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Research Article

THE HEMOSTATIC ACTIVITY OF BIS (2-AMINOETHAN-1-SULFONATE) CALCIUM

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ABSTRACT

Objective: It is known that local or systemic injection of hemostatic drugs is used to reduce blood loss and number of blood transfusions in patients with hypocoagulation and hemorrhagic diathesis. However, the analysis of literature data shows that the drugs applied for the control of bleeding, traditionally used in medical practice, are not effective enough. This study deals with the systemic hemostatic activity of bis (2-aminoethan-1-sulfonate) calcium in the experiment.

Methods: Experimental work *in vitro* is performed on the blood of healthy male donors, under conditions *in vivo* it is done on intraperitoneal injection in male rats. Thromboelastography was carried out with apparatus Thromboelastography (TEG) 5000 (Haemoscope Corporation, United States). The influence of first synthesized derivative and ethamsylate on functional activity of platelets was studied using a platelet aggregation analyzer "Biola 230LA" (Russia). Experimental evaluation of the system specific hemostatic activity *in vivo* was carried out using the model of parenchymatous bleeding in mature male rats. The interference came amid registration of bleeding stop time and extent of blood loss.

Results: Bis (2-aminoethan-1-sulfonate) calcium shows procoagulation and proaggregant activity both *in vitro* and *in vivo*. Proaggregatory effect of bis (2-aminoethan-1-sulfonate) calcium is successfully implemented in the systemic hemostatic activity in terms of parenchymal bleeding, surpassing the control group and the group of etamsylate.

Conclusion: The results of these studies reveal potentially high systemic hemostatic activity of bis (2-aminoethan-1-sulfonate) calcium, urging the need for further study on this compound and its analogs to create on their basis highly efficient, selective correctors of the hemostatic system.

Keywords: Taurate of calcium, Hemostasis system, Proaggregatory activity, Hemostatic activity.

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INTRODUCTION

It is known that local or systemic injection of hemostatic drugs is used to reduce blood loss and number of blood transfusions in patients with hypocoagulation and hemorrhagic diathesis. Examples include products based on fibrin or thrombin for local hemostasis in abdominal surgery or traumatology [1], proton-pump inhibitors to prevent recurrence of bleeding from the gastrointestinal tract [2], antifibrinolytic funds, and desmopressin for the system hemostasis [3]. However, drugs to stop bleeding, traditionally used in medical practice, are not always effective to lead to the effective reduction of blood loss which is dangerous due to the development of hemorrhagic shock, dilutional coagulopathy, or thrombosis and thromboembolic complications [4]. Overview of clinical recommendations shows that among synthetic systemic hemostatic drugs, the highly proved efficacy was confirmed only for tranexamic acid, aminocaproic acid, and etamsylate [5,6]. The results of the previous research show potentially high activity of some newly synthesized compounds relating to hemostasis system *in vitro* and *in vivo* [7]. This study deals with the systemic hemostatic activity of bis (2-aminoethan-1-sulfonate) calcium (compound I) (Fig. 1) in the experiment [8].

MATERIALS AND METHODS

Design

All researches are conducted in the following stages [9]:

1. The first stage examined the impact of compound I on the hemostasis system *in vitro*.
2. The second stage assessed the effect of compound I on hemostasis system *in vivo* after intraperitoneal injection to rats.
3. The third stage studied systemic hemostatic activity of compound I under model bleeding conditions following intraperitoneal injection to rats.

Under *in vitro* conditions, the study of the effect on the functional activity of platelets was carried out within concentration of 5×10^{-4}–2×10^{-3} mol/l, coagulation hemostasis component - 10^{-3}–10^{-5} g/l. Under *in vivo* conditions, rats were intraperitoneally injected with the test substances in equimolar concentration which for etamsylate was 38.1 mg/kg, for the compound I was 73.8 mg/kg.

Experimental work *in vitro* was performed on the blood of 65 male donors. The average age of donors was 20.4±2.2 years old. Informed consent for participation in the study was obtained from all participants before blood sampling. The study was approved by the Ethics Committee of the Bashkirian State Medical University (No. 1 dated January 2, 2018).

