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Comparative study of predisposition to thrombosis with administration of known systemic hemostatic agents and fibrin monomer in the experiment

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ABSTRACT

Aim. To compare predisposition to thrombosis caused by administration of known systemic hemostatic agents and fibrin monomer under the conditions of normal coagulation versus drug-induced hypocoagulation in the experiment.

Materials and methods. The prothrombotic effect of intravenous (IV) administration of various systemic hemostatic agents was compared in a series of *in vivo* experiments. These agents included fibrin monomer (FM) (0.25 mg / kg), prothrombin complex concentrate (PCC) (40 IU / kg) or recombinant factor VIIa (rFVIIa) (270 mcg / kg). The studies were conducted under the conditions of hypocoagulation induced by the administration of warfarin (*per os* at a dose of 0.4–0.5 mg / kg / day for 14 days) or dabigatran etexilate (*per os* at a single dose of 15–20 mg / kg). Hemostatic system parameters were evaluated using thromboelastometry and calibrated automated thrombography.

Results. It was found that PCC reversed anticoagulant effects and led to an overcompensated increase in the density characteristics of the blood clot along with an excessive increase in thrombin generation in the groups of animals with warfarin-induced coagulopathy. The use of PCC and rFVIIa in the groups of animals with dabigatran-induced hypocoagulation also resulted in an increase in blood thrombogenic properties. In the administration of PCC, it was manifested though an increased D-dimer level and in administration of rFVIIa – through an increase in the clot density characteristics. At the same time, replacement of these hemostatic agents with FM did not affect the hemostatic system parameters.

Conclusion. FM at a dose of 0.25 mg / kg, as opposed to PCC and rFVIIa, is safer in terms of the risk of thrombosis.

Keywords: thrombosis, hypocoagulation, prothrombin complex concentrate, Eptacog alfa (activated), fibrin monomer, warfarin, dabigatran etexilate

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Сравнительный анализ предрасположенности к тромбообразованию при применении известных системных гемостатических средств и фибрин-мономера в эксперименте

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РЕЗЮМЕ

Цель. Провести сравнительную оценку предрасположенности к тромбообразованию, обусловленную применением известных системных гемостатических средств и фибрин-мономера в условиях нормокоагуляции и на фоне фармакологически индуцированной гипокоагуляции в эксперименте.

Материалы и методы. В сериях экспериментов *in vivo* сопоставляли протромботический эффект внутривенного введения различных системных гемостатических препаратов. В их числе использовались фибрин-мономер (ФМ) (0,25 мг/кг), концентрат факторов протромбинового комплекса (КФПК) (40 МЕ/кг) или рекомбинантный фактор VIIa (rFVIIa) (270 мкг/кг). Исследования проводились на фоне гипокоагуляции, обусловленной приемом варфарина (*per os* в дозе 0,4–0,5 мг/кг/сут на протяжении 14 сут) или дабигатрана этексилата (*per os* в разовой дозе 15–20 мг/кг). Оценивали показатели системы гемостаза, включая проведение тромбоэластометрии и калиброванной тромбографии.

Результаты. Установлено, что в группах животных с индуцированной варфарином коагулопатией КФПК реверсировал эффекты антикоагулянта, но приводил к сверхкомпенсированному усилению плотностных характеристик сгустка крови наряду с избыточным усилением генерации тромбина. Использование КФПК и rFVIIa в группах животных с гипокоагуляцией, вызванной приемом дабигатрана, также приводило к нарастанию тромбогенных свойств крови. Это иллюстрировалось в случаях использования КФПК увеличением уровня D-димера, а применения rFVIIa – усилением плотностных характеристик сгустка. В то же время замена данных гемостатиков на ФМ не отражалась на показателях системы гемостаза.

Заключение. ФМ в дозе 0,25 мг/кг в сравнении с КФПК и rFVIIa более безопасен с позиции риска возникновения внутрисосудистого тромбообразования.

