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EVALUATION OF THROMBOGENICITY OF ß-PROPIOLACTONE/ULTRAVIOLET (ß-PL/UV) TREATED PPSB IN CHIMPANZEES

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ABSTRACT

The thrombogenicity of ß-PL/UV-treated PPSB (factor IX concentrate) was evaluated in chimpanzees. PPSB isolated from ß-propiolactone-treated and UV-irradiated plasma was injected into chimpanzees at a dose of approximately 100 units/kg body weight. An FDA licensed PPSB preparation served as the negative control, and a preparation containing activated as well as precursor clotting factors served as the positive control.

15 minutes, 1 h, 4 h, and 24 h after the PPSB application the following parameters were determined in the chimpanzee blood: factors II, VII, IX, X, VIII, fibrinogen, AT III, thrombin coagulase, Quick value, APTT and platelet count.

Neither the untreated control preparation, nor the PPSB isolated from ß-propiolactone-treated and UV-irradiated plasma, showed signs of thrombogenicity in the chimpanzee model. The positive control indicated that the chimpanzee is a suitable model for the thrombogenicity testing of activated clotting factors.

Key words: Factor IX concentrate, Thrombosis, ß-propiolactone/ ultraviolet treatment

INTRODUCTION

The treatment of citrated plasma with ß-propiolactone and UV irradiation leads to inactivation of viruses possibly present in the plasma (1, 2, 3, 4, 5, 6, 7, 8, 9). The present study was designed to determine if this treatment results in thrombogenicity of the treated plasma proteins.

In vitro tests were demonstrated to be insufficient for the determination of the thrombogenicity of PPSB (factor IX concentrate) preparations with peptide substrates or the TGt_{50} (the incubation time required to give a fibrinogen clotting time of 50 seconds) and the NAPTT (non-activated partial thromboplastin time) (10). Unequivocal determination of the thrombogenicity of PPSB preparations has so far been possible only in models in vivo (11). As an alternative to the proposed dog or hemophilia B dog models, we have determined the thrombogenicity of ß-PL/UV-treated PPSB in chimpanzees. PPSB isolated from ß-propiolactone-treated and UV-irradiated plasma was injected into chimpanzees at a dose of approximately 100 units/kg body weight. An FDA licensed PPSB preparation served as a control. To establish whether the chimpanzee is sensitive to thrombogenic material, an activated preparation of clotting factors was administered to a chimpanzee at a dose of approximately 75 units/kg body weight.

MATERIAL AND METHODS

The following preparations were used:

1. PPSB concentrate from Biotest, which was prepared from ß-propiolactone-treated and UV-irradiated plasma. The starting plasma, from which the cryoprecipitate had been separated, was fresh frozen. The plasma was adsorbed with DEAE Sephadex A 50. The eluted PPSB preparation contained approximately 25 units of factor IX/ml as well as 5 I.U. of heparin/ml.

2. Control PPSB: As control PPSB preparation manufactured by Hyland was used.

3. Anti-inhibitor coagulant complex (Autoplex®) from Hyland. The Autoplex® lot 0650R044AA contained 660 units of factor VIII correctional activity in 30 ml.

Table 1

Tested PPSB Preparations

		Biotest		Hyland
Lot No.		492010	493029	790315A 121A
Volume (ml)		20	20	30
Units per bottle	factor II	580	660	360
	factor VII	360	520	2580
	factor IX	540	400	570
	factor X	540	640	570

Table 1 shows the lot numbers and activities of the test preparations.

Experimental

Five chimpanzees, aged 4 to 7 years, were used. Their number, sex, age and weight are given in table 2 together with the lot numbers of the test material they each received.

Table 2

Chimpanzees and Tested PPSB Preparations

Number	Age (years)	Sex*	Weight (kg)	Test material
2	7	m	22.8	Biotest; 493029
19	6 1/2	m	31.8	Biotest; 492010
22	5 1/2	m	21.0	Biotest; 493029
84	6	f	25.3	Biotest; 492010
150	4 1/4	m	20.2	Hyland; 79031A 121A
19**	7	m	36.0	Hyland; 0650R044AA (Autoplex®)

* m = male; f = female

** Chimpanzee No. 19 was used twice. In the first experiment it received PPSB as shown, and in the second experiment, the preparation containing activated clotting factors.

Four bottles of the test material were used for each animal. The total amount given is calculated on the basis of units per kg body weight.

Table 3

Total number of PPSB factor units applied per animal, and units per kg body weight

Chimpanzee	II		VII		IX		X	
Number	u	u/kg	u	u/kg	u	u/kg	u	u/kg
22	2640	125	2080	99	2000	95	2560	121
2	2640	116	2080	91	2000	88	2560	112
84	2320	91	1440	57	2160	86	2160	85
19	2320	73	1440	45	2160	68	2160	68
150	1440	71	10320	510	2280	112	2280	112

The chimpanzees were anaesthetized with ketamine-HCl for the PPSB infusion and for the blood withdrawals.