Experimental studies *in vivo* were performed *in vivo* on 70 laboratory rats in compliance with national legislation and International recommendations of the European Convention for the protection of vertebrate animals for experimental animals, regulations for laboratory practice in preclinical studies. The animals were kept in standard conditions of animal quarters with natural lighting, air temperature of 20±2°C, and humidity of 55–60% in plastic cages. 24 h before the research, the feeding was stopped without limiting the access to water.

Blood sampling from donor volunteers was carried out from cubital

vein using the systems of vacuum blood sampling BD Vacutainer® (Dickinson and Company, United States). The stage of working with laboratory animals included their anesthesia with diethyl ether and blood sampling from the jugular vein. Venous blood was stabilized by 3.8% sodium citrate solution in a ratio of 9:1.

Research on influence of compound I and comparators on platelets aggregation was carried out with a laser analyzer of platelet aggregation "Biola 230LA" ("Biola," Russia). For aggregation inductor was used adenosine diphosphate (ADP) with a concentration of 20 μg/ml and collagen - 5 mg/ml produced by "Technology Standard" (city of Barnaul, Russia).

A study of the influence of compound I and comparator on coagulation component of hemostasis was conducted by widely accepted clotting tests on hemocoagulometer Solar CGL 2110 ("SOLAR," Belarus). The research included the indicators of activated partial thromboplastin time, thrombin time, prothrombin time, and fibrinogen concentration [10]. The research applied reagents produced by "Technology Standard" (Barnaul, Russia).

Thromboelastography was carried out with TEG 5000 device (Haemoscope Corporation, United States). The analysis of the thromboelastograms defined general tendency of coagulation (R), functional activity of platelets and fibrinogen (MA, Angle), activity of fibrinolysis (clot lysis time [CLT]), and the physicomechanical properties of the formed clots (G). For TEG activator was used 0.2 mol $CaCl_2$ ("Technology Standard," Russia).

The experimental evaluation of the specific systemic hemostatic activity *in vivo* was carried out on viripotent male rats weighing 200–220 g. The drugs were injected intraperitoneally 1 h before parenhimatosis bleeding simulation. Then, under ether anesthesia was performed laparotomy, using longitudinal section along the white line of the abdomen. Intestines were directed into the wound, limiting them with drapes moistened with warm saline, and the anterior surface of the liver. With the help of a special device - a plastic limiter with a round hole in the center - the overlapped part of the liver was resected with a razor blade. The resected segment in a vertical projection had the form of a circle or ellipse, its size and shape were constant. Resulting evenly the bleeding wound with smooth edges and a uniform curvature had a total area of about 1.5 cm^2 and a depth of about 0.3 cm. The interference came amid registration of bleeding stop time and extent of blood loss. The amount of blood loss was determined by the gravimetric method, weighing the blood-soaked gauze material on electronic scales [11].

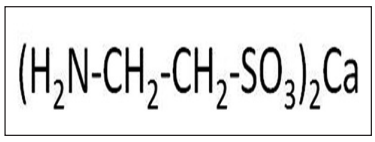

Fig. 1: The structure of compound I

The study of systemic hemostatic agents in experimental hemorrhage induced by heparin was carried out by introducing heparin into the tail vein of the experimental animal at a dose of 500 ME/kg 30 min before the start of the experiment.

The findings are processed using the statistical package Statistica 10.0 (StatSoft Inc., USA). The normality of the distribution of actual data was checked using the criterion of Shapiro-Wilka. The groups were described using the median and interquartile interval. Variance analysis was performed using the criterion of Kruskal–Wallis test (for independent observations) and Friedman (for repeated observations). Critical level of p significance for statistical criteria was taken equal to 0.05.

RESULTS

Results of studies *in vitro*

The findings determined that the compound I shows hemostatic properties exceeding level wise the values of etamsylate (Table 1).

MA indicator, which characterizes the functional activity of platelets, in the presence of compound I has been increased by 24.7% ($p<0.001$) and 15.9% ($p=0.001$) compared to the control and etamsylate, respectively. The index R that characterizes the enzymatic portion of coagulation is reduced on adding the compound I in 2.7 times ($p=0.002$) compared to control. This leads to a statistically significant increase of the total coagulation potential toward hypercoagulation - the TMA indicator reduced by 1.8 times ($p<0.001$) and 1.5 times ($p=0.003$) and TPI increased by 34.1% ($p<0.001$) and 16.7% ($p=0.001$) in comparison with the control and etamsylate. The values of the clot strength increase - the E and G indexes increase in the group of compound I by 1.5 times compared to the control. Etamsylate had no effect on indicators of the clot strength. Indicators that are responsible for fibrinolysis system (CLT, CL30, and LY30) remain at the level of the control values.

