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Experimental study of the systemic hemostatic effects of fibrin monomer in inhibition of platelet aggregation

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ABSTRACT

The aim of the study was to examine the hemostatic effects of fibrin monomer in post-traumatic parenchymal hemorrhage against pharmacologically conditioned inhibition of platelet aggregation function.

Materials and Methods. In the *in vivo* experiments in male rabbits, the hemostatic effects of fibrin monomer (FM) (0.25 mg/kg) were evaluated in comparison with tranexamic acid (TXA) (15 mg/kg) in post-traumatic parenchymal hemorrhage against the background of preliminary inhibition of platelet aggregation function with acetylsalicylic acid (2.0 mg/kg) and clopidogrel (8.0 mg/kg). Volume and rate of blood loss as well as the parameters of the hemostatic system were estimated.

Results. In comparison to placebo it has been established that FM when administered intravenously 1 hour before the injury can prevent severe bleeding associated with taking antiplatelets. The volume of blood loss after FM administration decreased by 6.0 times, the rate of blood loss reduced by 5.9 times, and when using TXA it was reduced by 2.4 ($P_{FM-TA}<0.02$) and 4.8 times, respectively. The hemostatic effects of TXA were realized when the hemostatic balance was shifted towards the increased fibrin formation (an increase in D-dimer plasma level). The use of FM was not accompanied by any significant changes in the blood coagulation system.

Conclusion. The fibrin monomer at a dose of 0.25 mg/kg IV is capable of preventing severe post-traumatic parenchymal bleeding caused by the combined use of drugs exhibiting different antiplatelet action. The phenomenon is not completely clear and needs to be analyzed in further research. The mechanism of hemostatic effects associated with FM is currently being studied.

Key words: fibrin monomer, acetylsalicylic acid, clopidogrel, tranexamic acid, hemostatic effect.

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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Conformity with the principles of ethics. The study was approved by the local Ethics Committee at Altai State Medical University (Protocol No. 12 of 12.11.2015). Animal studies were carried out in accordance with the Directive 86/609/EEC, the Declaration of Helsinki, and the "Rules of work with experimental animals".

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Системные гемостатические эффекты фибрин-мономера при ингибировании агрегационной функции тромбоцитов в эксперименте

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РЕЗЮМЕ

Цель. Изучить гемостатические эффекты фибрин-мономера при посттравматическом паренхиматозном кровотечении на фоне фармакологически обусловленного угнетения агрегационной функции тромбоцитов.

Материалы и методы. В экспериментах *in vivo* на кроликах-самцах оценивали гемостатические эффекты фибрин-мономера (ФМ) (0,25 мг/кг) в сравнении с транексамовой кислотой (ТК) (15 мг/кг) при посттравматическом паренхиматозном кровотечении на фоне предварительного угнетения агрегационной функции тромбоцитов ацетилсалициловой кислотой (2,0 мг/кг) и клопидогрелом (8,0 мг/кг). Оценивали объем и темп кровопотери, а также показатели системы гемостаза.

Результаты. Установлено, что ФМ в сравнении с плацебо при внутривенном введении за 1 час до травмы способен профилактировать тяжелое кровотечение, связанное с приемом антиагрегантов. Объем кровопотери после введения ФМ снижался по медиане в 6,0 раз, темп кровопотери – в 5,9 раза, при использовании ТК – в 2,4 ($p_{ФМ\text{-}ТА}<0.02$) и 4,8 раза, соответственно. Гемостатические эффекты ТК реализовывались при смещении гемостатического равновесия в сторону усиления фибринообразования (увеличение уровня D-димера в плазме крови). Применение ФМ не сопровождалось сколько-нибудь значимыми изменениями в системе свертывания крови.

Заключение. Фибрин-мономер в дозе 0,25 мг/кг, способен при в/в введении профилактировать тяжелое посттравматическое паренхиматозное кровотечение, вызываемое сочетанным приемом препаратов, обладающих различными механизмами антиагрегантного действия. Механизм гемостатических эффектов, связанных с ФМ, в настоящее время изучается.

Ключевые слова: фибрин-мономер, ацетилсалициловая кислота, клопидогрел, транексамовая кислота, гемостатический эффект.

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

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INTRODUCTION

Hemorrhagic syndrome can be life-threatening and may require emergency measures to preserve the patient's life and health in case of injuries, extensive surgery and iatrogenic exposure [1]. Moreover, reduction of bleeding associated with intensive drug-induced inhibition of platelet aggregation can be a serious problem for cardiac patients among others [2]. In this case, various therapeutic approaches are recommended to reduce blood loss, including donor platelet transfusion, the use of desmopressin (contributing to Von Willebrand factor expression by endotheliocytes) and/or administration of tranexamic acid that inhibits fibrinolytic reactions [3–5].

In the previous studies in our laboratory, when a controlled liver injury *in vivo* was inflicted, the phenomenon of significant hemostatic action of low-dose FM (des-AABB-fibrinogen) was found [6, 7]. This effect was also observed in intravascular thrombin inhibition induced by oral administration of dabigatran etexilate [8]. It should also be noted that in the above-mentioned experiments, FM was administered IV at a dose of 0.25 mg/kg that corresponds to its physiological blood plasma level of healthy people (less than 7.8 μg/ml) [9].

The aim of this study was to examine the hemostatic effects of fibrin monomer in post-traumatic parenchymal bleeding against pharmacologically conditioned inhibition of platelet aggregation function.

MATERIALS AND METHODS

The studies were performed on 49 healthy male rabbits of the Chinchilla breed weighing 3.0-4.5 kg, kept in standard vivarium conditions. Four groups of the animals were formed by block randomization (Fig. 1).

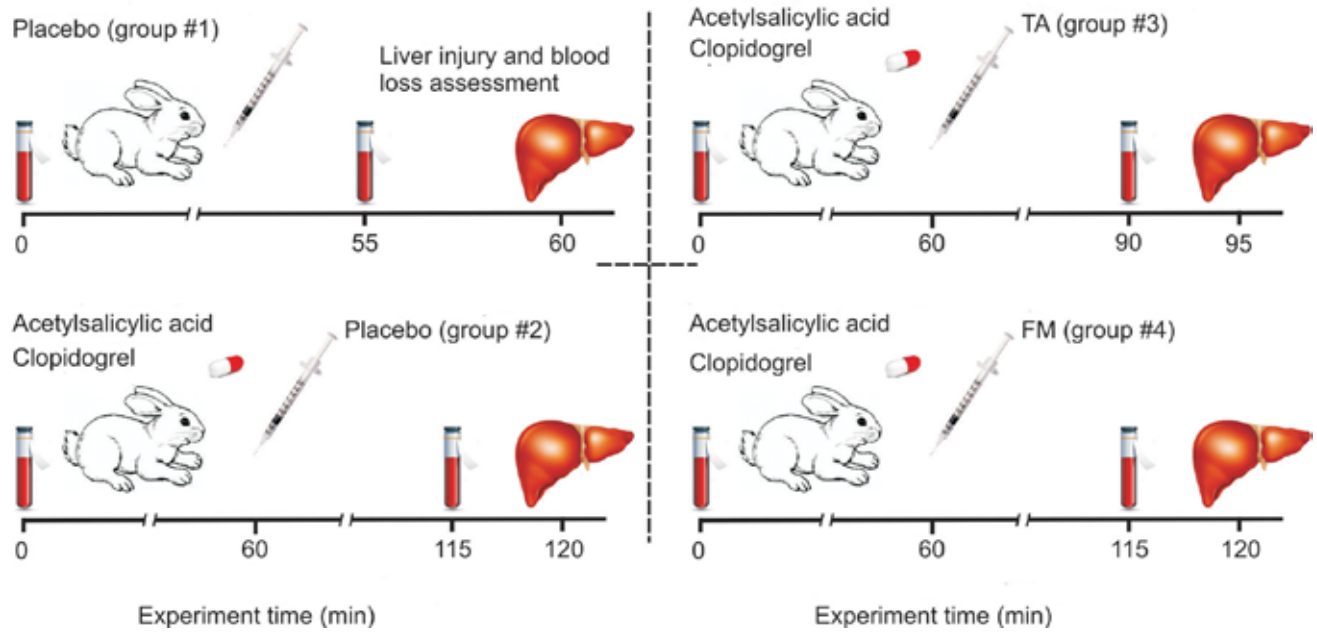

Fig. 1. Design of the study with a controlled liver injury.

