MORPHOLOGICAL DETERMINANTS FOR THE LOCAL HEMOSTATIC EFFECT OF EXOGENOUS FIBRIN MONOMER IN ITS SYSTEMIC ADMINISTRATION AFTER INJURY WITH INHIBITION OF PLATELET AGGREGATION IN THE EXPERIMENT

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ABSTRACT

Background. *In our previously published studies, we demonstrated a high hemostatic activity of a low dose of exogenous fibrin monomer during its systemic administration in a model of dosed liver injury with preliminary inhibition of platelet aggregation. However, the analysis of platelet involvement in the mechanisms of local fibrin formation has not been analyzed.*

The aim of the study. *To conduct a comparative analysis of the cellular composition of venous and wound blood, as well as blood in the wound vessels to assess the contribution of platelets to the hemostatic effect of exogenously administered fibrin monomers in dosed liver injury under conditions of pharmacologically determined thrombocytopathy.*

Methods. *In a model of dosed liver injury in rabbits after inhibition of platelet aggregation by acetylsalicylic acid in combination with clopidogrel, the effect of the administration of fibrin monomer was evaluated in comparison with the use of tranexamic acid. We studied the number of platelets in venous and wound blood smears, as well as in the contents of wound vessels.*

Results. *It has been established that with the systemic administration of exogenous fibrin monomer, the number of platelets in wound blood smears decreases by 17.2 % in comparison with free circulating venous blood. Platelets in wound blood form aggregates and are in an activated state. In the wound vessels, the number of these cells was maximum (150 per lower field) compared with the number of platelets in the placebo and tranexamic acid groups (55 and 84 per lower field, respectively). Also in the wound blood, erythrocytes with altered forms (echinocytes, schistocytes, stomatocytes and ovalocytes) were found.*

Conclusion. *Systemic administration of exogenous fibrin monomer affects the redistribution of platelets between the systemic circulation, wound vessels and wound blood, determining its hemostatic effect and local wound fibrin formation in dosed liver injury. The presence of receptor-mediated platelets recruitment due to fibrin monomer in the wound vessels with the participation of damaged erythrocytes is assumed.*

Key words: *fibrin monomer, acetylsalicylic acid, clopidogrel, tranexamic acid, platelets, hemorrhage control*

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МОРФОЛОГИЧЕСКИЕ ПРЕДПОСЫЛКИ ЛОКАЛЬНОГО ГЕМОСТАТИЧЕСКОГО ЭФФЕКТА ЭКЗОГЕННОГО ФИБРИН-МОНОМЕРА ПРИ ЕГО СИСТЕМНОМ ВВЕДЕНИИ ПОСЛЕ ТРАВМЫ ПРИ ПОДАВЛЕНИИ АГРЕГАЦИОННОЙ ФУНКЦИИ ТРОМБОЦИТОВ В ЭКСПЕРИМЕНТЕ

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РЕЗЮМЕ

Обоснование. *В опубликованных нами ранее исследованиях была продемонстрирована высокая гемостатическая активность низкой дозы экзогенного фибрин-мономера (ФМ) при его системном введении на модели дозированной травмы печени при предварительном угнетении агрегационной функции тромбоцитов. Однако анализ участия тромбоцитов в механизмах локального фибринообразования не анализировался.*

Цель исследования. *Провести сравнительный анализ клеточного состава венозной и раневой крови, а также крови в прираневых сосудах для оценки вклада тромбоцитов в обеспечение гемостатического эффекта экзогенно введённого ФМ при дозированной травме печени в условиях фармакологически обусловленной тромбоцитопатии.*

Методы. *На модели дозированной травмы печени у кроликов после угнетения агрегационной функции тромбоцитов ацетилсалициловой кислотой в сочетании с клопидогрелом оценивали влияние введения ФМ в сравнении с применением транексамовой кислоты (ТК). Изучали количество тромбоцитов в мазках венозной и раневой крови, а также в содержимом прираневых сосудов.*