The findings of the effect of compound I and etamsylate on platelet aggregation (Table 2) show that the injection of 2×10^{-3} mol/l of compound I leads to increased platelet aggregation on average by 5.5% relative to the control values for ADP and collagen, which is higher than the comparison drug by 45.6% and 39.2%, respectively.

Analysis on "dose-effect" relationship shows that at a concentration of 5×10^{-4} mol/l of compound I the platelet aggregation grows at an average by 2.2% for both inductors. This concentration does not enable to register proaggregant activity of etamsylate. The results of the study showed that pre-incubation of the comparator was followed by spontaneous platelet aggregation, characterized by a clear dependence of the effect on the concentration. This effect did not show in the study of compound I. This allows concluding that, at the moment of induced aggregation, the compound I has a more selective effect on activated platelets in contrast to the comparator, the main effect of which falls on intact platelets, thereby potentially increasing the risk of blood clot formation, on the one hand, and remaining ineffective at the moment of bleeding, on the other hand.

Table 1: The indicators of thromboelastography under the impact of compound I and etamsylate *in vitro*

Indicator	Control	Etamsylate	Compound I	p_2
R, m	12.4 (9.8–12.8)	7.9 (7.4–9.8)$^{\alpha}$	4.3 (3.7–5.2)$^{\alpha}$	0.002
Angle, deg	42.6 (41.8–45.6)	47.5 (45.8–50.8)$^{\beta}$	60.3 (58.7–64.9)$^{\beta}$	0.004
MA, mm	55.7 (53.8–57.2)	61.3 (59.4–63.8)$^{\alpha}$	69.7 (66.2–73.5)$^{\beta}$	0.001
TMA, min	34.8 (33.7–36.5)	29.1 (25.8–31.1)$^{\beta}$	20.1 (18.7–22.4)$^{\beta}$	0.003
G, dine/cm^2	6.2 (5.8–6.6)	6.7 (6.1–7.2)	9.3 (8.7–10.5)$^{\beta}$	0.001
E, dine/cm^2	126.5 (117.8–139.2)	131.4 (128.7–132.4)	147.2 (140.5–150.4)$^{\beta}$	0.001
TPI/s	14.7 (13.8–15.6)	16.3 (15.9–18.2)$^{\alpha}$	18.9 (17.3–20.2)$^{\beta}$	0.001
CL30, %	96.4 (92.5–98.3)	95.6 (93.8–97.5)	94.6 (93.8–98.5)	0.4
LY30, %	0.5 (0.3–0.6)	0.0 (0.0–0.0)	0.0 (0.0–0.0)	0.7
CLT, min	36.8 (34.8–39.6)	35.4 (33.8–36.9)	37.8 (35.4–39.6)	0.6
CI,	0.5 (0.3–1.2)	1.7 (1.5–1.8)$^{\beta}$	2.9 (2.6–3.5)$^{\beta}$	0.002

$^{\alpha}p<0.05$; $^{\beta}p<0.001$ - etamsylate or compound I versus control; p2 - etamsylate versus compound I. CLT: Clot lysis time

The findings followed by the impact of hemostasis on the coagulation component (Table 3) show that compound I speeds up clot formation in the extrinsic coagulation pathway by 21.6% (p=0.001).

There were no registered cases when etamsylate had impact on the standard coagulogram rates.

Results of studies *in vivo*

The next stage examined the impact of compound I and etamsylate on functional activity of platelets at intraperitoneal injection into rats (Table 4).

Maximal platelet aggregation on injecting compound I exceeded indicators of the control by more than 40% (p≤0.001), and the values of etamsylate - by an average of 15% (p≤0.001); platelet aggregation rate increased by 30.0% (p≤0.001) in respect to the control and by 25.5% (p≤0.001) in comparison with the etamsylate for both inductors of aggregation. The average radius of platelet aggregates in the presence of compound I and ADP and for collagen is higher than in the control group by 2.3 times (p≤0.001) and in group of etamsylate - by 1.6 times (p≤0.001).