Abbreviations and symbols: TXA – tranexamic acid; FM – fibrin monomer;

– blood sampling to assess hemostasis; – drug administration

The animals of group 1 (n = 11) were injected into the marginal ear vein with a HMD Cathy I. V. Cannulas (needle catheter Cathy (HMD Company)) with placebo (4.0 M urea solution corresponding to its concentration in the FM solution) in the volume of 0.5 ml. After one hour, a laparotomy was performed and a standard liver injury was inflicted in accordance with the available guidelines [10] under general anesthesia with Telazol (Zoetis, Spain). To suppress platelet aggregation at the beginning of the experiment, the animals of groups 2 to 4 were administered *per os* a mixture of acetylsalicylic acid (Thrombo ACC®, Lannacher Heilmittel GmbH, Austria) at a dose of 2.0 mg/kg and clopidogrel (Plavix®, Sanofi Winthrop Industrie, France) at a dose of 8.0 mg/kg dissolved in water.

An hour after taking these antiplatelets, animals were injected intravenously with solutions of the following drugs: in group 2 (n = 10) – placebo, in group 3 (n = 18) – tranexamic acid (Tranexam®, FSUE Moscow endocrine plant, Russia) at a dose of 15 mg/kg, in group 4 (n = 10) – FM at a dose of 0.25 mg/kg. Used in the experiment FM was obtained by previously registered technology [11]. A standard liver injury was performed under general anesthesia one hour after administration of placebo and FM to the animals of groups 2 and 4, and after 30 minutes, to the animals of group 3. After inflicting an injury, the nature of parenchymal bleeding was evaluated by means of gauze wipes using such criteria as the volume of blood loss as a percentage of the estimated circulating blood volume (% CBV) taking into account animal body weight, and the rate of blood loss per unit of time (mg/s) [10]. To evaluate the hemostasis system, blood was obtained by making an incision of the marginal ear vein (gravity flow) twice before drug administration and liver injury (Fig. 1). Blood was placed into the tubes with the appropriate stabilizers: 0.25 ml was placed into tubes with potassium salt of ethylenediaminetetraacetic acid (AQUISEL® K3E/EDTA 3K, Aquisel S. L. Company, Spain) to count the platelet number blood, and 5.0 ml was placed into polystyrene graduated centrifuge tubes with polyvinylchloride caps containing 0.11 M (3.8 %) sodium citrate solution (blood-stabilizer ratio 9:1) to study other parameters.

Platelet-rich plasma and platelet-poor plasma were obtained according to the common method.

The study of hemocoagulation involved the assessment of the number of platelets in venous blood and their aggregation induced by adenosine diphosphate of disodium salt (ADP), taken at the concentration of 10 μM), activated partial thromboplastin time (APTT) and prothrombin time (PT), as well as fibrinogen concentration and the D-dimer level. The results of APTT and PT assessment were presented in the form of a ratio calculated by the formula: Ratio = $CT_{experiment}/CT_{control}$, where: Ratio is a ratio; $CT_{experiment}$ is coagulation time in experimental plasma (s); $CT_{control}$ is coagulation time in control plasma (s). The platelet number was determined using the hematology analyzer Drew-3 (Drew Scientific Inc., England). Platelet aggregation function was evaluated using the Chrono-Log 490-2D aggregometer (CHRONO-LOG Corporation, USA), the coagulometric parameters were defined with Thrombostat 2coagulation analyzer (Behnk Electronik, Germany) using reagent kits by the company "Technologia Standart" (Russia), the D-dimer level was measured with the analyzer-reflectometer NycoCard Rader II (Axis-Shield PoC AS, Norway) and the test system NycoCard® D-Dimer (Axis-Shield PoC AS).

Distribution of the characteristics was evaluated by Shapiro-Wilk test, the group differences depending on distribution were assessed by Student's t-test, Mann – Whitney U-test, Fisher's exact test, the correlation was evaluated by Spearman's rank correlation coefficient (rS). The differences were considered statistically significant at p J 0.05. The results were processed by MedCalc Version 17.9.7 (license BU556-P12UT-BBS55-YAH5M-UBE51). The data are presented as median (Me), 25th and 75th percentiles Me [Q_{25}÷Q_{75}]).

RESULTS

The study established a high mortality rate of the animals in group 2 (4 out of 10), associated with cardiorespiratory arrest against the background of ongoing bleeding. In contrast, in the other groups no mortality was observed (in groups 1 and 4; $p_{1\text{-}2}$ = 0.035; $p_{2\text{-}4}$ = 0.035) or it was lower as in group 3 (3 out of 18), $p_{2\text{-}3}$ = 0.208).

