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Antithrombotic Activity of a Novel Diazepino[1,2-α] Benzimidazole Derivative on Arterial Thrombosis Model in Rats without Concomitant Pathology and in Rats with Experimental Myocardial Infarction

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Antithrombotic activity of a novel tricyclic derivative of diazepino[1,2-α]benzimidazole (DAB-15) was examined on the model of arterial thrombosis developed in rats without concomitant pathology and in rats with experimental myocardial infarction. DAB-15 demonstrated high antithrombotic efficacy in modeled thrombosis of carotid artery in rats without the concomitant pathology surpassing that of the reference drugs acetylsalicylic acid and clopidogrel by 5.1 and 4.8 times, respectively. In rats with experimental noncoronary myocardial infarction, DAB-15 increased the thrombus formation time by 86.2% in comparison with experimental control level in non-treated rats with similar myocardial infarction.

Key Words: *DAB-15 agent; thrombosis; myocardial infarction; antithrombotic action*

In 2016, WHO documented more than 25,000,000 thrombotic complications such as coronary heart disease, acute coronary syndrome, ischemic stroke, and abnormalities of peripheral circulation in the extremities [5,12]. In light of this, prevention and treatment of these diseases are the most urgent problems of modern medicine.

Intravascular thrombosis is provoked by adhesion and aggregation of the platelets; these processes play the key role in provoking this pathology. To prevent and/or treat thrombosis, the clinicians widely use drugs that block activation of the platelet phase of hemostasis [7]. Of them, especially popular are acetylsalicylic acid (ASA), a platelet cyclooxygenase inhibitor, and clopidogrel, a blocker of platelet $P2Y_{12}$ receptors [9,11]. However, these drugs do not always exert the necessary action, and sometimes they provoke various side effects such as gastrointestinal toxicity, hemorrhage, *etc*. [6].

Previously, the search for biological activity of heterocyclic compounds yielded agents with high antiaggregant activity [1,4,13]. Of them, a novel derivative of diazepino[1,2-α]benzimidazole (DAB-15) that presumably inhibits the synthesis of thromboxane A_2 demonstrated a higher antithrombotic activity both *in vivo* and *in vitro* in comparison with the reference drugs ASA and clopidogrel [2].

This work was designed to study the antithrombotic action of DAB-15 on the model of carotid arterial thrombosis in rats without concomitant pathology and in animals with experimental noncoronary myocardial infarction.

MATERIALS AND METHODS

Experiments were carried out on male random-bred albino rats (n=72, body weight 250-300 g). The animals were maintained under vivarium conditions with a natural day/night cycle at 22-24°C and relative air

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humidity of 40-50% in compliance to GOST R 50258-92 and received standard food. All procedures were carried out in strict adherence to The Principles of Good Laboratory Practice during Preclinical Studies in the Russian Federation (GOST R 51000.3-96 and 51000.4-96), European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986); and Directive No. 267 of Ministry of Health of Russian Federation (On Establishing the Rules of Laboratory Practice; June 19, 2003). The rats were sacrificed in compliance with The International Guiding Principles for Biomedical Research Involving Animals (US National Research Council, 1997). The experimental protocols were approved by Bioethics Committee of Volgograd State Medical University (protocol No. 94-2015). The study was carried out in compliance to Methodical Recommendations on Testing The Hemostasis-Affecting Drugs [3]. During the study, the rats were healthy; they did not modify behavior, the sleep-wakefulness regimen, or appetite. Prior to experiments, the rats were fasting for 24 h with water *ad libitum*.

At the first stage, the antithrombotic activity of DAB-15 was examined on the model of arterial thrombosis in rats without concomitant pathology. The model was developed by surface application of 50% ferric chloride onto carotid artery [8]. The reference drugs were ASA (Sigma) and clopidogrel (Dr. Reddy's). All tested agents were dissolved in distilled water and administered intragastrically in a single dose 2 h prior to experiments. DAB-15 was given in the doses of 9, 17, and 33 mg/kg (the latter is equimolar to 19 mg/kg ASA). ASA was used in the doses of 19, 100, and 150 mg/kg. Clopidogrel was employed in the doses of 44 mg/kg (equimolar to 19 mg/kg ASA), 88, and 122 mg/kg. The control rats were administered with equivalent volume of the solvent. The rats were narcotized with chloral hydrate (400 mg/kg intraperitoneally), thereupon the carotid artery was isolated. A cotton disc moistened with 50% ferric chloride was placed onto the artery. The blood flow was measured with ultrasonic dopplerography using a Minimax-Doppler-K system (Minimax). The ultrasound transducer was mounted at the distance of 1 cm from the cotton disk. The blood flow was recorded until complete occlusion of the artery occurred. The antithrombotic activities of DAB-15 and the reference drugs were assessed with thrombus formation time (TFT) equal to application (exposure) time needed for artery occlusion.