Результаты. *Установлено, что при системном введении экзогенного ФМ количество тромбоцитов в мазках, полученных из раневой крови, снижается на 17,2 % в сравнении со свободно циркулирующей венозной кровью. Тромбоциты в раневой крови образуют агрегаты и находятся в активированном состоянии. В прираневых сосудах количество этих клеток было максимальным (150 в поле зрения) по сравнению с числом этих клеток в группах плацебо и с применением ТК (55 и 84 в поле зрения соответственно). Также в раневой крови встречались эритроциты с изменёнными формами – эхиноциты, шизоциты, стоматоциты и овалоциты.*

Заключение. *Системное введение экзогенного ФМ влияет на перераспределение тромбоцитов между системным кровотоком, прираневыми сосудами и раневой кровью, определяя его гемостатический эффект и локальное прираневое фибринообразование при дозированной травме печени. Предполагается наличие рецепторно-опосредованного привлечения тромбоцитов за счёт ФМ в прираневых сосудах с участием повреждённых эритроцитов.*

Ключевые слова: *фибрин-мономер, ацетилсалициловая кислота, клопидогрел, транексамовая кислота, тромбоциты, остановка кровотечения*

Для цитирования: Вдовин В.М., Сузопов Е.В., Шахматов И.И., Бобров И.П., Орехов Д.А., Долгатов А.Ю., Момот А.П. Морфологические предпосылки локального гемостатического эффекта экзогенного фибрин-мономера при его системном введении после травмы при подавлении агрегационной функции тромбоцитов в эксперименте. *Acta biomedica scientifica*. 2023; 8(4): 155-164. doi: 10.29413/ABS.2023-8.4.17

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OBJECTIVES

The concept of the hemostasis system as a multicomponent cascade reaction aimed at the formation of fibrin strands with the participation of coagulation factors, endothelial cells and formed elements of blood has undergone certain changes in recent years. First of all, it concerns the regulation of this complex process.

Modern approaches to the study of blood coagulation regulation are based on studies of spatial thrombogenesis *in vitro* [1], the "cellular theory" of M. Hoffman and D.M. Monroe [2–4], within the framework of which new data obtained by analyzing the effect of one of the fibrinogen derivatives, des-AABB-fibrinogen (fibrin monomer (FM)) – on the hemostasis system under physiological conditions, as well as with pharmacologically determined suppression of blood coagulation reactions. Previous studies carried out in our laboratory have shown the presence of unique hemostatic and hemostasiological effects with systemic administration of FM without activation of blood coagulation reactions and pathological thrombosis [5, 6].

From our point of view, the detected regulatory effects of FM are realized outside the set of coagulation reactions leading to the thrombin formation, but preceding fibrin formation. It is indicated by studies conducted under conditions of mediated inhibition of thrombin by unfractionated heparin [7, 8], as well as with direct suppression by dabigatran [9]. In both experimental models, FM demonstrated the effect of minimizing post-traumatic blood loss. At the same time, it contributed to local fibrin formation without correction of hypocoagulation caused by the administration of anticoagulants.

Based on the results presented above, our research group suggested that the mechanisms of the hemostatic effect of FM are realized with the participation of blood cells, primarily platelets, despite the pharmacological suppression of their functional activity. The latter is indicated by the results of studies conducted using dual antiplatelet therapy and correction of post-traumatic bleeding with tranexamic acid (TA) or FM [10]. In this work, a high local hemostatic activity of FM comparable to the effects of TA with a concomitant 16-fold decrease in the intensity of ADP-induced platelet aggregation compared with control values was demonstrated. In the same series of experiments, the authors carried out morphological analysis of tissues in the area of injury, where high activity of wound fibrin formation was revealed [11]. In the above materials, no sufficient attention was paid to assessment of the regularity of platelet redistribution between venous and wound blood, which seems necessary for a possible decoding of the mechanisms of the hemostatic effect of FM *in vivo*.

THE AIM OF THE STUDY

To conduct a comparative analysis of the cellular composition of venous and wound blood, as well as blood in the wound vessels to assess the contribution of platelets to the hemostatic effect of exogenously administered fibrin monomers in dosed liver injury under conditions of pharmacologically determined thrombocytopathy.