Ключевые слова: тромбообразование, гипокоагуляция, концентрат факторов протромбинового комплекса, эптаког альфа (активированный), фибрин-мономер, варфарин, дабигатрана этексилат

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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INTRODUCTION

Currently, a whole range of systemic hemostatic agents with a known mechanism of action is available in clinical practice [1]. These include antiplatelet agents, fibrinogen, as well as cryoprecipitate enriched with fibrinogen, prothrombin complex concentrate (PCC), factors VIII and IX, eptacog alfa (activated) also known as recombinant factor VIIa (rFVIIa), anti-inhibitor coagulant complex (FEIBA), tranexamic acid, etc. The mentioned hemostatic agents are in demand in practical medicine for prevention of bleeding disorder or management of bleeding in injuries and major surgeries, including thromboprophylaxis. It is noted that their use in general leads to a shift in the hemostatic balance toward increased blood coagulation, which provides a hemostatic effect [2].

Safety of drugs and their effectiveness are priority conditions for the selection of certain drugs that affect the hemostatic system. It is known that the use of some systemic hemostatic agents at recommended doses is associated with the risk of developing venous or arterial thrombosis, as they may lead to an excessive hemostatic potential.

Previously, we conducted original studies that showed the presence of independent hemostatic activity of exogenous fibrin monomer (FM) in the experimental model of liver injury [3]. Similar results were obtained using the same model with drug-induced hypocoagulation [4, 5]. According to the results of the above studies, FM was as efficient as both rFVIIa and PCC. These publications emphasized the comparison of the listed drugs with FM in terms of their effectiveness, while safety issues (in terms of the risk of thrombosis) were not analyzed and discussed. Obviously, this serious aspect should be considered in an additional analysis, which involves assessing the odds for the so-called thrombotic preparedness, characterized by corresponding changes in the hemostatic system [6].

In this regard, the aim of this study was to conduct a comparative assessment of predisposition to thrombosis due to the use of known systemic hemostatic agents and FM under the conditions of normal coagulation and drug-induced hypocoagulation in the experiment.

MATERIALS AND METHODS

The data were collected from 94 healthy male Chinchilla rabbits weighing 3.0-4.5 kg, kept in standard vivarium conditions. The animals were divided into 7 groups by block randomization. Animal experiments were carried out in accordance with the European Convention and Directives for the Protection of Vertebrate Animals Used in the Experiment 86/609/EEC, as well as the Declaration of Helsinki and the "Rules for Conducting Work with the Use of Experimental Animals". The study was approved by the local Ethics Committee at Altai State Medical University (Protocol No. 12 of 12.11.2015).

At the beginning of the experiment, animals in groups 1 (n = 13), 2 (n = 14), and 3 (n = 16) received warfarin dissolved in water (Nycomed, Denmark) *per os* at a dose of 0.4–0.5 mg / kg / day for 14 days until international normalized ratio (INR) values of 2.0 and above were reached. After this period, blood was collected from the marginal ear vein of the animals (by the free flow technique) to study the hemostatic system. Then, these animals received a placebo administered intravenously at a dose of 0.5 ml (3.75 M urea solution corresponding to its concentration in the FM solution), PCC (Prothromplex 600, Baxter, Italy) at a dose of 40 IU / kg, or FM at a dose of 0.25 mg / kg, respectively. The FM-based agent was obtained using the original technology [7]. One hour after the intravenous administration of a placebo or a systemic hemostatic agent, blood was sampled again.

At the beginning of the experiment, the animals in groups 4 (n = 10), 5 (n = 14), 6 (n = 14), and 7 (n = 13) received dabigatran etexilate dissolved in water (Pradaxa®, Boehringer Ingelheim, Germany) *per os* at a dose of 15–20 mg / kg. To achieve a sufficient anticoagulant effect, the dose of the drug for the animals was determined taking into account the correction factor for dose conversion between animals and humans [8] and the recommendations specified in the medication guide (Pradaxa®, registration certificate No. LSR-007065/09). After two hours, blood was taken from these animals to study hemostasis, and then a placebo was injected intravenously at a dose of 0.5 ml, PCC (Protromplex 600, Baxter, Italy) was administered at a dose of 40 IU / kg, rFVIIa (NovoSeven, Novo Nordisk A/C, Denmark) was administered at a dose of 270 mcg / kg or FM was administered at a dose of 0.25 mg / kg, respectively. The doses for PCC and rFVIIa were determined according to the current guidelines [9–11]. One hour after the intravenous administration

of a placebo or a hemostatic agent, blood was taken again.