The mortality of the animals in group 2 was comparable with the severity of blood loss (Fig. 2).

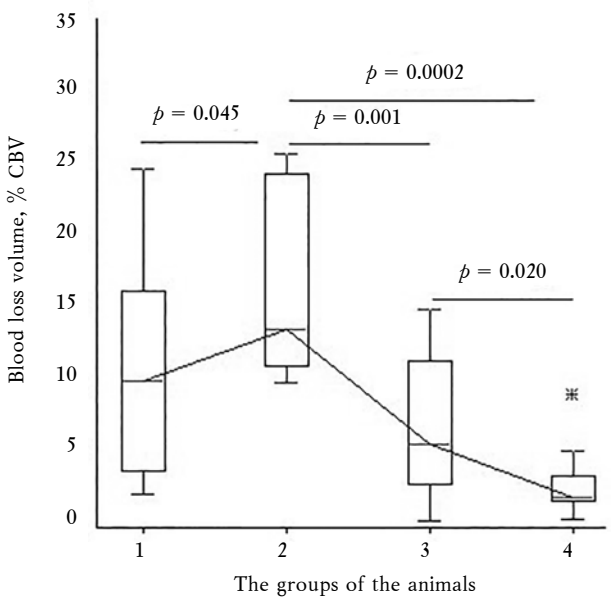

Fig. 2. The parameters of blood loss in the experimental groups: the values are presented as median (*Me*), the horizontal line inside the rectangle; and as the values corresponding to the 2.5th and 97.5th percentiles, the lower and upper vertical bars.

The volume of blood loss in the group of the animals treated with antiplatelets and placebo (group 2) was 1.4 times higher (13.6 [11.3 ÷ 22.0] % of CBV) compared to the same rate in the group of the animals treated with placebo only (group 1) (10.1 [4.1 ÷ 13,5] % CBV). However, the rate of blood loss in the animals in these groups did not differ. These observations indicate the reproduction of hemorrhagic syndrome caused by double antiplatelet therapy in group 2.

It was further found that the volume of blood loss in the groups of the animals pre-treated with antiplatelets after intravenous administration of TXA (group 3) (5.7 [3.1 ÷ 10.6] % CBV) and FM (group 4) (2.0 [1.8 ÷ 3.2] % CBV) was 2.4 times and 6.0 times less, respectively, in comparison with group 2 (placebo). Similar patterns were observed when assessing the rate of blood loss that decreased by 4.8 times after TXA administration (group 3) (6.2 [4.5 ÷ 8.3] mg/s) and by 5.9 times after FM administration (group 4) (5.0 [4.3 ÷ 6.8] mg/s) compared to the placebo group (group 2) (29.9 [11.9 ÷ 34.3] mg/s), table.

Table

Hemostatic characteristics in the experimental groups against the background of antiplatelets, hemostatic agents, and placebo *Me* [Q_{25}÷Q_{75}]