METHODS

The study included 48 healthy mature male Chinchilla rabbits weighing 3.0–4.5 kg. Animal experiments were carried out in accordance with the European Convention and Directives for the Protection of Vertebrates Animals used in the experiment 86/609/EEC, as well as the World Medical Association Declaration of Helsinki and the "Regulations for Animal Use in Biomedical Research". The work was approved by the local Ethics Committee of the Altai State Medical University (Protocol No. 12 dated November 12, 2015).

To suppress platelet aggregation function, a mixture of acetylsalicylic acid (Thrombo ACC®; Lannacher Heilmittel GmbH, Austria) at a dose of 2.0 mg/kg and clopidogrel (Plavix®; Sanofi Winthrop Industry, France) at a dose of 8.0 mg/kg dissolved in water was administered to all animals *per os*. As is known, acetylsalicylic acid causes inhibition of platelet cyclooxygenase-1 and a subsequent decrease in the production of A_2 thromboxane. Clopidogrel is a prodrug, which is transformed into its active form through metabolism in the liver and acts as an antagonist of $P2U_{12}$ platelet receptors [12].

Next, all the animals were divided into three groups using the random number method. In 1 hour after taking these antiplatelet drug, animals were injected intravenously (IV) into the marginal vein of the ear with aqueous solutions of the following drugs: group No. 1 (n = 13) – placebo solution (3.75 M urea solution corresponding to its concentration in FM solution) with a volume of 0.5 ml; group No. 2 (n = 22) – tranexamic acid solution (Tranexam®; Moscow Endocrine Plant, Russia) at a dose of 15 mg/kg; group No. 3 (n = 13) – FM at a dose of 0.25 mg/kg (Technologiya-Standart LLC, Russia). All animals underwent standard liver injury under general anesthesia with Telazol (Zoetis, Russia; 10 mg/kg IV) in accordance with available recommendations: groups No. 1 and No. 3 – 1 hour after administration of placebo and FM, animals of group No.2 – 30 minutes after administration [13]. As it was shown earlier, FM showed its maximum effects when administered 1 hour before injury [6]. According to the manufacturer, with a high risk of bleeding, tranexamic acid is injected into the systemic circulation 20-30 minutes before the intervention (Instructions for use, ЛСР-001709/07).

To determine the morphology of erythrocytes, the number and aggregation function of platelets in animals, blood was obtained after incision of the marginal vein of the ear (by gravity) twice – before administration of hemostatic drugs or placebo and before liver injury. To assess the aggregation function of platelets, blood was placed in plastic tubes containing 0.11 M (3.8 %) sodium citrate solution (blood-stabilizer ratio 9:1). The production of platelet-rich blood plasma was carried out ac-

cording to a generally accepted method. Platelet aggregation in it was evaluated using a Chronolog 490-2D aggregometer (CHRONO-LOG Corporation, USA) when using adenosine diphosphate (ADP) aggregation agonist at an initial concentration of 10 μm.

To count the number of platelets and evaluate the morphology of erythrocytes, smears of blood from the marginal vein of the ear and blood from the wound surface 3–5 minutes after injury were obtained. The smears were stained according to Romanovsky–Giemse followed by oil immersion microscopy on a Nikon Eclipse E-200/F binocular microscope with a Nikon Digital Sight 1000 camera (Nikon, Japan) at a total optical magnification of ×1000. The number of platelets by Phonio ($\times 10^9$/L), their morphological characteristics (shape, presence of granules and inclusions), as well as the presence of aggregates of these cells in the smears were assessed. Along with this, the morphology of erythrocytes (size and shape) was evaluated.

After spontaneous cessation of post-traumatic bleeding, liver tissue, including its wound part and a fragment of an intact surface, was taken as part of histological studies, followed by fixation in a 10 % solution of neutral formalin according to Lilly. Material histologic processing was carried out using isopropyl alcohol using a TISSUE-TEK VI PTM6 carousel-type tissue processor (Sakkura, Japan). Paraffinization was carried out using the TISSUE-TEK TEC 5 embedding console (Sakkura, Japan). Histological sections 4–5 μm were obtained using a semi-automatic rotary microtome Accu-Cut SRM (Sakkura, Japan), the preparations were stained with hematoxylin and eosin in a TISSUE-TEK Prisma slide stainer with film encapsulation in TISSUE-TEK Film machine (Sakkura, Japan). The count of platelets (the number in the field of view) was carried out on microslides, including large vessels (venous or arterial), in five fields of view at magnification × 1000, under oil immersion, followed by calculation of the average number of cells in the fields of view. Microphotography was performed using Leica DM 750 E200 microscope with Leica EC3 digital video camera (Leica Microsystems CMS GmbH, Germany). The image analysis was performed using the "Image Tool 3.0" software.

The distribution of features in the samples was evaluated according to the Shapiro – Wilk test. Depending on the distribution of features, the Student's t-test and Mann – Whitney U-test or Wilcoxon test were used. Differences in the mortality rate of animals in the groups were established using the Fisher's exact test. The differences were considered statistically significant at $p < 0.05$. The experimental data were processed using the statistical software MedCalc 17.9.7 (license BU556-P12UT-BBS55-YAH5M-UBE51). The data obtained are presented in the form of median (Me), 25th and 75th percentiles (Q): Me [$Q_{25} \div Q_{75}$].

RESULTS

The use of dual antiplatelet therapy in animals led to an expected decrease in the intensity of ADP-induced aggregation of venous blood platelets in study groups No. 1, No. 2 and No. 3 by 4.5, 3.0 and 16.6 times, respectively (Table 1).

At the same time, in the control group (group No. 1), the number of platelets in the systemic bloodstream after administration of antiplatelet agents and placebo decreased moderately (by 19.8 %) compared with the baseline value (Table 2).

These cells were usually in an inactive state, their aggregates were absent (Fig. 1a). In rare cases, there were single activated platelets (up to 1–3 in the field of view). The appearance of erythrocytes in the systemic circulation was featureless. The number of platelets in wound blood was 32.8 % less than in circulating venous blood before traumatic exposure, and 46.0 % less than the same indicator before the introduction of antiplatelet agents. In the wound blood, platelets were visualized in an activated, partially aggregated state. The granules of the blood plates were tightly "condensed" (tightly fitting to each other), which also indicated the activation of these cells. The number of platelets in the aggregates varied from 6 to 10 cells (Fig. 1b). During the assessment of the morphology of erythrocytes in wound blood

TABLE 1

INDICATORS OF PLATELET AGGREGATION IN THE EXPERIMENTAL ANIMAL GROUPS, ME [Q25÷Q75]

Indicators	Group No. 1 Antiplatelet agents and placebo		Group No. 2 Antiplatelet agents and TA		Group No. 3 Antiplatelet agents and FM	
	before administration (1a)	after administration (1b)	before administration (2a)	after administration (2b)	before administration (3a)	after administration (3b)
ADP-induced platelet aggregation, %	20.1 [18.4÷45.9]	4.5 [0.6÷7.0] **$p_{1a-1b} = 0.001$; $\Delta_{1a-1b} = -4.5$ times**	24.0 [19.0÷46.5]	8.0 [4.9÷10.1] **$p_{2a-2b} < 0.001$; $\Delta_{2a-2b} = -3.0$ times**	19.9 [13.3÷20.1]	1.2 [1.0÷2.0] **$p_{3a-3b} = 0.001$; $\Delta_{3a-3b} = -16.6$ times**

Note. p is the level of statistical significance of the differences in the compared indicators; Δ is the difference in indicators.

the presence of echinocytes up to 5–8 pieces (in the field of view), single schizocytes, stomatocytes and ovalocytes were noted. Histological analysis of the liver parenchyma revealed that the count of platelets was about 55 cells in the field of view in the vessels next to the wound surface.