The findings of systemic hemostatic activity on the model of parenhimatosis hemorrhage in rats are presented in Table 5.

Table 5 data show that on intraperitoneal injection into rats the etamsylate reduced bleeding time by 26.5% (p=0.002) in comparison with the control group without significant impact on the total amount of blood loss. Compound I reduces the bleeding time by 34.9% (p=0.0003) and 11.5% (p=0.001) compared to the control and etamsylate, respectively. This effectively decreased the volume of blood loss by 2.2 (p=0.0002) and 1.9 (p=0.0001) times in comparison with the control group and that of etamsylate, respectively.

Preliminary intravenous injection of heparin in the control group resulted in increased bleeding time and blood loss by 1.5 (p≤0.001)

Table 2: Indicators of ADP- and collagen-induced platelet aggregation under the influence of etamsylate and compound I *in vitro*

No	Sample	Concentration, mol/l	Spontaneous aggregation of platelets (% to the control)	Enhancement of ADP-induced platelet aggregation (% to the control)	Enhancement of collagen-induced platelet aggregation (% to the control)
1	Etamsylate	2×10^{-3}	23.2 (20.5–24.6)	3.3 (2.6–4.3)	3.2 (2.4–4.5)
		10^{-3}	9.8 (7.2–12.7)	2.2 (1.6–3.3)	2.4 (1.2–2.7)
		0.5×10^{-3}	2.7 (1.3–5.4)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
2	Compound I	2×10^{-3}	0.0 (0.0–0.0) p=0.0001	5.7 (4.7–7.2) p=0.001	5.1 (4.8–6.4) p=0.003
		10^{-3}	0.0 (0.0–0.0) p=0.001	4.1 (3.8–6.3) p=0.002	3.9 (2.7–4.1) p=0.001
		0.5×10^{-3}	0.0 (0.0–0.0) p=0.001	2.2 (1.6–3.2) p=0.0001	2.1 (1.9–2.6) p=0.0001

p - difference in groups etamsylate versus compound I

Table 3: Influence of compound I and etamsylate on coagulogram *in vitro*

Indicator	Control	Etamsylate	p_1	Compound I	p_2	p_3
APTT, s	23.1 (21.6–24.7)	23.4 (22.7–24.8)	0.7	18.1 (17.5–19.3)	0.001	0.002
TT, s	27.2 (26.4–28.9)	28.3 (27.5–29.6)	0.2	26.5 (25.7–28.9)	0.4	0.3
PT, s	12.4 (11.5–13.9)	12.9 (11.5–14.3)	0.3	12.7 (11.8–14.5)	0.5	0.3
Fibrinogen, s	24.3 (22.5–26.7)	25.9 (24.7–26.3)	0.2	24.6 (23.5–25.9)	0.9	0.5

APTT: Activated partial thromboplastin time, TT: Thrombin time, PT: Prothrombin time, p_1 - etamsylate versus control, p_2 - compound I versus control, p_3 - etamsylate versus compound I

Table 4: Indicators of ADP- and collagen-induced platelet aggregation in rats following intraperitoneal injection of etamsylate and compound I into rats

No	Indicator	Control	Etamsylate	Compound I	p_2
1	Collagen, mm	54.8 (53.6–57.6)	65.8 (63.5–68.7) p_1=0.006	77.8 (74.9–79.1) p_1=0.002	0.0001
2	MPA (collagen), r.u.	6.4 (6.1–6.9)	9.2 (7.6–10.6) p_1=0.001	14.8 (12.7–15.2) p_1=0.0001	0.002
3	tg α.(collagen), s	36.7 (34.9–39.4)	35.9 (35.6–38.4) p_1=0.02	46.9 (45.7–49.8) p_1=0.0003	0.0001
4	ADP, mm	53.6 (51.8–58.5)	68.3 (66.2–69.7) p_1=0.002	79.4 (75.3–80.1) p_1=0.0002	0.0008
5	MPA (ADP), r.u.	6.2 (6.1–7.0)	8.7 (7.3–10.5) p_1=0.002	13.8 (11.6–14.7) p_1=0.0001	0.001
6	tg α (ADP), s	43.2 (40.6–44.1)	46.8 (45.4–48.3) p_1=0.001	57.9 (58.3–59.4) p_1=0.0002	0.004

p_1 - etamsylate or compound I versus control; p_2 - etamsylate versus compound I. r.u.: Relative units