Blood from all animals in the study was placed in plastic tubes with EDTA potassium salt to determine platelet count and with 0.11 M (3.8%) sodium citrate solution (the ratio of blood and stabilizer was 9:1) to identify other parameters. Platelet-poor plasma in all samples was obtained according to the generally accepted method. In venous blood samples, the platelet count was assessed on the automatic hematology analyzer Drew-3 (Drew Scientific Inc., UK – USA). In the blood plasma, the international normalized ratio (INR), the echitoxic time (ET) of coagulation, and fibrinogen concentration according to the Clauss assay were determined on the Thrombostat 2 coagulometer (Behnk Electronik, Germany) using reagents from Technology-Standard Ltd. (Russia). D-dimer level was determined using the NycoCard Reader II (Axis-Shield PoC AS, Norway) and NycoCard® D-Dimer test systems (Axis-Shield PoC AS, Norway).

Thromboelastometry of the blood stabilized with citrate was performed on the ROTEM® Gamma thromboelastometer (Pentapharm GmbH, Germany) with the star-TEM reagent in the NATEM assay. The following parameters were determined: CT – coagulation time; CFT – clot formation time; α angle – clot amplitude; MCF – the maximum clot firmness; A10 – clot amplitude after 10 minutes. To assess thrombin generation, the calibrated automated thrombography according to N.S. Hemker (2003) was used on the Fluoroskan Ascent FL microplate fluorometer (ThermoFisher SCIENTIFIC, Finland) with Thrombinoscope™ 3.0.0.26 software and reagent kits from Thrombinoscope® (Netherlands) (PPP-Reagent, Thrombin Calibrator, FluCa-Kit) with tissue factor at a concentration of 5.0 pM. The following parameters were taken into account: lag time – initiation of thrombin generation; ETP – endogenous thrombin potential; peak thrombin – peak thrombin concentration; ttPeak – time to reach peak thrombin concentration; V – the rate of thrombin generation [12].

The distribution of characteristics in the samples was evaluated using the Shapiro – Wilk test. Depending on the distribution of the characteristics, Student's *t*-test, Mann – Whitney *U*-test, or Wilcoxon *W*-test were used. The differences were considered statistically significant at $p \leq 0.05$. The results were processed using the MedCalc software version 17.9.7 (license BU556-P12YT-BBS55-YAH5M-UBE51). The data were presented as the median and the interquartile range (Me [Q_{25}÷Q_{75}]).

RESULTS

When systemic hemostatic drugs including PCC (group 2) and FM (group 3) were used in the groups of animals with warfarin-induced coagulopathy (verified in group 1 – placebo), the achieved effects in the hemostatic system differed (Table 1). In particular, the administration of PCC led to the normalization of INR and a statistically significant decrease in the platelet count in the peripheral blood. It was accompanied by an overcompensated increase in the density characteristics of the blood clot (according to the thromboelastometry data, for MCF (+21%, $p < 0.005$) and A10 (+49%, $p < 0.006$)) in comparison with placebo [13] and excessive thrombin generation (according to the calibrated automated thrombography data, for ETP, peak thrombin and thrombin generation V) (Table 1). It should be noted that in the group of warfarinized animals who received FM (group 3), despite a sharp decrease in blood loss (by 9.1 times compared with placebo – in group 1) [5], high INR and a hypocoagulation shift according to calibrated automated thrombography were not corrected toward normal physiological values.

In the following groups of animals, in which dabigatran was used for direct thrombin inhibition, the hemostatic system parameters also differed after the administration of PCC, rFVIIa, and FM: in groups 5, 6, and 7, respectively (Table 2). The administration of PCC or rFVIIa to animals receiving dabigatran etexilate led to an increase in D-dimer by 2.8 and 8.0 times, respectively, which was not specific for the experimental group that received FM. In addition, the use of PCC in groups 5 and 2 was accompanied by a statistically significant decrease in the platelet count in peripheral blood (by 17.0 and 6.1%, respectively), which was not characteristic of the FM effects. According to the data obtained in group 6, where rFVIIa was used as a systemic hemostatic agent, a shift to hypercoagulability was seen in such thromboelastometry parameters as CT, MCF, CFT and A10, which was not noted in animals receiving FM (group 7). According to several parameters, the density characteristics of the clot (α angle (+28.2%, $p < 0.001$), CFT (–44.7%, $p < 0.001$) and A10 (+30.2%, $p < 0.019$)) exceeded those in the placebo

group [13]. As it was shown earlier, the use of FM reduced blood loss by 2.9 times compared with group 4, while the administration of PCC and rFVIIa did not affect blood loss [14].

