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Antithrombotic Activity of the Antiplatelet Agent Angipur on the Model of Arterial Thrombosis in Rats with Isoproterenol-Induced Myocardial Infarction

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We studied the effect of Angipur on the process of experimental thrombosis induced by damage to the carotid artery wall by surface application of 50% ferric chloride (III) solution in rats without comorbidities and with isoproterenol-induced myocardial infarction. In animals without comorbidities, Angipur administered intravenously was 1.2 times less effective, in terms of ED_{50}, than the well-known inhibitor of GPIIb/IIIa platelet receptors tirofiban. However, under conditions of non-coronary myocardial infarction, Angipur significantly prolonged the time of thrombus formation and exhibited 1.4-fold higher activity than the reference drug tirofiban.

Key Words: *arterial thrombosis; Angipur; tirofiban; isoproterenol-induced myocardial infarction*

Antiplatelet agents are used for the treatment of acute cardiovascular diseases [14]. The GPIIb/IIIa receptor is the final common platelet aggregation pathway independent of the agonist and is an ideal therapeutic target for blocking arterial thrombosis. Multiple randomized clinical trials have demonstrated that GPIIb/IIIa platelet receptor inhibitors abciximab, eptifibatide, and tirofiban significantly reduce the risk of death or recurrent myocardial infarction [7]. The clinical effectiveness of these drugs is associated with the prevention of aggregation of activated platelets mediated by fibrinogen. This mechanism of antiplatelet action is very attractive for preventing thrombotic complications.

It is known that heterocyclic compounds exhibit anti-thrombogenic properties [1,2,6]. Angipur is a new synthetic purine derivative that exhibits the properties of a platelet GPIIb/IIIa receptor antagonist [4]. However, its antithrombotic activity in intact animals and animals with non-coronary myocardial infarction, when the thrombogenic potential of the blood increases, has not been studied.

This research is aimed at evaluation of antithrombotic activity of the antiplatelet compound Angipur on the model of arterial thrombosis of the carotid artery in rats without comorbid pathologies and in animals with isoproterenol-induced myocardial infarction.

MATERIALS AND METHODS

The experiments were carried out on 60 sexually mature outbred male rats weighing 250-300 g obtained from the nursery of Scientific Center of Biomedical Technology of the Federal Medical-Biological Agency of Russia). The animals were kept in a vivarium at 22-24°C, relative humidity 40-50%, and natural light conditions and received standard diet (GOST R 50258-92). In working with animals, we were guided by the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, 1986). Studies of the drugs were carried out by the requirements [3].

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The effects of the study compound Angipur (3-methyl-8-(piperazine-1-yl)-7-(thietane-3-yl)-1-ethyl-1H-purine-2,6(3H,7H)-dione hydrochloride) were compared to those of tirofiban (Correvio), a synthetic non-peptide inhibitor of GIIb/IIIa platelet receptors for intravenous administration. All the drugs were dissolved in 0.9% NaCl.

For modeling myocardial infarction, the rats were injected with L-isoproterenol (Sigma) subcutaneously in the inguinal fold area in a dose of 85 mg/kg twice with an interval of 24 h [11]. Twenty minutes before administration of the drugs, the rats were anesthetized with chloral hydrate (400 mg/kg, intraperitoneally). Angipur and tirofiban were administered intravenously in doses equal to ED_{50} for antithrombotic activity determined in animals without comorbidities; in 5 min, the antithrombotic effect was evaluated using a rat model of carotid artery thrombosis induced by surface application of 50% ferric chloride (III) solution [10].

Arterial thrombosis of the carotid artery in rats was modelled by surface application of a 50% solution of ferric chloride (III) [10]. Angipur and tirofiban were administered to rats (6 animals per group) intravenously once 5 min before induction of thrombosis in pharmacologically active doses of 0.9 and 0.5 mg/kg, respectively (which provide a 50% antiplatelet effect on the ADP-induced aggregation model *in vivo*). