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Effects of tranexamic acid and exogenous fibrin monomer on the liver injury area and systemic circulation in pharmacological suppression of platelet function in an experiment

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Abstract

Aim. To identify and compare the morphological, hemostatic and hemostasiological consequences of intravenous administration of tranexamic acid and fibrin monomer in controlled liver injury against drug-induced thrombocytopathy.

Methods. The morphological features of fibrin formation in the area of liver injury after spontaneous bleeding arrest combined with the indicators of blood loss in the animals treated with intravenous placebo, tranexamic acid or fibrin monomer was studied in 69 male rabbits. The effects of these drugs were assessed against thrombocytopathy associated with the combined use of acetylsalicylic acid and clopidogrel. Platelet number and function (adnosine diphosphate-induced aggregation), the data of thromboelastometry and calibrated automated thrombogram, fibrinogen concentration and D-dimer level were considered in the blood test. The feature distribution in the samples was assessed using the Shapiro–Wilk test. Depending on the distribution, Student's t-test, Mann–Whitney U test or Wilcoxon signed-rank test were used to test for a significant difference between the features. Differences in mortality rate were established by using Fisher's exact test. The differences were considered statistically significant at $p < 0.05$.

Results. A model of thrombocytopathy which showed decreased platelet aggregation function (by 4.5 times), increased blood loss (by 40%), and high mortality (53.9%) was reproduced. Only a small accumulation of thrombotic material was noted on the injured surface of such animals. The use of tranexamic acid led to decreased post-traumatic bleeding (2.5 times) and animal mortality (20%). The latter was provided on the wound surface by increasing the thickness of both thrombotic deposits and fibrin strands. When fibrin monomer was used, the phenomenon of an overcompensated decrease in blood loss (by 6.7 times) accompanied by zero mortality was noted despite a pronounced decrease in platelet aggregation. The maximum increase in the thickness of thrombotic material and fibrin strands was morphologically determined in the injury area compared with other animal groups.

Conclusion. Morphological features of traumatic hemostatic effect at the injured area when using tranexamic acid and fibrin monomer have a number of differences despite the similarity of the achieved results in minimizing blood loss.

Keywords: fibrin monomer, acetylsalicylic acid, clopidogrel, tranexamic acid, post-traumatic bleeding, hemostatic effect, fibrin formation.

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Background. Hemostaseology is one of the branches of hematology that has been actively developing over a long period of time as new knowledge is acquired. Modern studies of the regulation of blood coagulation at the cellular and molecular levels [1–4], as well as the aspects of spatial thrombogenesis *in-vitro* [5] provide new prerequisites for studying the mechanisms of the hemostasis system *in-vivo* [6].

Studies performed earlier in our laboratory have demonstrated the presence of unique hemostatic and hemostaseological effects in low doses

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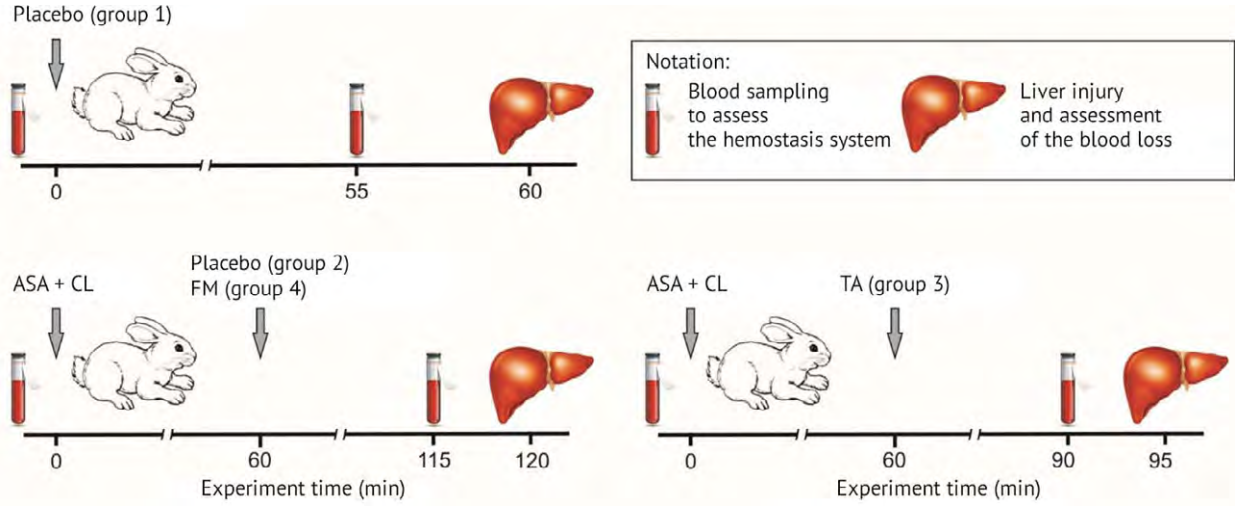

Fig. 1. Design of experiments with dosed liver injury; ASA — acetylsalicylic acid; CL — clopidogrel; TA — tranexamic acid; FM — fibrin monomer.

of the fibrin monomer (FM, structurally *desAABB* fibrinogen) administered into the bloodstream [7, 8]. They also revealed that the hemostatic effect of exogenous FM is manifested after the trauma to the parenchymal organ not only in the group of intact animals, but also in animals against the inhibition of platelet aggregation function associated with the use of double antiplatelet therapy [9]. It was noted that the FM hemostatic activity was comparable to that in the cases of using the tranexamic acid (TA), a drug frequently used to minimize microcirculatory bleeding, including post-traumatic blood loss [10, 11].