DISCUSSION

Researchers around the world published findings indicating the risk of thrombotic complications in patients receiving rFVIIa or PCC for prevention or relief of overt bleeding. A number of foreign researchers expressed their concern regarding this issue. So, in the study by A. Girolami et al., cases of arterial and venous thrombosis with unspecified localization were observed in patients with several bleeding disorders (deficiency of FVII and FXI, dysfibrinogenemia, von Willebrand disease, Glanzmann thrombasthenia) when they received rFVIIa [15]. Cases of thrombosis after the use of rFVIIa in cardiac surgical patients were also described in the literature [16, 17]. The review by M. Levi et al. is of particular interest, as it presented the results of safety analysis of rFVIIa in 35 randomized trials involving 4,468 patients. It was shown that thromboembolic events were documented in 9.0% of patients included in the study; these events took place mainly in the arterial bed [18].

Many authors also associated the use of PCC with various types of intravascular coagulation. S.G. Yates and R. Sarode noted that the risk of thromboembolic complications (TEC) after the PCC administration in the treatment of bleeding associated with coumarin intake remained an important clinical problem [19]. A number of authors described various thrombotic events associated with PCC, namely, superficial thrombophlebitis, deep vein thrombosis, pulmonary embolism, arterial and cavitary thrombosis, and disseminated intravascular coagulation (DIC) [20–24]. At the same time, the risk of thrombosis increases in patients with cardiovascular diseases and the elderly, as well as in the combined use of rFVIIa and PCC [25].

Previously published studies showed that the administration of exogenous FM was accompanied by an increase in D-dimer, a marker of coagulation and fibrinolysis, by 7.0 and 8.0 times in groups of animals receiving this agent at doses of 2.5 and 5.0 mg / kg [3]. This was accompanied by an increase in the density characteristics of the blood clot (based on thromboelastometry findings) [13] and consumption of platelets with a 1.5-fold decrease in their number in the peripheral blood (with FM at a dose of 5.0 mg / kg) [3]. At the same time, the use of FM at a dose of 0.25 mg / kg did not lead to changes characteristic of a shift to hypercoagulability [3, 13]. It should be noted that, according to the calibrated automated thrombography data, the intensity of thrombin generation did not increase, regardless of the used FM dose [13].

In these studies, both rFVIIa and PCC recipients showed a trend toward intravascular thrombosis. When using rFVIIa, thromboelastometry detected an increase in the density of the blood clot (for α angle, CT, CFT, MCF, and A10) and an 8-fold increase in D-dimer compared with the baseline value before the administration of this agent ($p < 0.005$). The use of PCC was also accompanied by a rise in D-dimer by 2.8 times compared with the baseline value ($p < 0.003$), an overcompensated increase in the density of the blood clot (based on MCF and A10 parameters of thromboelastometry), as well as excessive thrombin generation (in terms of ETP, peak thrombin, and V). At the same time, the replacement of the above systemic hemostatic agents with FM did not lead to intravascular coagulation, according to the methods used to assess the hemostastic system.

