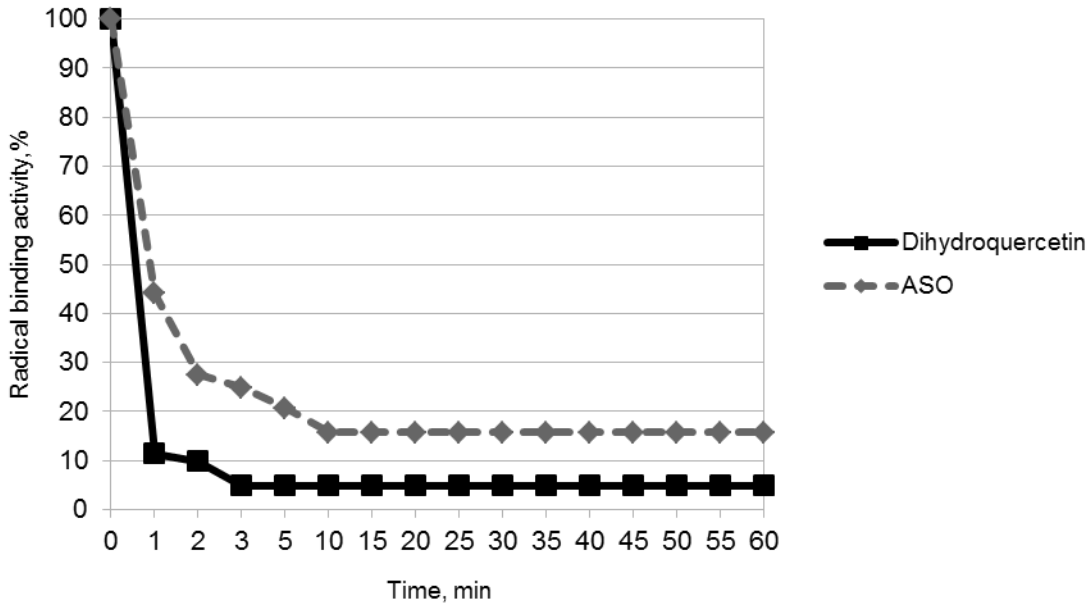

Figure 2. The kinetic curves for the reaction of DPPH with active substance of natural origin (ASO) and comparison drug dihydroquercetin

Intensity of ASO's (0.1 mg/ml) radical-binding activity was similar to the reference substance (dihydquercetin). Amount of time which took DPPH to react with the substance up to 50% was 2 minutes for ASO, similar as for dihydquercetin (Figures 2).

4. Discussion

Currently the role of NO as modulator of myocardium contractility is to generate negative inotropic effect mediated mainly by cGMP-bound mechanism (Zaripova, 2016; Ziolo, 2008). According to the expressed hypothesis it was supposed that decrease of contraction force and end diastolic pressure of isolated heart in case of injection of composition of HAs to perfusion solution is being realized by the mentioned signaling pathway. In postischemia period contraction force exceeded corresponding reference quantity under simultaneous weakening of postishemic contracture.

It is admitted fact that the inhibition of the pumping function of the heart, the growth of the end diastolic pressure during ischemia and reperfusion is largely due to Ca^{2+}-overload of cardiomyocytes (Metelitsa, 1996; Turer, 2010). Since the mentioned phenomena were also mentioned, there were reasons to believe that Ca^{2+} overload took place in the experiments. At the same time in po preventive application of ASO, a significant weakening of reperfusion contracture was observed. Perhaps more effective contracture restoration compared to one that as observed in control example could be caused by GK-induced decrease in Ca^{2+}-overload of cardiomyocytes during ischemia, which ensured more efficient restoration of the contractile function of the heart during reperfusion.

Based on the data above, it can be assumed that the cardioprotective effect of ASO could also be a result of increased synthesis of NO and, as a consequence, cGMP in the myocardium. It is known that an important role in ensuring of the hear immunity to the damaging effect of coronary occlusion and subsequent reperfusion is played by a system that depends on cGMP (Qin, 2014). Majority of researches confirm a positive role of nitric oxide in case of heart ischemia (Bell, 2003; Konorev, 1995; Qin, 2004). Thus, in the experiments by American physiologists on the model of 30-minute regional ischemia and 120-minute reperfusion, the cardioprotective effect of the nitric oxide donor S-nitroso-N-acetylpenicillamine (SNAP) was shown, which was expressed in a significant decrease in the size of the necrosis zone of the myocardium (Qin, 2004). Besides, the application of nitric oxide donor S-nitrosoglutathione (GSNO) in case of 35-minute ischemia prevents postischemic contractive dysfunction of myocardium, growth of reperfusion contracture of cardiomyocytes (Konorev, 1995). It was supposed that decrease in necrotic death of cardiomyocytes, improvement of contractility and decrease of reperfusion contracture after the preventive application of ASO could be a result of activation of NO-synthase signal mechanism by these compounds.

As another possible mechanism of cardioprotective application of ASO it could be suggested its anti-oxidant action. It is known that one of the key phases of pathogenesis of ischemic and reperfusion damage of cardiomyocytes' membranes is increase in production of active oxygen forms (Turer, 2010). Inhibition of lipo-peroxide reactions can, on the contrary, help to reduce the number of irreversibly damaged cardiomyocytes during anoxia-reoxygenation. In this regard, it should be noted that humic substances have definite antioxidant properties, which are convincingly demonstrated in the literature (Belousov, 2014; Buzlama, 2010) and results of this paper.

Taking into account the obtained data, it is necessary to note that many pharmacological agents possess cardioprotective properties: calcium channel inhibitor, β-adrenoreceptor blocking agent, nitrovasodilators. However, all these drugs inhibit the pumping function of the heart (Metelitsa, 1996, 2005; Syrkin, 2003). The studied ASO is favorably compared with the mentioned above

drugs because they do not reduce the force of the heart contractions and weaken the reperfusion contracture of the heart, which indicates the prospect of preclinical study of the drug on the basis of the studied ASO as a new effective and safe cardioprotector.

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Disclosure

The authors have no conflicts of interest to disclose.

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