The PPSB preparations were infused at a rate of approximately 6 ml/minute and Autoplex® at a rate of approximately 2 ml/minute. Blood samples were taken before and approximately 15 min, 1 h, 4 h, and 24 h after the PPSB application. Each time, 30 ml of blood were withdrawn. 8 parts of blood were mixed with 2 parts of a 3.8 % sodium citrate solution. The citrate-stabilized blood was centrifuged within 2 hours after the blood withdrawal, the plasma was separated from the cells, filled in plastic tubes and frozen immediately at - 70 °C. It was maintained at this temperature until shipped on dry ice to the Biotest laboratory for examination.

Platelet and coagulation studies

Standard: A pool of citrated plasma from 10 chimpanzees served as standard for the determination of the activity of the coagulation factors II, VII, VIII, IX and X. A normal, human, plasma pool served as reference for the Quick value, the APTT, the staphylococcal clumping test, the AT III and fibrinogen concentration and the thrombin coagulase time.

The activity of the factors II, VII and X was determined with reagents supplied by Behring. The activity of the factors VIII and IX, as well as the APTT and the Quick value were determined with reagents supplied by Dade. AT III was determined on immune diffusion plates (M-Partigen®, Behring). For the determination of fibrinogen degradation products (fdp) the thrombin coagulase time and the staphylococcal clumping test, reagents from Boehringer, Mannheim, were used. Protein was determined by the Biuret method (12). Fibrinogen was precipitated with thrombin, the washed fibrinogen clot was dissolved in NaOH and the fibrinogen concentration calculated from the protein determined by the Biuret method.

RESULTS

The results of the tests done on the chimpanzee blood samples are given in figures I, II, III and IV. Because of the limited space the results for antithrombin III and the factor VIII activity are not given. There was only little variation for these values between single blood withdrawals.

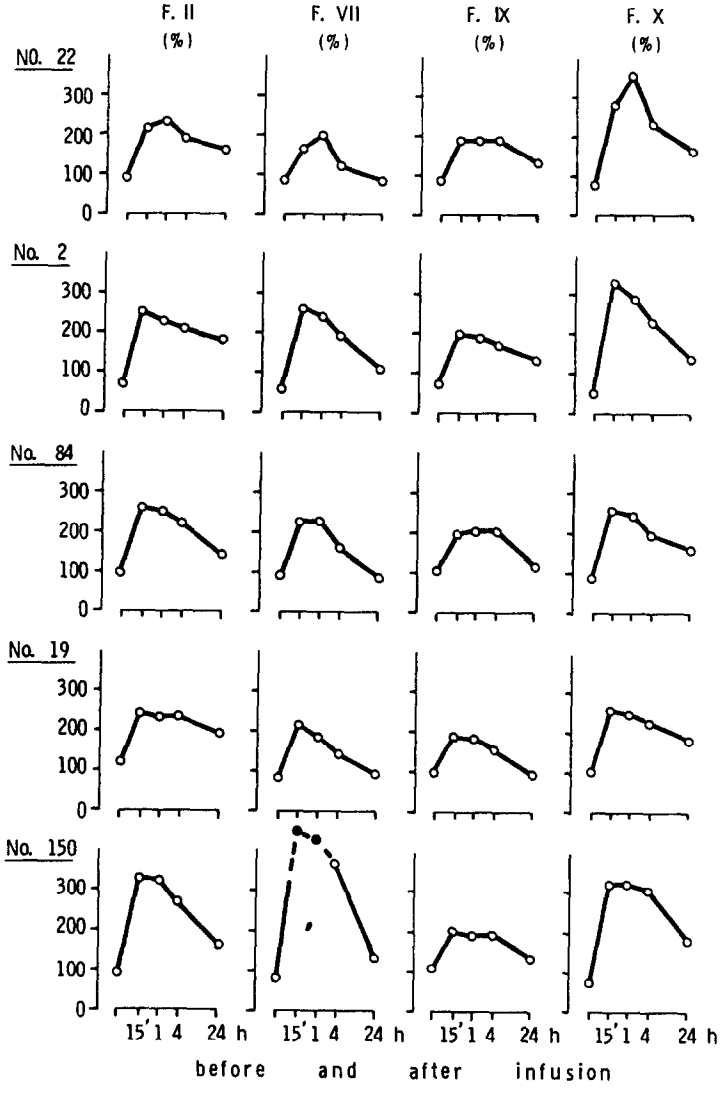

Fig. I

Activity of the PPSB factors in the chimpanzee plasma before and after PPSB application. Chimpanzees Nos. 22, 2, 84 and 19 received ß-PL/UV-treated PPSB, while chimpanzee No. 150 received untreated, control PPSB.