We chose the FM dose (0.25 mg/kg) from several possible ones ranging from 0.1 to 5.0 mg/kg [7], and the selection criterion was a combination of high hemostatic properties with the absence of a laboratory detectable prothrombogenic effect on the hemostatic system. It is noteworthy that this dose of FM when injected into the bloodstream is, according to the calculations, close to its physiological level in blood plasma of the healthy people (<7.8 μg/ml) [12].

The hemostatic effects of protamine sulfate and FM in heparinized animals were also studied previously with an assessment of the morphological presentation in the area of the injury [13]. These substances turned out to be very close to each other, and comparable in histological aspects of thrombogenesis in the wound area. Meanwhile, in the same experiments, it was revealed that protamine sulfate minimizes bleeding in the presence of systemic normo-coagulation (due to the binding of heparin), while FM implements its effect in the presence of permanent heparin-induced hypocoagulation.

To search for new facts that would enable to approach the deciphering the mechanisms of the hemostatic action of FM, it seemed interesting to perform the same analysis in the case of a decrease in the hemostatic potential associated with the suppression of the functional activity of platelets.

In this regard, **this study aimed** to identify and compare the morphological, hemostatic, and hemostaseological consequences of intravenous administration of TA and FM in dosed liver injury in presence of pharmacologically caused thrombocytopathy.

Materials and methods of research. The study was performed on 69 healthy adult male chinchilla rabbits weighing about 3.0–4.5 kg. Experiments on animals were performed 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 the experimental animals." The work was approved by the local ethical committee of the Altai State Medical University of the Ministry of Health of the Russian Federation (protocol No. 12 of November 12, 2015).

The animals were divided into four groups (Fig. 1).

An aqueous solution of placebo (3.75 M urea solution, corresponding to its concentration in the FM solution) in a volume of 0.5 ml was injected in the animals of the group 1 (n = 21) into the marginal ear vein (intravenously). Then, after one hour, laparotomy was performed under general anesthesia with telazol (Zoetis, Russia, intravenous 10 mg/kg), and a standard liver injury was made in accordance with the available recommendations [14].

To suppress the platelet aggregation function at the beginning of the experiment, the animals of groups 2–4 received per os a mixture of acetylsali-

cylic acid dissolved in the water (Thrombo ASS®, 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. As it is known, the mechanism of action of acetylsalicylic acid is associated with the irreversible inhibition of platelet cyclooxygenase-1, and a subsequent decrease in the synthesis of thromboxane A_2. Clopidogrel, on the other hand, is a prodrug; through metabolism in the liver, it turns into its active form and acts as an antagonist of platelet P_2Y_{12} receptors [15].

One hour after the administration of these antiplatelet agents, the animals were injected intravenously with 0.5 ml aqueous solution of placebo in the group 2 (n = 13), TA (tranexam®, Moscow Endocrine Plant, Russia) at a dose of 15 mg/kg in the group 3 (n = 22), and FM at a dose of 0.25 mg/kg (Technology-Standard) in the group 4 (n = 13).

Liver standard injury was made under general anesthesia with telazol in animals of the group-2 and-4, one hour after the administration of placebo and FM, and in the group 3 animals, 30 minutes later, and the nature of parenchymal bleeding was assessed by using the gauze napkins by the volume of blood loss (% of the calculated volume of circulating blood) with taking into account the body weight of the animal, as well as the rate of blood loss per unit of time (mg/s) [14].

To assess the hemostasis system, blood was obtained after the incision of the marginal ear vein (by gravity) twice the before drug administration and before liver injury (Fig. 1). Blood was collected in tubes with appropriate stabilizers, namely with the potassium salt of ethylenediaminetetraacetic acid (AQUISEL® K3E/EDTA 3K, Aquisel SL, Spain) in a volume of 0.25 ml for counting the platelets, and with 0.11 M (3.8%) sodium citrate solution (blood to stabilizer ratio 9:1) in a volume of 5.0 ml for studying the other parameters. The production of platelet-rich and platelet-depleted blood plasma was performed according to the generally accepted method.

The study of the hemostasis system included an assessment of the number of platelets in the venous blood using a Drew-3 hematology analyzer (Drew Scientific Inc., UK-USA), and their aggregation function using a Chronolog 490-2D aggregometer (CHRONO-LOG Corporation, USA) using adenosine diphosphate (ADP) at a concentration of 10 μM, determination of fibrinogen concentration on a Thrombostat 2 coagulometer (Behnk Electronik, Germany) with the reagent kits from Tekhnologiya-Standart (Russia), as well as the level of D-dimer using a NycoCard Rader II reflectometer analyzer (Axis-Shield PoC AS, Norway) and Nyco Card® D-Dimer test systems (Axis-Shield PoC AS, Norway).

To assess the generation of thrombin, we used the method of calibrated automated thrombography according to the N.S. Hemker (2003) using a Fluoroskan Ascent tablet fluorometer (ThermoFisher SCIENTIFIC, Finland) with Thrombinoscope™ 3.0.0.26 software and Thrombinoscope® bv reagent kits (Netherlands) (PPP-Reagent, Thrombin Calibrator, FluCa-Kit) with tissue factor in the concentration of 5.0 pM. The test parameters Lag-time (time of initiation of thrombin formation), ETP (endogenous thrombin potential), Peak thrombin (peak thrombin concentration), ttPeak (time to the peak thrombin concentration), and V (rate of thrombin formation) were considered.

Thromboelastometry of the blood stabilized with sodium citrate was performed on a ROTEM® Gamma thromboelastometer (Pentapharm GmbH, Germany) with the Startem reagent in Natem mode. The parameters CT (time of the onset of coagulation), CFT (clot formation time), angle α (clot amplitude), MCF (maximum clot firmness), and A_{10} (amplitude of the clot after 10 minutes) were determined.

After stopping the bleeding for histological examination, liver tissue was taken from the animals, including the entire wound part and a fragment of the intact surface, followed by the fixation by 10% solution of the neutral formalin according to Lilly. The material was processed using isopropyl alcohol using a TISSUE-TEK VI PTM_6 carousel automatic processing machine (Sakkura, Japan). Paraffinization was performed using a TISSUE-TEK TEC 5 paraffin embedding station (Sakkura, Japan).

Histological sections 4–5 μm thick were obtained using a semi-automatic rotary microtome Accu-Cut SRM (Sakkura, Japan), the specimens were stained with hematoxylin and eosin in the automatic staining machine TISSUE-TEK Prisma (Sakkura, Japan), and enclosed under a film in an automatic unit for automatic enclosing of micro-preparations TISSUE-TEK Film (Sakkura, Japan).

To determine the morphological structure of fibrin in the tissues, the staining of the prepared sections was performed by the ORB method (orange G, acid red 2C, and water blue) according to D.D. Zerbino and L.L. Lukasevich [16] using a set of reagents for determining the age of fibrin (BVS, Russia).

The platelets were counted during the morphological studies in the venous or arterial large vessels, in five fields of view at a magnification of ×1000, under the oil immersion of a microscope, followed by the calculation of the average number of cells. Microphotography was performed using a Leica DM 750 E_{200} microscope with a Leica EC_3

Table 1. Indicators of morphometric study of histological specimens of liver wounds

Indicators	Group 1. After the placebo administration	Group 2. After the administration of antiplatelet agents and placebo	Group 3. After the administration of antiplatelet agents and TA	Group 4. After the administration of antiplatelet agents and FM
Thickness of thrombotic masses, μm	66.2 [62.7–83.5]	48.8 [38.7–65.6]	140.5 [132.6–170.8]	297.6 [279.4–314.2]
		p_{1-2}=0.002; Δ×1.6	p_{1-3}=0.00004; Δ×2.1 p_{2-3} <0.000001; Δ×2.9	p_{1-4}=0.000001; Δ×4.5 p_{2-4} <0.000001; Δ×6.1 p_{3-4} <0.000001; Δ×2.1
Fibrin strand thickness, μm	0.83 [0.72–0.93]	1.34 [1.18–1.45]	2.24 [1.61–2.80]	3.44 [2.52–3.90]
		p_{1-2}=0.003; Δ×1.6	p_{1-3} <0.000001; Δ×2.7 p_{2-3}=0.00001; Δ×1.7	p_{1-4} <0.000001; Δ×4.1 p_{2-4} <0.000001; Δ×2.6 p_{3-4}=0.000007; Δ×1.5
Platelet count, number/f.v.	73.5 [61.0–90.8]	55.0 [50.8–60.0]	84.0 [82.0–89.5]	150.0 [113.5–201.0]
		p_{1-2}=0.016; Δ×1.3	p_{1-3}=0.091 p_{2-3}=0.0003; Δ×1.5	p_{1-4}=0.005; Δ×2.0 p_{2-4}=0.001; Δ×2.7 p_{3-4}=0.029; Δ×1.8

Note: *p* — level of statistical significance of the differences between the indicators compared; f.v. — field of vision; TA — tranexamic acid; FM — fibrin monomer; Δ — difference in indicators.