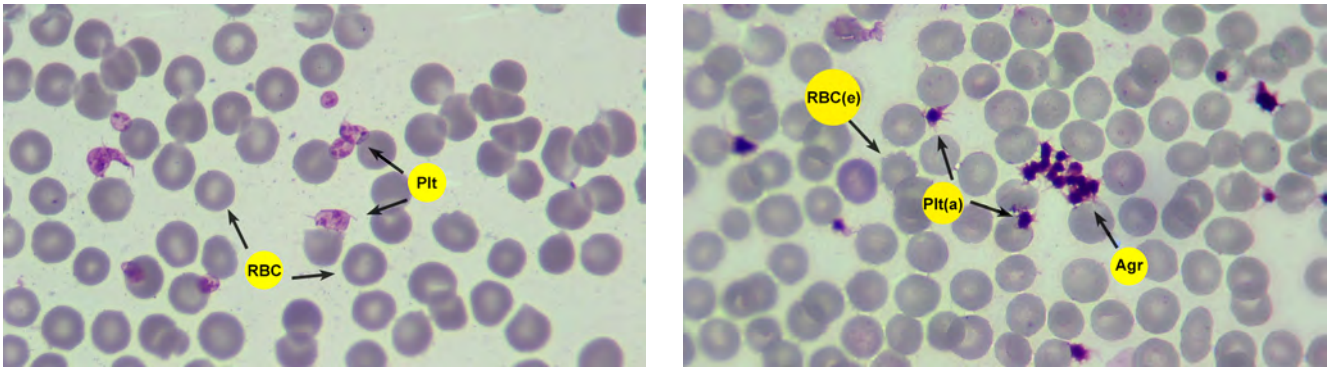
In the group of animals that received a fibrinolysis inhibitor along with antiplatelet agents (group No. 2), the number of platelets in the systemic bloodstream remained stable both before and after TA (Table 2). Platelets under microscopy were predominantly in an inactive state, without visible aggregation (Fig. 2a). The appearance of erythrocytes also corresponded to the physiological norm, although there were single echinocytes in the field of view. In wound blood, the number of platelets did not significantly differ from the count of blood plates in circulating venous blood taken before the injury. During visualization, activated platelets were noted in the wound blood, and their granules were densely "condensed". Along with this, platelet aggregates (2–20 cells) were detected (Fig. 2b). Chang-

TABLE 2

INDICATORS OF PLATELET COUNT IN SYSTEMIC BLOOD, INJURY BLOOD AND IN THE VESSELS NEAR THE WOUND SURFACE, ME [Q25÷Q75]

Groups	Platelet count in venous blood (smear)		Platelet count in wound blood (smear), count/FOV (c)	Platelet count in large vessels near the wound (morphometry on the section), count/FOV (d)
	before the administration of drugs, count/FOV (a)	after the administration of drugs, count/FOV (b)		
Group No. 1 Antiplatelet agents and placebo	605.0 [571.3÷648.5]	485.0 [436.0÷540.0] **p_{1a-1b} = 0.028; Δ = –19.8 %**	327.0 [273.0÷419.5] **p_{1b-1c} = 0.008; Δ = –32.6 %** **p_{1a-1c} = 0.028; Δ = –46.0 %**	55.0 [50.8÷60.0]
Group No. 3 Antiplatelet agents and tranexamic acid	574.5 [531.3÷648.5]	597.0 [451.8÷708.5] p_{2a-2b} = 0.754 p_{b-2b} = 0.109	602.0 [403.0÷841.5] p_{2b-2c} = 0.695 p_{2a-2c} = 0.388	84.0 [82.0÷89.5] **p_{1g-2g} = 0.0003; Δ = +52.7 %**
Group No. 3 Antiplatelet agents and fibrin monomer	514.0 [477.8÷560.5]	530.0 [470.5÷546.0] p_{3a-3b} = 0.756 p_{1b-3b} = 0.403	439.0 [427.0÷443.0] **p_{3b-3c} = 0.021; Δ = –17.2 %** **p_{3a-3c} = 0.028; Δ = –13.8 %**	150.0 [113.5÷201.0] **p_{1d-3d} = 0.001; Δ = +172.7 %** **p_{2d-3d} = 0.029; Δ = +78.5 %**