At the second stage, the antithrombotic activity of DAB-15 was examined on a similar model of arterial thrombosis in rats with noncoronary myocardial infarction. This infarction was modeled with isoprenaline (85 mg/kg) injected two times subcutaneously into the region of inguinal fold with the interinjection time of 24 h [10]. A single dose of DAB-15 was administered intragastrically 2 h prior to provoking arterial thrombosis but not earlier than 24 h after the last injection of isoprenaline in the dose of 5.6 mg/kg equal to ED_{50} for *in vivo* antiaggregant activity established in previous studies [2]. The control rats received the equivalent volume of the solvent.

The data were analyzed statistically using Mann—Whitney *U* test for paired data, Microsoft Office Excel 2007, and GraphPad Prism 5.0 software. ED_{50}, which suppressed platelet aggregation by 50%, was calculated with regression analysis. The results are summarized as m±SEM.

RESULTS

At the first stage, the antithrombotic activity of DAB-15 and the reference drugs was examined on the model of arterial thrombosis in rats without experimental myocardial infarction. When used in the doses of 9, 17, or 33 mg/kg, DAB-15 significantly increased TFT in carotid artery by 8.2, 40.4, or 62.3%, respectively (Table 1). ED_{50} of DAB-15 was 23.4 mg/kg.

The reference drug ASA significantly increased TFT by 16.8, 35.7, or 68.4% when administered in the doses of 19, 100, or 150 mg/kg, respectively (Table 1). ED_{50} of ASA was 119 mg/kg. Similarly, clopidogrel (44, 88, or 122 mg/kg) significantly increased TFT by 12.5, 35.7, or 54.6%, respectively (Table 1).

At the second stage, we examined antithrombotic activity of DAB-15 under the conditions of experimental noncoronary myocardial infarction. The experimental infarction *per se* decreased TFT from the control value of 19.4 min to 14.5 min attesting to enhanced thrombogenic potential of the blood in animals with myocardial infarction. In such rats, a single intragastric administration of DAB-15 (5.6 mg/kg) increased TFT by 86.2% (Table 2).

An important part of preclinical examination of antiaggregant drugs is the study of their specific activity not only in healthy animals, but also in those with experimental pathology. The model of carotid arterial thrombosis induced by ferric chloride is based on platelet activation. The development of thrombosis in this model results from oxidative stress characterized by severe damage to endotheliocytes. Damaged endotheliocytes release physiological activators of platelets, thereby elevating the thrombogenic potential of the blood.

In the employed model of carotid arterial thrombosis induced by ferric chloride, DAB-15 surpassed the reference drugs ASA and clopidogrel in the value of EC_{50} by 5.1 and 4.8 times, respectively (Table 1).

TABLE 1. Antithrombotic Activity of DAB-15, ASA, and Clopidogrel in Rat Model of Carotid Artery Thrombosis Induced by Ferric Chloride (m±SEM, *n*=6)

Substance	Dose, mg/kg	TFT, min	TFT increment, %	ED_{50}, mg/kg
Solvent (control)	—	19.4±0.4	—	
DAB-15	9	21.0±0.6*	8.2±2.9*	23.4
	17	27.3±1.0*	40.4±5.3*	
	33	31.5±0.6*	62.3±3.3*	
ASA	19	20.8±1.3*	16.8±6.2*	119
	100	26.3±1.4*	35.7±7.5*	
	150	32.7±3.2*	68.4±16.4*	
Clopidogrel	44	21.8±0.6*	12.5±3.3*	113.9
	88	26.3±0.6*	35.7±3.3*	
	122	30.0±0.4*	54.6±2.1*	

Note. Here and in Table 2: *n* is the amount of rats in each group; *$p \leq 0.05$ in comparison with the control.

TABLE 2. Antithrombotic Activity of DAB-15 in Rat Model of Carotid Artery Thrombosis Induced by Ferric Chloride under Experimental Noncoronary Myocardial Infarction (m±SEM, *n*=6)

Group	Dose, mg/kg	TFT, min	TFT increment, Δ%
Solvent (rats without myocardial infarction)	—	19.4±0.4	—
Myocardial infarction+solvent	—	14.5±0.7	—
Myocardial infarction+(DAB-15)	5.6	27.0±1.0*	86.2±6.7

In rats with experimental noncoronary myocardial infarction, the complete occlusion of carotid artery induced by a thrombotic agent (ferric chloride) became more rapidly than in the rats without concomitant pathology attesting to enhanced thrombogenic potential of the blood in animals with experimental myocardial infarction.

Thus, under the conditions of noncoronary myocardial infarction DAB-15 (5.6 mg/kg) increased TFT by 86.2% thereby demonstrating a greater antithrombotic activity in comparison with that observed in the rats without concomitant pathology.

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