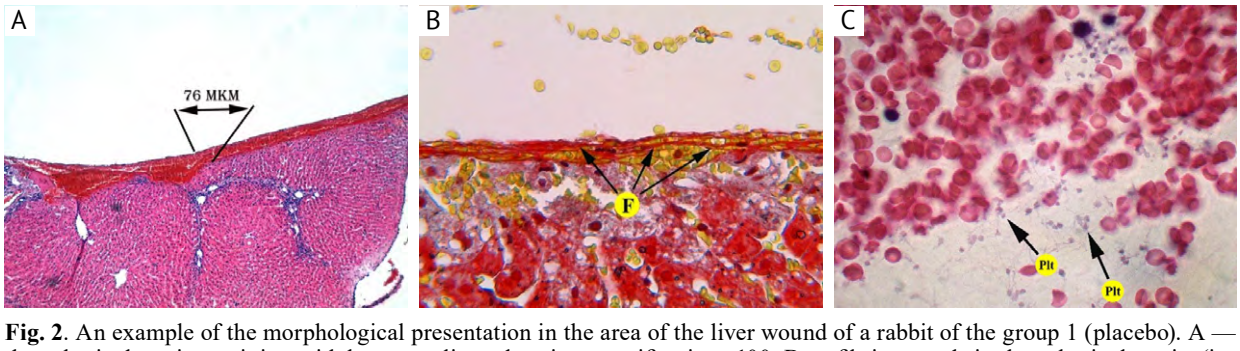

Fig. 2. An example of the morphological presentation in the area of the liver wound of a rabbit of the group 1 (placebo). A — thrombotic deposits, staining with hematoxylin and eosin, magnification ×100; B — fibrin strands in thrombotic deposits (indicated by arrows, F — fibrin), staining for fibrin by ORB (orange G, acid red 2S, and water blue), magnification ×400; C — platelets in the lumen of large vessels in the wound area (indicated by arrows, Plt — platelets), stained with the hematoxylin and eosin, magnification ×1000.

digital video camera (Leica Microsystems CMS GmbH, Germany). Morphometric measurements were performed using a licensed package of morphometric programs Video Test — Morphology 5.2 (VideoTest, Russia).

The animals were sacrificed by an overdose of the anesthetics.

The distribution of signs in the samples was assessed using the Shapiro–Wilk test. Depending on the distribution of signs, Student's t-test, Mann–Whitney U-test, or Wilcoxon's W-test were used. Differences in the mortality rate of the animals in the groups were established using the Fisher's exact test. Differences were considered statistically significant at $p < 0.05$. The experimental data were processed using the statistical software MedCalc Version 17.9.7 (license BU556-P12YT-BBS55-YAH5M-UBE51). The data obtained are presented as a median (Me), 25_{th} and 75^{th} percentiles (Q): Me [Q25÷Q75].

Results. To assess the morphological presentation after the use of the agents with systemic hemostatic action (TA and FM), sections of the wound surface of the liver, obtained immediately after the spontaneous arrest of wound bleeding, were examined.

We have previously described the morphological changes in the area of liver injury in animals without the pharmacological effects on the hemostatic system [17]. In this work, these data formed the basis for the formation of the group 1 (control), where the animals received only placebo. As noted above, thrombotic formations on the wound surface in this group were identified, as thin pink overlays with a smooth surface (Fig. 2, A), about 66 μm thick (Table 1). The composition of these overlays was mainly represented by the rarely anastomosed thin fibrin strands located parallel to the wound surface (indicated by arrows in Fig. 2, C). In addition, the inclusion of intact erythrocytes

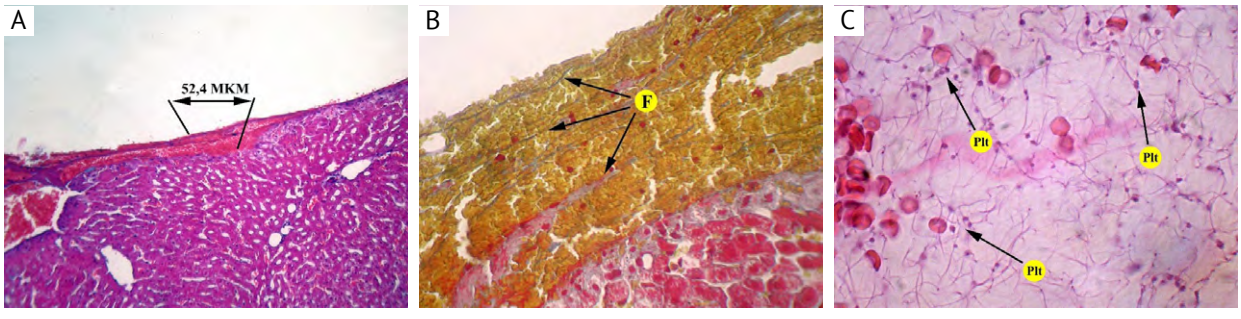

Fig. 3. An example of the morphological presentation in the area of the rabbit liver wound of the group 2 (antiplatelet agents and placebo). A — thrombotic deposits, staining with hematoxylin and eosin, magnification × 100; B — fibrin strands in thrombotic deposits (indicated by arrows, F — fibrin), ORB staining for fibrin (orange G, acid red 2C, and water blue), magnification × 400; C — platelets in the lumen of large vessels in the wound area (indicated by arrows, Plt — platelets), stained with the hematoxylin and eosin, magnification × 1000.