Table 1

The results of the hemostatic system evaluation in the experimental animals with administration of warfarin, *Me* (Q_{25}÷Q_{75})

Parameters	Group 1 (placebo)		Group 2 (PCC)		Group 3 (FM 0.25 mg / kg)	
	before placebo administration (1a)	after placebo administration (1b)	before FM administration (2a)	after PCC administration (2b)	before FM administration (3a)	after PCC administration (3b)
Platelet count, $\times 10^9$ / l	555.0 [471.0÷591.0]	512.0 [474÷700.0] $p_{1a–1b} = 0.382$	425.0 [392.8÷531.3]	399.0 [334.0÷454.5] $p_{2a–2b} = 0.049$ Δ –6.1%	509.0 [417.8÷578.0]	479.5 [408.3÷551.5] $p_{3a–3b} = 0.328$
INR, ratio	2.4 [2.0÷4.0]	2.5 [2.2÷4.6] $p_{1a–1b} = 0.650$	2.1 [1.7÷6.2]	1.1 [1.0÷1.2] $p_{2a–2b} = 0.002$ Δ –47.6%	2.0 [1.6÷3.6]	2.0 [1.5÷2.9] $p_{3a–3b} = 0.063$

Table 1 (continued)

Parameters	Group 1 (placebo)		Group 2 (PCC)		Group 3 (FM 0.25 mg / kg)	
	before placebo administration (1a)	after placebo administration (1b)	before FM administration (2a)	after PCC administration (2b)	before FM administration (3a)	after PCC administration (3b)
Fibrinogen, g / l	2.8 [2.6÷4.3]	3.0 [2.6÷4.4] $p_{1a–1b}$ = 0.814	3.3 [2.8÷4.1]	2.9 [2.5÷3.6] $p_{2a–2b}$ = 0.260	3.1 [2.7÷3.5]	3.0 [2.5÷3.3] $p_{3a–3b}$ = 0.065
D-dimer, ng / ml	150.0 [100.0÷200.0]	150.0 [100.0÷200.0] $p_{1a–1b}$ = 0.351	100.0 [100.0÷100.0]	100.0 [100.0÷200.0] $p_{2a–2b}$ = 0.180	200.0 [100.0÷250.0]	200.0 [150.0÷400.0] $p_{3a–3b}$ = 0.075
Thromboelastometry						
CT, sec	2,122.5 [1,328.3÷2,464.8]	2,095.0 [1,052.0÷2,398.0] $p_{1a–1b}$ = 0.530	1,573.5 [948.3÷2,394.0]	494.0 [355.0÷626.0] $p_{2a–2b}$=0.002 Δ –3.2 times	1,459.0 [783.5÷2,198.8]	1,559.5 [734.0÷1,918.8] $_{3a–3b}$ = 0.221
α angle, degrees	n.r. in 9 cases	n.r. in 7 cases	48.0 [39.5÷52.0] n.r. in 8 cases	68.0 [59.0÷71.0]	39.5 [30.3÷60.8] n.r. in 6 cases	37.0 [32.8÷55.3] n.r. in 4 cases $p_{3a–3b}$ = 0.767