Table 5: Indicators of hemostatic activity of etamsylate and compound I following intraperitoneal injection into rats

Model	Indicator	Control	Etamsylate	Compound I
I	Bleeding time, s	96.8 (94.3–98.7)	70.4 (70.1–74.6) p_1=0.002	63.7 (61.8–66.2) p_1=0.0003 p_2=0.001
	Δ weight of drapes, g	7.3 (7.1–8.4)	6.7 (5.9–7.3) p_1=0.2	3.5 (3.4–4.2) p_1=0.0002 p_2=0.0001
II	Bleeding time, s	137.4* (135.6–143.8)	137.9 (136.2–138.1) p_1=0.2	112.4 (110.5–113.8) p_1=0.002 p_2=0.001
	Δ weight of drapes, g	10.5* (8.7–11.3)	9.9 (8.5–11.1) p_1=0.3	6.4 (5.5–7.2) p_1=0.001 p_2=0.002

*p≤0.05 - Group I model versus Group II model for control. p_1 - etamsylate or compound I versus control; p_2 - etamsylate versus compound I. Model: I - intact rats, II - model of hypocoagulation induced by heparin injection

and 1.3 ($p \leq 0.001$) times, respectively. There was no record of positive impact of etamsylate on the reduction of test indicators in a systemic hypocoagulation. Compound I reduced the bleeding time and blood loss on average by 19.5% ($p \leq 0.001$) and 39.0% ($p \leq 0.001$) compared with groups control and etamsylate.

DISCUSSION

Etamsylate is a chemically 2,5-dihydroxybenzenesulfonic acid with N-ethylethanamine. Molecular formula is $C_{10}H_{17}NO_5S$, and its molecular weight is 263.33 g/mol. Etamsylate is completely soluble in water, methanol, and ethanol but partially soluble in methylene chloride [12].

Etamsylate is long enough used as a means to prevent and treat diapedetic hemorrhages, hemoptysis, melena, hematuria, and menorrhagia [13,14]. In addition, etamsylate is effective in the prevention and treatment of bleeding during operational period on interventions in the well-vascularized tissues, in such realms as otolaryngology, gynecology, obstetrics, urology, ophthalmology, plastic, and reconstructive surgery [6].

The main hemostatic effect of etamsylate is realized due to proaggregatory activity and potentiating of platelets adhesion. The research made by Sack and Dujovne shows that etamsylate provokes aggregation of platelets in platelet-enriched plasma, but such platelet aggregation is minor and reversible [15]. The study of biochemical prerequisites of proaggregation platelets effect of Okum *et al*. helped to determine that etamsylate enhances platelet aggregation and ATP release induced by arachidonic acid, thromboxane A_2, collagen, and calcium ionophore A23187 but not ADP and/or adrenaline [16].

The own findings established that the main effect of etamsylate falls on intact platelets. The gain of adhesive functions of platelets contributes to the increased risk of thrombosis, whereas etamsylate remains ineffective at the time of bleeding not associated with damage of parenchymatous organs [17,18].

The findings of the experimental work proved that the new bis(2-aminoethan-1-sulfonate) calcium shows hemostatic activity that exceeds the values of etamsylate both *in vitro* and *in vivo*. The compound I activity data *in vivo* fully corresponds to the data obtained at the stage *in vitro*. Compound I showed proaggregatory activity after intraperitoneal injection in laboratory rats, more efficient than etamsylate by reducing the volume of blood loss and bleeding time on the models of parenchymal bleeding in the standard terms and conditions heparin-induced hypocoagulation. It should be noted that at the time of induced platelet aggregation, the compound I has a more selective effect on activated platelets, increasing the overall hemostatic potential due to the existing procoagulation effect, that is, particularly effective under the original existing hypocoagulation.

CONCLUSION

The results of the preclinical studies reveal the potentially high systemic hemostatic activity of bis(2-aminoethan-1-sulfonate) calcium and urge further study of this compound and its analogs to create on their basis highly effective systemic hemostatic agents.

AUTHORS' CONTRIBUTION

The article is a product of the intellectual environment of the whole team and that all members have contributed in various degrees to the analytical methods used, to the research concept, and to the experiment design.

CONFLICTS OF INTEREST

All authors have none to declare.

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