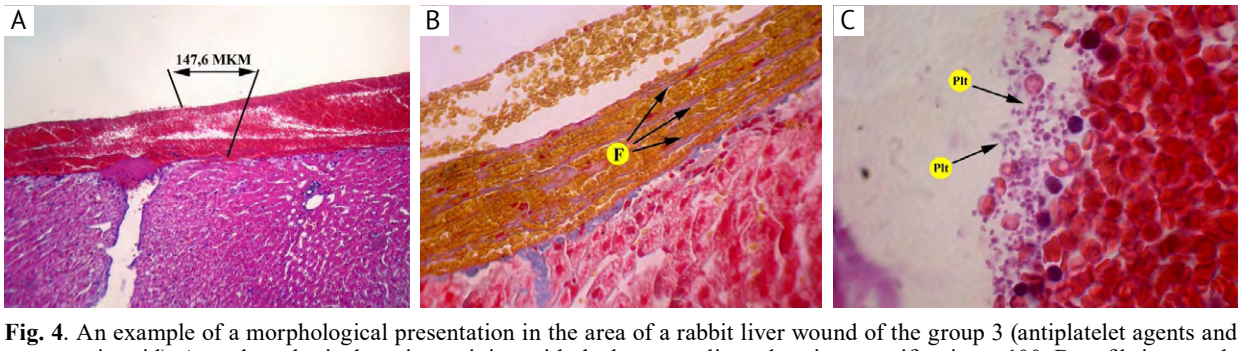

Fig. 4. An example of a morphological presentation in the area of a rabbit liver wound of the group 3 (antiplatelet agents and tranexamic acid). A — thrombotic deposits, staining with the hematoxylin and eosin, magnification × 100; B — fibrin strands in thrombotic deposits (indicated by arrows, F — fibrin), ORB staining for fibrin (orange G, acid red 2C, and water blue), magnification × 400; C — platelets in the lumen of large vessels in the wound area (indicated by arrows, Plt — platelets), stained with the hematoxylin and eosin, magnification × 1000.

between the fibrin strands were recorded, which in general became a prerequisite for classifying such thrombotic formations as thrombi of a mixed type (fibrin-erythrocytic) [16]. Additionally, the count of the platelets in the lumen of large vessels was determined (indicated by arrows in Fig. 2, C), the number of which was about 73 cells in the field of view (Table 1).

In the group of animals with pharmacological suppression of the platelet function, that received placebo (group 2), less noticeable thin, smooth, pinkish thrombotic masses were revealed in the area of the wound (Fig. 3, A), which thickness was about 48 μm (Table 1). These masses consisted of thin pink fibrin strands (indicated by the arrows in Fig. 3, B), which were located mainly parallel to the wound surface of the liver, with rare anastomoses between the strands. At the same time, the thickness of the fibrin fibers increased by 1.6 times (compared with the group 1, Table 1). In addition, a small number of intact erythrocytes was found in thrombotic masses.

In general, the aspects of thrombotic deposits in the group 2 enabled to classify them, as in the group 1, as a mixed type of the thrombus (fibrin-erythrocytic).

When counting platelets in the lumen of the large vessels (indicated by arrows in Fig. 3, C), their count was 1.3 times less than the group 1, and it was about 55 cells in the field of vision (Table 1).

In the group of animals that received TA (group 3), the thickness of thrombotic deposits was comparatively greater (Fig. 4, A) than the groups 1 and 2 (by 2.1 and 2.9 times, respectively, Table 1) and was about 140 microns. The thrombotic mass had a brownish color and a hummocky surface, consisted of many predominantly hemolyzed erythrocytes and fibrin strands (indicated by arrows in Fig. 4, B). The latter were thickened and were located mainly parallel to the wound surface of the liver in the various directions, and formed a small number of anastomoses.

Overall, this enabled to classify the trauma-related thrombotic masses in the group 3, as in the above-described groups, as thrombi of a mixed type. We also note that along with an increase in the thickness of the thrombotic masses, an increase in the thickness of the fibrin strands was also found to be 1.7 times greater compared with the group 2 (Table 1).

In the lumens of large vessels, a greater number of platelets was noted (indicated by arrows in

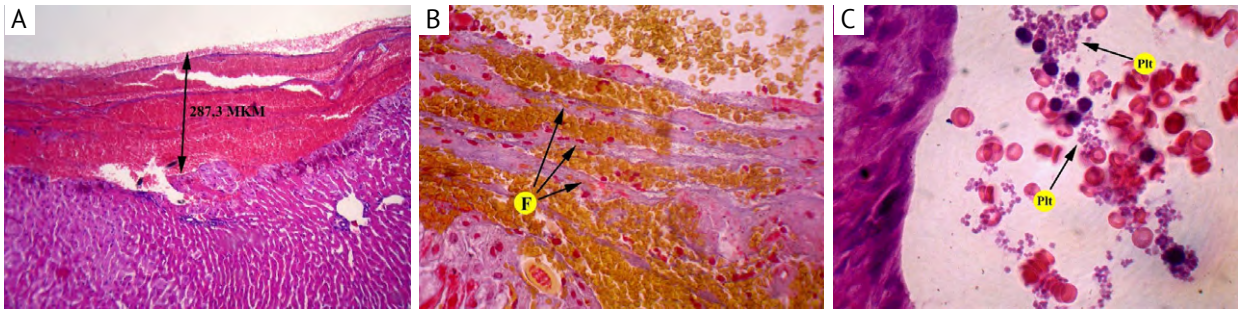

Fig. 5. An example of a morphological presentation in the area of a rabbit liver wound of the group 4 (antiplatelet agents and fibrin monomer). A — thrombotic deposits, staining with the hematoxylin and eosin, magnification × 100; B — fibrin strands in thrombotic deposits (indicated by arrows, F — fibrin), ORB staining for fibrin (orange G, acid red 2C, and water blue), magnification × 400; C — platelets in the lumen of large vessels in the wound area (indicated by arrows, Plt — platelets), stained with the hematoxylin and eosin, magnification × 1000.