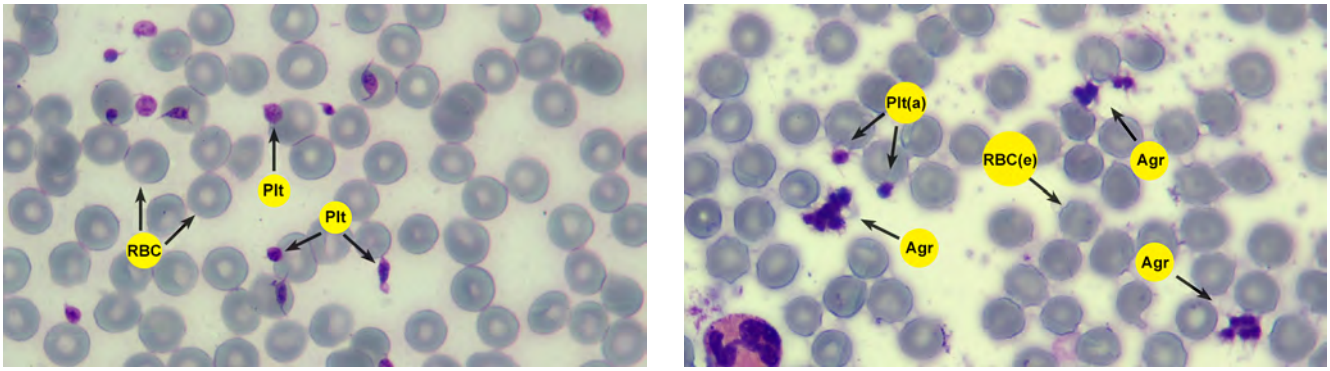
Note. *p* is the level of statistical significance of the differences in the compared indicators; FOV is the field of view; Δ is the difference in indicators.



a ***b***

FIG. 1.

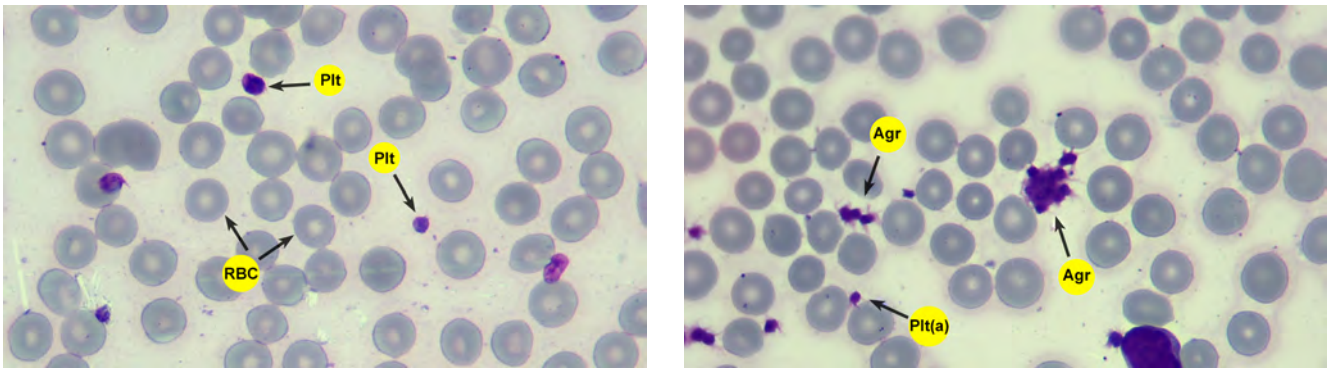
Visualization of rabbit blood from group No. 1 (antiplatelets and placebo): ***a*** *– systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells);* ***b*** *– injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets; RBC(e) – lysed red blood cell (echinocyte)). Hereinafter: Romanovsky – Giemsa staining; light microscopy, magnification × 100*



a *b*

FIG. 2.