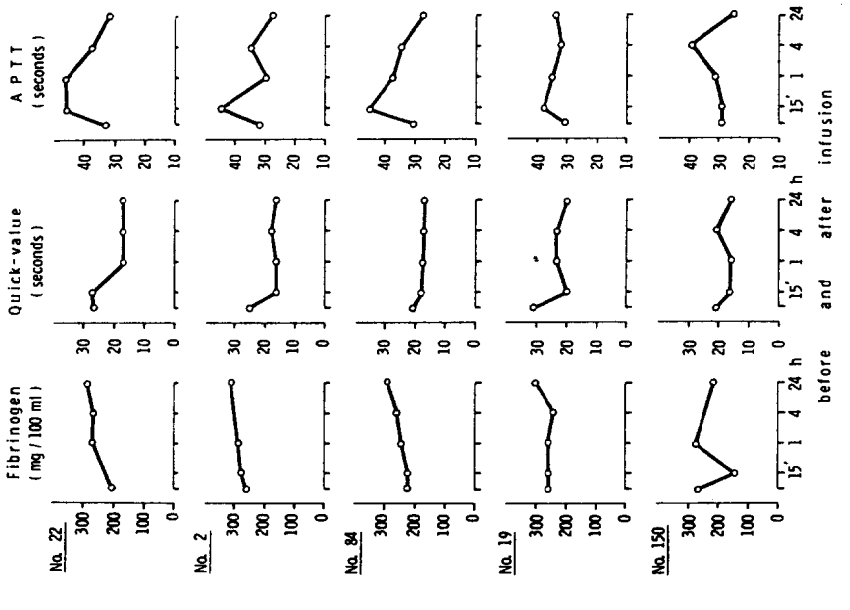

Fig. II

Coagulation analysis in chimpanzees Nos. 22, 2, 82, 19 and 150.

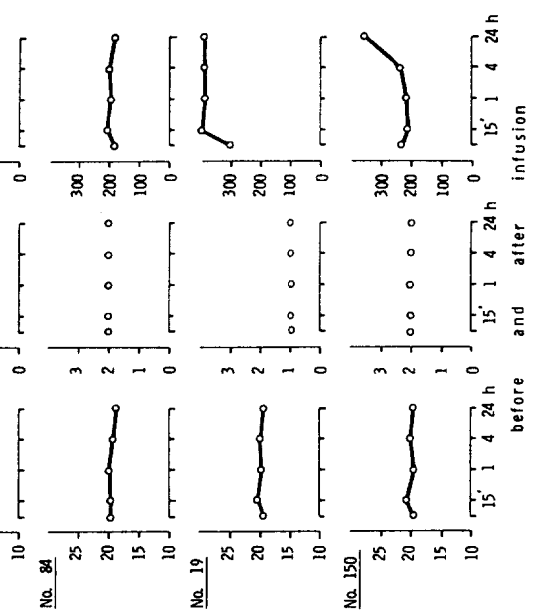

Fig. III

Thrombin coagulase time in seconds, fibrinogen degradation products in µm/ml measured with the staphylococcal clumping test and platelet counts in chimpanzees Nos. 22, 2, 19 and 150.