Fig. 4, C), compared with the group 2, when counted, their number was about 84 cells in the field of view (Table 1).

In the animals that received FM (group 4), in the area of the injury, the most pronounced, compared with the other study groups (Fig. 5, A), tuberous, brown thrombotic deposits were determined, with their thickness of about 297 μm (Table 1). Thrombotic masses consisted of many hemolyzed erythrocytes and thick coarse fibrin strands, which were located mainly parallel to the wound surface, and formed a significant number of anastomoses (indicated by arrows in Fig. 5, C). An increase in the thickness of fibrin strands was noted compared with the groups 1, 2, and 3 (4.5, 6.1, and 2.1 times, respectively) (Table 1).

This morphological presentation, as in the previous groups, enabled to classify thrombotic formations in the wound area as a mixed type of thrombus.

In the specimens studied, the number of platelets in the lumens of large vessels (indicated by the arrows in Fig. 5, C) was maximum compared with the other groups, and amounted to about 150 cells in the field of view (Table 1).

The intergroup differences revealed in the morphological presentation of the liver tissue in the area of the wound surface were combined with changes in the parameters of the hemostasis system in venous blood and its plasma. The corresponding results obtained in the study groups are presented in Table 2.

The use of dual antiplatelet therapy led to the expected decrease in platelet (ADP-induced) aggregation in the groups 2, 3, and 4 by 4.5, 3.0, and 16.6 times, respectively. The follow-up data in the groups 2 and 4 were also accompanied by a decrease in both chronometric (CT, CFT) and density indicators during the clot formation (angle α, MCF, and A_{10}) during the blood thromboelastometry, which, however, was not typical for the group 3, where TA was used as a hemostatic drug. In the same group, a 10-fold increase in the level of D-dimer was noted, which is not entirely understandable, since an increase in the thrombin generation was not detected according to the calibrated thrombography test in the venous blood plasma in all the experiments.

Discussion. According to the results of the study, in the group 2 of animals that received antiplatelet drugs, an increased blood loss (by 40% compared with the group 1, p = 0.040), and a high mortality rate of animals (53.9%; Table 3) were noted, which was quite expected due to a decrease in the aggregation function of platelets (4.5 times), and several indicators of thromboelastometry (Table 2). The morphological presentation in the area of the injury is the formation of small thrombotic masses consisting of fibrin threads and unchanged erythrocytes, with a reduced number of platelets in the lumens of the large vessels near the injury. In this case, it corresponded to the increase in blood loss.

In the case of using TA, which is clinically indicated in case of bleeding in thrombocytopathy/thrombocytopenia [18], despite the pharmacologically conditioned suppression of platelet function, a decrease in the post-traumatic blood loss was revealed both in volume (2.5 times from 14.1 to 5.7%; $p = 0.005$), and in terms of rate (2.7 times from 16.4 to 6.2 mg/s; $p = 0.006$) compared with the group 2. Based on the data of the morphological study of the liver, the latter, most probably, was provided by an increase in the thickness of both thrombotic deposits and the fibrin strands themselves on the wound surface.

It is noteworthy that the administration of TA and a decrease in blood loss in these animals were accompanied by close to the initial (normal) thromboelastometry parameters. In this case, we are probably dealing with a change in the hemostatic equilibrium toward thrombogenesis due

Table 2. Results of assessment of the hemostasis system in the groups of experimental animals.