*Visualization of rabbit blood from group No. 2 (antiplatelets and tranexamic acid): **a** – systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells); **b** – injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets; RBC(e) – lysed red blood cell (echinocyte))*



a *b*

FIG. 3.

*Visualization of rabbit blood from group No. 3 (antiplatelets and FM): **a** – systemic blood smear after drug administration (non-lysed red blood cells and platelets: Plt – non-activated platelets; RBC – normal red blood cells); **b** – injury blood smear (modified cell morphology: Agr – platelet aggregate; Plt(a) – activated platelets)..*

es in the morphology of erythrocytes concerned the formation of a large number of echinocytes (25–30 cells in the field of view). Morphometry of liver tissue revealed that in the vessels next to the wound surface, the count of platelets was about 84 cells in the field of view.

In the group of animals that received exogenous fibrin monomer along with antiplatelet agents (group No. 3), the number of platelets in the systemic bloodstream remained unchanged regardless of pharmacological effects (Table 2). They were mostly in an inactive state, and aggregates of blood plates were not observed (Fig. 3a). The appearance of erythrocytes in the systemic bloodstream practically did not differ from the physiological norm, although 10–15 echinocytes per the field of vision were found among erythrocytes. The number of platelets in the systemic circulation was 17.2 % higher than in wound blood. Activated and aggregated platelets with densely "condensed" granules were also found there. The number of platelets in the aggregates varied from 5 to 9 cells (Fig. 3b). Single echinocytes, ovalocytes and schistocytes (up to 3 in the field of view) were found among erythrocytes. During microscopy of histological preparations about 150 platelets were noted inside the wound vessels in the field of view.

DISCUSSION

As a result of the study, we reproduced a model of coagulopathy caused by oral administration of two antiplatelet agents with different mechanisms of action. It was confirmed by a decrease in ADP-induced platelet aggregation in animals in all experimental groups.

After the use of antiplatelet agents, the number of platelets in the systemic venous bloodstream in animals treated with TA or FM did not change. On the contrary, in the control group (without the use of systemic hemostatics), the number of blood plates in the systemic circulation decreased statistically significantly from the initial level, which corresponds to the data obtained in the work of O.D. Ostroumova et al. [14]. Moreover, platelets of the systemic blood flow in all groups were in an inactive state, the formation of aggregates in the smears was not noted.

An important aspect necessary for studying the mechanisms of local hemostasis when using FM, in our opinion, was the study of the cellular composition of wound blood and wound vessels. It was determined that in the TA group, the number of platelets in wound blood remained unchanged, compared with the number of these cells in the systemic circulation. On the contrary, in the placebo and FM groups, wound blood contained fewer blood plates compared to systemic blood flow. Wound blood platelets in all the observed groups were in an activated state, with densely "condensed" granules, and formed aggregates, which is natural for traumatic effects. As is known, the process of convergence of granules (condensation) of platelets leads to a change in the shape of platelets, flattening of their cytoplasm and signals readiness for thrombus formation [15]. A relatively higher number of cells in aggregates were observed in the group with TA.

From our point of view, the number of platelets in the wound vessels during histological examination of microslides of the wound surface of the liver may reflect the degree of involvement of cells from the general circulation to the injury area. The smallest number of blood plates in these vessels was observed in the control group, while their maximum value was recorded in the FM group – 2.7 times.

On the other hand, a decrease in the number of platelets in wound blood may be explained by their involvement in the process of local thrombosis. To explain this phenomenon, we can refer to the results of our previous studies [11]. Thus, in the placebo and antiplatelet agents groups, we observed a low-intensity formation of thrombotic masses in the area of injury, consisting of fibrin strands and unchanged erythrocytes. On the contrary, the use of TA and FM against the background of pharmacologically induced thrombocytopathy led to an increase in the thickness of thrombotic wound deposits (with a maximum value in the group with FM) and fibrin strands in their composition.