CFT, sec	n.r. in 10 cases	n.r. in 8 cases	356.0 [307.5÷794.5] n.r. in 8 cases	166.0 [110.0÷181.0]	452.5 [218.5÷522.5] n.r. in 8 cases	367.0 [187.0÷404.8] n.r. in 6 cases p_{3a-3b}=0.735
MCF, mm	n.r. in 8 cases	n.r. in 7 cases	22.5 [9.0÷49.5] n.r. in 4 cases	70.0 [67.0÷76.0] $p_{2a–2b}$=0.008 Δ –3.1 times	32.5 [15.8÷50.5] n.r. in 6 cases	44.0 [32.0÷49.5] n.r. in 4 cases $p_{3a–3b}$ = 0.139
A10, mm	n.r. in 9 cases	n.r. in 8 cases	8.5 [4.0÷34.5] n.r. in 4 cases	64.0 [55.0÷68.0] $p_{2a–2b}$=0.007 Δ –7.5 times	24.5 [18.8÷38.0] n.r. in 6 cases	32.0 [27.0÷41.0] n.r. in 5 cases $p_{3a–3b}$ = 0.260
Calibrated automated thrombography						
Lagtime, min	3.5 [2.7÷4.5] n.r. in 2 cases	4.4 [3.4÷5.6] n.r. in 3 cases $p_{1a–1b}$ = 0.592	5.0 [4.3÷5.3] n.r. in 6 cases	1.7 [1.5÷2.0] $p_{2a–2b}$ Δ –2.9 times	4.5 [4.5÷5.3]	6.0 [5.9÷6.3] n.r. in 2 cases $p_{3a–3b}$ = 0.593
ETP, nmol × min	150.2 [92.3÷183.9] n.r. in 2 cases	103.0 [60.9÷158.8] n.r. in 3 cases $p_{1a–1b}$ = 0.109	97.8 [68.2÷104.9] n.r. in 6 cases	582.0 [444.9÷806.4] $p_{2a–2b}$ Δ +6.0 times	131.7 [81.3÷145.2]	149.3 [111.3÷189.6] n.r. in 2 cases $p_{3a–3b}$ = 0.514
Peak thrombin, nmol	28.2 [18.9÷56.2] n.r. in 2 cases	12.5 [7.5÷21.8] n.r. in 3 cases $p_{1a–1b}$ = 0.041 Δ –2.3 times	10.9 [7.3÷14.8] n.r. in 6 cases	65.4 [41.3÷74.5] $p_{2a–2b}$ Δ +6.0 times	10.5 [10.3÷13.6]	13.3 [10.9÷21.9] n.r. in 2 cases $p_{3a–3b}$ = 0.285
ttPeak, min	6.5 [4.7÷7.2] n.r. in 2 cases	9.2 [8.3÷10.5] n.r. in 3 cases $p_{1a–1b}$ = 0.108	9.5 [7.9÷9.9] n.r. in 6 cases	9.5 [8.8÷9.6]	10.5 [10.2÷11.0]	10.8 [10.3÷11.1] n.r. in 2 cases $p_{3a–3b}$ = 0.922
V, nmol / min	9.4 [7.1÷25.9] n.r. in 2 cases	3.4 [2.0÷6.5] n.r. in 3 cases $p_{1a–1b}$ = 0.085	2.4 [1.6÷4.7] n.r. in 6 cases	7.8 [6.4÷11.8] $p_{2a–2b}$ Δ +3.3 times	2.3 [1.6÷3.0]	2.8 [2.3÷5.2] n.r. in 2 cases $p_{3a–3b}$ = 0.592