Indicators	Group 1		Group 2		Group 3		Group 4	
	Before the placebo administration $_{(1a)}$	After the placebo administration $_{(1b)}$	Before the administration of antiplatelet agents and placebo $_{(2a)}$	After the administration of antiplatelet agents and placebo $_{(2b)}$	Before the administration of antiplatelet agents and TA $_{(3a)}$	After the administration of antiplatelet agents and TA $_{(3b)}$	Before the administration of antiplatelet agents and FM $_{(4a)}$	After the administration of antiplatelet agents and FM $_{(4b)}$
Platelet count, $\times 10^9$/L	477.5 [405.8÷621.5]	480.5 [412.3÷555.0] $p_{1a-1b} = 0.151$	438.0 [419.0÷484.0]	452.0 [435.0÷501.0] $p_{2a-2b} = 0.136$	433.5 [373.0÷532.0]	427.5 [356.3÷491.0] $p_{3a-3b} = 0.189$	468.0 [419.0÷498.0]	426.0 [408.0÷467.0] $p_{4a-4b} = 0.458$
ADP-induced platelet aggregation, %	20.5 [19.0÷28.7]	22.0 [19.2÷31.4] $p_{1a-1b} = 0.598$	20.1 [18.4÷45.9]	4.5 [0.6÷7.0] $p_{2a-2b} = 0.001$. $\Delta_{2a-2b} \times 4.5$	24.0 [19.0÷46.5]	8.0 [4.9÷10.1] $p_{3a-3b} = 0.000001$ $\Delta_{3a-3b} \times 3.0$	19.9 [13.3÷20.1]	1.2 [1.0÷2.0] $p_{4a-4b} = 0.001$. $\Delta_{4a-4b} \times 16.6$
Fibrinogen, g/l	3.3 [2.8÷4.4]	3.7 [2.8÷4.5] $p_{1a-1b} = 0.811$	3.3 [3.0÷3.5]	3.4 [3.2÷3.8] $p_{2a-2b} = 0.758$	3.5 [2.9÷3.9]	3.2 [3.0÷3.8] $p_{3a-3b} = 0.753$	3.5 [3.2÷4.1]	3.4 [3.0÷4.1] $p_{4a-4b} = 0.872$
D-dimer, ng/ml	100.0 [100.0÷100.0]	100.0 [100.0÷200.0] $p_{1a-1b} = 0.201$	100.0 [100.0÷175.0]	100.0 [100.0÷200.0] $p_{2a-2b} = 0.180$	300.0 [200.0÷400.0]	1000.0 [525.0÷1350.0] $p_{3a-3b} = 0.010$. $\Delta_{3a-3b} \times 3.3$	150.0 [100.0÷275.0]	175.0 [100.0÷300.0] $p_{4a-4b} = 0.463$
Thrombogram (calibrated thrombography)								
Lag-time, min	2.2 [2.0–2.7]	2.0 [1.8–2.7] $p_{1a-1b} = 0.068$	2.7 [2.7–3.0]	2.7 [2.7–3.0] $p_{2a-2b} = 0.999$	3.3 [3.3–3.6]	3.4 [3.3–3.8] $p_{3a-3b} = 0.285$	2.4 [2.3–2.5]	2.6 [2.5–2.8] $p_{4a-4b} = 0.109$
ETP, nmol × min	373.9 [338.7–500.4]	484.8 [360.6–622.5] $p_{1a-1b} = 0.224$	333.5 [307.7–453.0]	418.0 [307.7–422.2] $p_{2a-2b} = 0.988$	337.1 [288.0–406.1]	362.6 [335.3–396.5] $p_{3a-3b} = 0.999$	427.2 [406.7–481.4]	412.5 [362.4–461.8] $p_{4a-4b} = 0.068$
Peak thrombin, nmol/l	76.2 [40.7–90.9]	81.7 [34.3–138.8] $p_{1a-1b} = 0.128$	55.2 [43.5–85.5]	56.5 [51.8–73.6] $p_{2a-2b} = 0.348$	38.8 [27.5–57.6]	50.2 [42.2–57.7] $p_{3a-3b} = 0.465$	77.9 [68.3–93.0]	70.4 [55.1–86.8] $p_{4a-4b} = 0.144$
ttPeak, min	5.8 [5.0–7.3]	5.4 [4.6–6.3] $p_{1a-1b} = 0.143$	6.7 [5.7–6.7]	6.3 [6.0–7.3] $p_{2a-2b} = 0.138$	8.1 [7.4–8.6]	8.4 [7.7–9.0] $p_{3a-3b} = 0.109$	5.8 [5.6–6.4]	6.2 [5.5–7.5] $p_{4a-4b} = 0.285$
V, nmol/min	25.3 [9.2–29.1]	26.8 [7.8–62.2] $p_{1a-1b} = 0.102$	18.4 [11.4–28.8]	12.9 [9.6–23.2] $p_{2a-2b} = 0.117$	20.7 [18.0–22.2]	19.4 [16.5–23.7] $p_{3a-3b} = 0.999$	22.7 [21.7–28.1]	20.4 [13.7–28.9] $p_{4a-4b} = 0.144$

Thromboelastometry indicators								
CT, s	605.5 [453.8÷801.5]	628.0 [479.0÷856.0]	508.0 [391.0÷892.5]	642.0 [140.0÷1190.0]. in two cases n.c.	673.0 [484.5÷784.0]	587.0 [386.0÷854.0]	431.5 [246.0÷962.8]	828.0 [452.5÷1154.0]
		$p_{1a–1b} = 0.821$		$p_{2a–2b} = 0.616$		$p_{3a–3b} = 0.384$		$p_{4a–4b} = 0.221$
Angle α, degrees	57.0 [46.5÷62.0]	55.0 [49.0÷65.0]	70.0 [50.0÷78.0]	62.0 [44.0÷73.0]. in two cases n.c.	54.0 [45.0÷64.5]	58.0 [43.0÷61.0]	72.0 [45.8÷76.8]	46.0 [39.5÷64.0]
		$p_{1a–1b} = 0.207$		$p_{2a–2b} = 0.813$		$p_{3a–3b} = 0.902$		$p_{4a–4b} = 0.017$. $\Delta_{4a–4b} \times 1.6$
CFT, s	182.5 [148.8–269.3]	206.0 [146.0–254.0]	152.5 [112.5–326.3]	233.0 [116.0–315.0]. in two cases n.c.	217.0 [152.0–298.0]	238.0 [183.3–336.8]	166.5 [111.0–273.5]	276.0 [147.8–346.5]
		$p_{1a–1b} = 0.288$		$p_{2a–2b} = 0.999$		$p_{3a–3b} = 0.267$		$p_{4a–4b} = 0.029$. $\Delta_{4a–4b} \times 1.7$
MCF, mm	59.5 [56.0÷64.3]	58.0 [54.0÷64.0]	54.0 [51.5÷65.0]	50.0 [46.0÷61.0]? in two cases n.c.	61.0 [55.0÷70.0]	64.0 [58.0÷71.0]	63.0 [58.0÷66.3]	54.5 [50.5÷62.3]
		$p_{1a–1b} = 0.956$		$p_{2a–2b} = 0.156$		$p_{3a–3b} = 0.342$		$p_{4a–4b} = 0.079$
A_{10}, mm	44.0 [40.8÷52.5]	43.0 [39.0÷50.0]	42.0 [34.0÷64.5]	39.0 [35.0÷52.0]? in two cases n.c.	46.0 [37.0÷56.5]	48.0 [35.0÷56.5]	56.0 [41.5÷60.0]	40.5 [34.8÷49.0]
		$p_{1a–1b} = 0.422$		$p_{2a–2b} = 0.240$		$p_{3a–3b} = 0.925$		$p_{4a–4b} = 0.041$. $\Delta_{4a–4b} \times 1.4$