Thus, the use of modern antiplatelet agents was not accompanied by the attraction of platelets to the wound surface and their inclusion in the process of local thrombus formation. A similar conclusion can be drawn for the TA group. As is known, TA is used for increased bleeding associated with thrombocytopathy/thrombocytopenia [16, 17]. It is also known that the hemostatic effect of TA is mediated by a change in the hemostatic balance towards thrombosis due to a disruption of the plasminogen activation in plasmin [18]. Consequently, against the background of the use of TA, the role of platelets in local thrombosis (taking into account the suppression of their function by antiplatelet agents) is insignificant. This is also evidenced by their stable content in wound blood (in comparison with systemic blood flow) and a moderate increase in their number in the wound vessels.

A new result obtained in the course of this study is the fact of platelet redistribution in the case of FM application. Probably, a decrease in the number of these cells in wound blood with an increase in their number in the wound vessels indicates their targeted attraction to the site of injury when exogenous FM is introduced into the bloodstream.

The assumption of the inclusion of platelets in wound fibrin formation with the introduction of FM is supported by studies that demonstrate the independent ability of this fibrinogen derivative to enhance the aggregation activity of platelets [19–23] and erythrocytes [24]. As is known, FM is a derivative of fibrinogen with a similar molecular structure, differing only in the absence of four fibrinopeptides (2A and 2B), as a result of the action of thrombin. Fibrinogen is able to bind to GP IIb/IIIa platelet receptors to form molecular bonds between cells during their aggregation. The binding centers with these receptors are present in the FM molecule and are localized at the C-end of γ-chain (conservative sequence of Liz-Gln-Ala-Gli-Asp-Val at position 400–411) [25, 26] and α-chain (tripeptide Arg-Gli-Asp at position 572–574) [27, 28]. Consequently, FM, like fibrinogen, has the ability to bind to platelets through GP IIb/IIIa receptors and form fibrin-like bridges between cells to stimulate their aggregation function. In addition, it is known that platelets in the presence of collagen of damaged vessels and thrombin are able to produce microvesicles enriched with a large number of receptors, including IIb/IIIa, necessary for interaction with fibrin, fibrinogen and fibronectin [29, 30].

Thus, we suggest that in animals with pharmacologically determined thrombocytopathy, the use of exogenous FM may be accompanied by receptor-mediated involvement of blood plates in the mechanisms of local thrombosis.

Another important aspect of this study was the assessment of the morphology of erythrocytes to determine the contribution of these cells to the hemocoagulation process. The appearance of erythrocytes in the systemic circulation in the observation groups corresponded to the physiological norm. In cases involving the use of TA and FM, the presence of single echinocytes was noted, which may be represented by artifacts formed during the preparation of blood smears [31].

There were erythrocytes with altered forms in the wound blood of animals – echinocytes, schistocytes, stomatocytes and ovalocytes. After passing the formed elements through the damaged vessels of the wound surface, a disruption of the structure of their cell membrane is noted. Previous studies have shown that thrombotic formations in the placebo group included unchanged erythrocytes, forming mixed-type thrombi (fibrin-erythrocyte) [11]. On the contrary, thrombotic masses in the wound when using TA and FM consisted of a large number of predominantly hemolysed erythrocytes. In general, it can be assumed that damage to the cell membranes of erythrocytes, up to hemolysis, creates an additional condition for the activation of blood coagulation, including against the background of the use of TA and FM.

CONCLUSION

The results obtained on the model of pharmacologically conditioned thrombocytopathy demonstrated the effect of platelet redistribution between the systemic

bloodstream, wound vessels and wound blood when exogenous FM is injected into the bloodstream. It is assumed that the targeted attraction of injected FM to the injury site has a receptor-mediated mechanism for local thrombosis, despite the action of antiplatelet agents. The study also showed a positive contribution of erythrocytes to the formation of wound thrombotic masses. The detailed mechanisms of exogenous FM vector accumulation on the wound surface will have to be clarified in subsequent studies.

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Conflict of interest

The authors declare that there is no conflict of interest in the submitted article.

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