Note: in tables 1 and 2: PCC – prothrombin complex concentrate, FM – fibrin monomer, p – statistical significance of the differences in the compared parameters, Δ is the difference between the values, n.r. – not registered.

Table 2

The results of the hemostatic system evaluation in the experimental animals with direct thrombin inhibition, *Me* (Q_{25}÷Q_{75})

Parameters	Group 4 (Placebo)		Group 5 (PCC)		Group 6 (rFVII)		Group 7 (FM 0.25 mg / kg)	
	before placebo administration (4a)	after placebo administration (4b)	before FM administration (5a)	after PCC administration (5b)	before FM administration rFVII (6a)	after PCC administration rFVII (6b)	before FM administration (7a)	after PCC administration (7b)
Platelet count, ×10 ^{9} / l	541.5 [481.3÷553.0]	501.0 [421.5÷517.5] $p_{4a–4b}$ = 0.116	521.0 [458.0÷601.8]	432.5 [379.8÷501.5] $p_{5a–5b}$ = 0.050 Δ –17.0%	638.5 [535.3÷709.5]	638.0 [523.8÷717.5] $p_{6a–6b}$ = 0.972	559.0 [528.3÷566.5]	550.5 [499.8÷565.3] $p_{7a–7b}$ = 0.600
Echitoxic time, ratio	3.4 [3.1÷3.8]	3.5 [3.1÷4.1] $p_{4a–4b}$ = 0.333	2.1 [1.8÷2.5]	2.5 [2.0÷3.3] $p_{5a–5b}$ = 0.016 Δ +19.1%	2.5 [2.1÷3.7]	3.0 [2.2÷3.6] $p_{6a–6b}$ = 0.156	2.6 [2.2÷2.9]	2.5 [2.2÷2.7] $p_{7a–7b}$ = 0.600
Fibrinogen, g / l	2.9 [2.3÷4.2]	2.9 [2.4÷3.1] $p_{4a–4b}$ = 0.139	4.1 [3.4÷5.0]	3.6 [3.0÷4.7] $p_{5a–5b}$ = 0.033 Δ –12.2%	2.9 [2.4÷3.2]	2.8 [2.4÷3.4] $p_{6a–6b}$ = 0.969	3.2 [2.9÷3.5]	3.1 [2.9÷3.5] $p_{7a–7b}$ = 0.433
D-dimer, ng / ml	100.0 [100.0÷200.0]	100.0 [100.0÷200.0] $p_{4a–4b}$ = 0.480	200.0 [200.0÷300.0]	550.0 [400.0÷800.0] $p_{5a–5b}$ = 0.003 Δ 2.8 times	100.0 [100.0÷150.0]	800.0 [300.0÷1,100.0] $p_{6a–6b}$ = 0.005 Δ 8.0 times	100.0 [100.0÷200.0]	200.0 [100.0÷300.0] $p_{7a–7b}$ = 0.075
Thromboelastometry								
CT, sec	925.0 [619.0÷995.0]	713.0 [673.0÷853.0] $p_{4a–4b}$ = 0.515	1023.0 [776.0÷1404.8]	964.5 [768.5÷1087.8] $p_{5a–5b}$ = 0.037 Δ –5.7 %	675.5 [573.5÷996.8]	484.0 [274.0÷948.3] $p_{6a–6b}$ = 0.048 Δ –28.3%	660.0 [477.5÷907.0]	480.0 [348.3÷637.0] $p_{7a–7b}$ = 0.139
α angle, degree	59.0 [48.0÷66.0]	62.0 [60.0÷66.0] $p_{4a–4b}$ = 0.953	61.5 [54.3÷67.3] n.r. in 3 cases	57.0 [50.5÷62.0] n.r. in 2 cases $p_{5a–5b}$ =0.327	61.5 [56.3÷68.8]	70.5 [63.3÷75.0] $p_{6a–6b}$ = 0.059	64.5 [59.3÷75.3]	65.5 [59.8÷76.3] $p_{7a–7b}$ = 0.767
CFT, sec	169.0 [140.0–210.0]	151.0 [134.0–167.0] $p_{8a–8b}$ = 0.767	175.0 [143.0÷231.5] n.r. in 3 cases	231.5 [182.3÷425.8] n.r. in 1 case $p_{5a–5b}$ = 0.137	166.5 [112.0–196.0]	114.0 [84.0–147.8] $p_{6a–6b}$ = 0.035 Δ –31.5%	132.0 [82.5÷165.5]	150.0 [123.0÷165.0] $p_{7a–7b}$ = 0.859
MCF, mm	56.0 [54.0÷66.0]	62.0 [48.0÷66.0] p_{4a-4b} = 0.953	65.5 [58.3÷74.0] n.r. in 3 cases	69.5 [62.0÷70.8] n.r. in 3 cases $p_{5a–5b}$ = 0.889	59.5 [45.0÷64.8]	65.5 [56.0÷73.0] $p_{6a–6b}$ = 0.021 Δ +10.1%	63.0 [55.8÷67.8]	62.5 [58.0÷68.8] $p_{7a–7b}$ = 0.779
A10, mm	48.0 [47.0÷59.0]	52.0 [45.0÷60.0] $p_{4a–4b}$ = 0.374	57.5 [47.3÷65.8] n.r. in 2 cases	54.0 [48.0÷59.0] n.r. in 2 cases $p_{5a–5b}$ = 0.441	48.0 [39.8÷55.0]	56.0 [49.5÷65.8] $p_{6a–6b}$ = 0.034 Δ +16.6%	55.0 [48.0÷65.8]	54.0 [49.0÷67.0] $p_{7a–7b}$ = 0.889

Note: rFVII – recombinant factor VIIa.

CONCLUSION

The presented data revealed prothrombotic effects of the above hemostatic agents, namely, PCC and rFVIIa. The latter were tested in two experimental models with drug-induced hypocoagulation associated with warfarin or dabigatran. The effects were manifested through overcompensated changes in hemostatic system parameters (compared with intact animals), including an increase in D-dimer and a shift toward hypercoagulability according to thromboelastometry.

It should be noted that FM at a dose of 0.25 mg / kg resulted in significant reduction of blood loss without the above-described thrombogenic effects in the blood, which distinguished it from the known hemostatic agents. Therefore, we suppose that FM is safer in terms of adverse events, such as spontaneous thrombosis in the bloodstream.

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