Note: *p* — level of statistical significance of differences between the compared indicators; TA — tranexamic acid; FM — fibrin monomer; ADP — adenosine diphosphate; n.c. — no coagulation; Δ — difference in indicators; calibrated thrombography indices: Lag-time — time of initiation of thrombin formation; ETP — endogenous thrombin potential; Peak thrombin — peak thrombin concentration; ttPeak — time to reach the peak thrombin concentration; V — rate of thrombin formation; thromboelastometry indices: CT — coagulation onset time; CFT — clot formation time; angle α — clot amplitude; MCF — maximum clot firmness; A10 — amplitude of the clot after 10 minutes.

Table 3. Indicators of post-traumatic blood loss in groups of experimental animals.

Indicators	Group 1. After the placebo administration	Group 2. After the administration of antiplatelet agents and placebo	Group 3. After the administration of antiplatelet agents and TA	Group 4. After the administration of antiplatelet agents and FM
Volume of blood loss, % of the volume of circulating blood	10.1 [4.3÷16.3]	14.1 [12.0÷18.8]	5.7 [2.8÷11.3]	2.1 [1.8÷5.4]
		p_{1-2}=0.040. Δ×1.4	p_{1-3}=0.189 p_{2-3}=0.005; Δ×2.5	p_{1-4}=0.008; Δ×4.8 p_{2-4}=0.00002; Δ×6.7 p_{3-4}=0.058
Blood loss rate, mg/s	25.7 [7.1÷36.5]	16.4 [10.8÷33.7]	6.2 [4.5÷8.6]	7.0 [4.5÷8.2]
		p_{1-2}=0.685	p_{1-3}=0.012; Δ×4.1 p_{2-3}=0.006; Δ×2.7	p_{1-4}=0.032; Δ×3.7 p_{2-4}=0.002; Δ×2.3 p_{3-4}=0.959
Mortality, number of individuals	0 (0%) из 21	7 (53.9%) from 13	4 (20%) from 22	0 (0%) from 13
		p_{1-2}=0.0003	p_{1-3}=0.108 p_{2-3}=0.057	p_{1-4}=0.400 p_{2-4}=0.005 p_{3-4}=0.274

Notes: *p* — level of statistical significance of the differences between the indicators compared; TA — tranexamic acid; FM — fibrin monomer; Δ — difference in indicators.

to a decrease in the intensity of plasmin-dependent fibrinolysis. As indirect evidence of this position, we may take a multiple increase in the level of D-dimer, the origin of which is possibly associated with the non-plasmin mechanism of fibrinolysis caused by the elastase and cathepsin G. As shown earlier, the latter are capable of independently destroying fibrin, enhancing the effect of plasmin [19, 20].

In our opinion, the new knowledge in this article includes the phenomenon of local massive thrombogenesis in the wound under the action of FM administered intravenously at a dose of 0.25 mg/kg. This pattern led to an overcompensated decrease in the volume, and rate of blood loss (in terms of blood loss, from 14.1 to 2.1% compared with the group 2; $p = 0.00002$). The latter resulted in the absence of the lethal outcomes during the experiment. At microscopy, thrombotic masses, as in other groups of study, had a mixed nature (fibrin-erythrocytic type), with hemolyzed erythrocytes, however, in addition to thickening of these masses, in animals that received FM, a relatively greater increase in the thickness of fibrin strands rich in the anastomoses was found.

Another trend was the preservation of a relatively large number of platelets in the lumens of large vessels, which probably argues for their low "interest" in the ongoing processes of thrombogenesis in the wound. This could be supplemented with the fact that the intense thrombogenesis with the use of exogenous FM occurred in the presence of a relatively low activity (as measured by ADP aggregation and thromboelastometry indicators) of platelets in the venous circulation.

The disadvantage of this work is the fact that we could not convincingly explain the several facts (an increase in the amount of D-dimer during the action of TA, and its formation without an increase in the generation of thrombin in the corresponding group of animals), however, the established fact of the local hemostatic action of FM with its systemic administration, including in case of a decrease in the coagulation potential, remains evidence based.

CONCLUSION

The morphological aspects of the near-wound hemostatic effect of TA and low-dose FM have several differences, despite the similarity of the results achieved in minimizing the blood loss (under conditions of pharmacologically mediated suppression of platelet function).

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