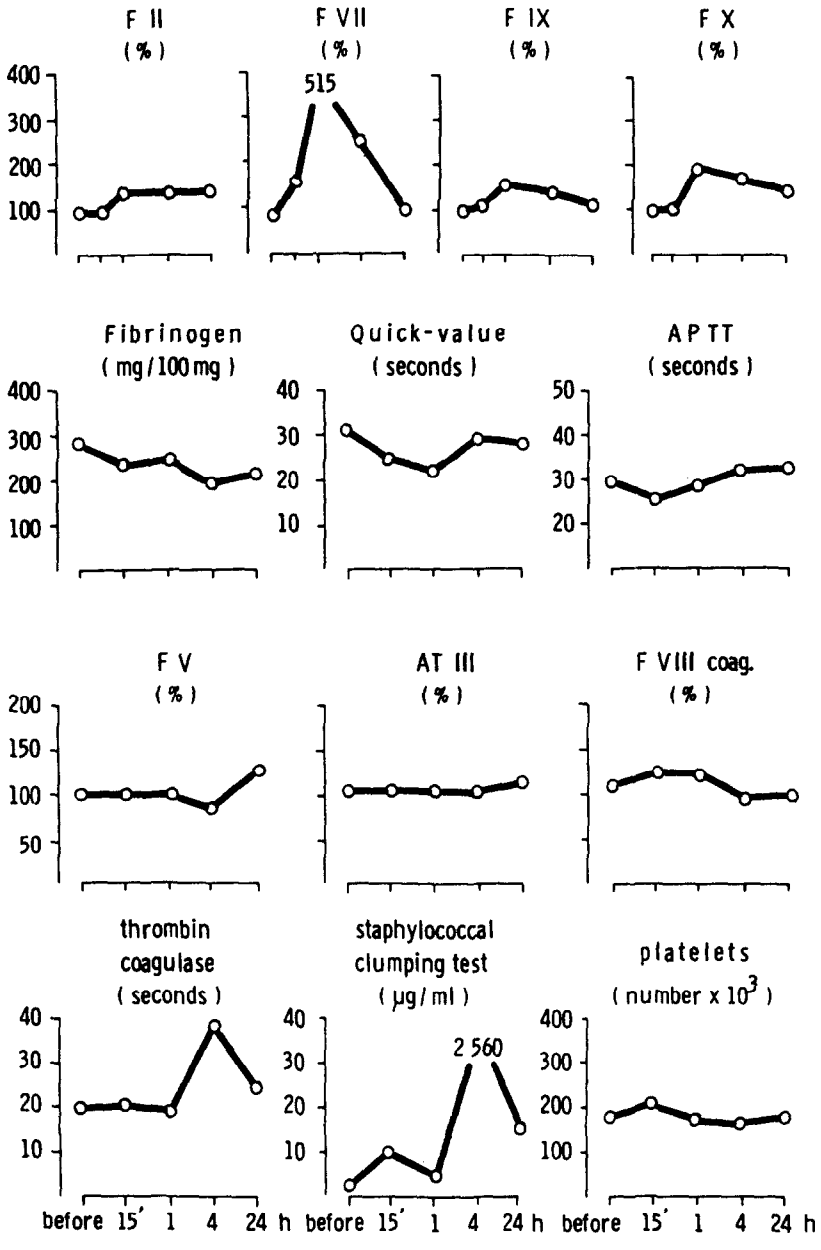

Fig. IV

Coagulation analysis in chimpanzee No. 19** (chimpanzee No. 19 in the second experiment, receiving Autoplex®).

The activity rise of the PPSB factors in the chimpanzee blood 15 minutes after the PPSB application is given in table 4.

Table 4

Activity increase of the PPSB factors in % of normal

Chimpanzee number	II	VII	IX	X
22	120	78	100	202
2	178	199	124	274
84	162	135	84	170
19	158	128	88	150
150	230	436	92	247

The efficacy of PPSB preparations is usually described as the increase of the factors in % calculated per unit per kg body weight.

The increase in % per kg results from the quotient of the measured factor increase and the amount of units applied per kg. These values are given in table 5.

Table 5

Calculated rise of the PPSB factors in % after application of 1 U/kg

Chimpanzee number	II	VII	IX	X
22	0.96	0.78	1.05	1.67
2	1.53	2.18	1.40	2.45
84	1.78	2.36	0.98	2.00
19	2.16	2.84	1.29	2.20
150	3.23	0.85	0.82	2.20

During the PPSB application and following blood withdrawals the pulse and blood pressure of the chimpanzees was recorded.

No remarkable changes for the blood pressure values and the puls rate were recorded, so that the detailed results can be obmitted here.

DISCUSSION

The thrombogenicity studies of PPSB preparations of Hedner and Nilsson (11, 13) in dogs demonstrated that PPSB in a dose of approximately 100 units of factor IX per kg body weight caused considerable changes in the coagulation parameters, such as those tested in this study. Especially critical was the four hour blood sample after the PPSB application. Instead of the dog model we used chimpanzees, since they are much closer to man.

To evaluate the susceptibility of chimpanzees towards thrombogenic blood products, a preparation containing activated as well as non-activated coagulation factors was tested. Application of this material to a normal chimpanzee confirmed that it can indeed lead to DIC phenomena, which demonstrates the validity of the chimpanzee as an animal model for studying the safety of the thrombogenicity of coagulation factor preparations.

The comparison of the PPSB control preparation with the preparation made from ß-propiolactone-treated and UV-irradiated plasma shows that both induced certain changes in the test parameters, but these changes were equivalent for both types of preparations. These changes can be expected, because they concern the rise in the activity of the PPSB factors in the chimpanzee plasma. This rise of the PPSB factors is paralleled by a shortening of the Quick value. Neither the untreated control preparation nor the PPSB from ß-propiolactone-treated and UV-irradiated plasma showed signs of a thrombogenic effect in this chimpanzee model.

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