Parameter	Group 1		Group 2		Group 3		Group 4	
	before (1a)	after (1б)	before (2a)	after (2б)	before (3a)	after (3б)	before (4a)	after (4б)
Platelet count, $\times 10^9$/л	477.5 [405.8÷621.5]	480.5 [412.3÷555.0] $p_{1a-1б}$= 0.151	430.5 [413.3÷479.8]	475.0 [438.5÷515.3] $p_{2a-2б}$= 0.121	429.5 [373.0÷504.8]	408.0 [347.3÷477.5] $p_{3a-3б}$= 0.102	475.5 [468.0÷561.0]	460.0 [411.5÷532.8] $p_{a-4б}$ = 0.312
ADP-induced aggregation, %	20.5 [19.0÷28.7]	22.0 [19.2÷31.4] $p_{1a-1б}$= 0.598	18.4 [17.0÷19.7]	1.3 [0.4÷6.4] $\Delta_{2a-2б}$ -14 раз (times) $p_{2a-2б}$= 0.005	20.8 [17.4÷37.6]	6.8 [4.0÷9.8] $\Delta_{3a-3б}$ – 3 раза (times) $p_{3a-3б}$= 0.0003	19.8 [11.7÷20.8]	1.1 [1.0÷1.9] $\Delta_{4a-4б}$ – 18 раз (times) $p_{4a-4б}$ = 0.00007
APTT, ratio	1.1 [1.0÷1.2]	1.1 [0.9÷1.2] $p_{1a-1б}$= 0.248	1.1 [0.9÷1.1]	1.0 [0.9÷1.1] $p_{2a-2б}$= 0.110	0.9 [0.8÷1.1]	0.9 [0.8÷1.0] $p_{3a-3б}$= 0.124	1.0 [0.9÷1.0]	0.9 [0.9÷1.0] $p_{4a-4б}$ = 0.614
PT, ratio	1.1 [0.6÷1.6]	0.9 [0.9÷1.3] $p_{1a-1б}$= 0.476	1.1 [1.0÷1.2]	1.1 [1.0÷1.1] $p_{2a-2б}$= 0.645	1.0 [0.8÷1.3]	0.9 [0.8÷1.4] $p_{3a-3б}$= 0.458	1.1 [1.0÷1.1]	1.0 [1.0÷1.1] $p_{4a-4б}$ = 0.251
Fibrinogen, g/l	3.3 [2.8÷4.4]	3.7 [2.8÷4.5] $p_{1a-1б}$= 0.811	3.3 [3.0÷3.5]	3.4 [3.2÷3.8] $p_{2a-2б}$= 0.758	3.5 [2.9÷3.9]	3.2 [3.0÷3.8] $p_{3a-3б}$= 0.753	3.5 [3.2÷4.1]	3.4 [3.0÷4.1] $p_{4a-4б}$ = 0.872
D-dimer, нг/мл	100.0 [100.0÷100.0]	100.0 [100.0÷200.0] $p_{1a-1б}$= 0.205	100.0 [100.0÷175.0]	100.0 [100.0÷200.0] $p_{2a-2б}$= 0.180	300.0 [200.0÷400.0]	1000.0 [525.0÷1350.0] $\Delta_{3a-3б}$ +3.3 times $p_{3a-3б}$=***0.010***	150.0 [100.0÷275.0]	175.0 [100.0÷300.0] $p_{4a-4б}$ = 0.463

Notes: p – the achieved level of statistical significance of the differences in the compared values; ADP – adenosine diphosphate; APTT – activated partial thromboplastin time; PT – prothrombin time; Δ – the difference of values.

A positive relationship between the volume and the rate of blood loss was determined in the animals treated with both TXA (group 3) $rS = 0.86$ ($p = 0.002$) and FM (group 4) rS = 0.79 ($p = 0.006$), that was absent in placebo groups (1 and 2).

Along with the assessment of blood loss, the rates of ADP-induced platelet aggregation and coagulogram were studied in order to visualize drug-induced thrombocytopathy and to comparatively assess the effects of TXA and FM (Table).

In the animals of groups 2, 3 and 4, inhibition of ADP-induced platelet aggregation function (in %) was reported to be inhibited by 3-18 times against the background of the combined use of antiplatelets, without any change in the platelet number, as well as without shifts in the chronometric values of the coagulogram and fibrinogen concentration.

DISCUSSION

The results obtained in the experiment indicate that the noticeable hemostatic effects of TXA were realized against the background of more than 3-fold (median) increase in the D-dimer plasma level with persistent pharmacologically conditioned thrombocytopathy which is considered to be an evidence of a shift in hemostatic balance towards increased fibrin formation. At the same time, the use of FM in platelet function inhibition was not accompanied by any significant coagulation activation which, however, is in contradiction with the observed systemic hemostatic effects (in terms of volume and rate of blood loss).

As it is known, acetylsalicylic acid irreversibly inhibits platelet cyclooxygenase-1, followed by a decrease in the formation of thromboxane A_2. Clopidogrel, in turn, is a prodrug and turns into its active form as an antagonist of $P2Y_{12}$-platelet receptors through metabolism in the liver [12]. When FM was used, correction of platelet function, reduced under the antiplatelet action, was not reported which currently does not allow to explain the mechanism of noted hemostatic effects associated with FM.

CONCLUSION

Fibrin-monomer (des-AABB-fibrinogen) administered intravenously at a dose of 0.25 mg/kg s able to prevent severe posttraumatic parenchymal bleeding caused by combined use of drugs with different mechanisms of antiplatelet action. The nature of this phenomenon is not quite clear, and more research is required.

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Authors contribution

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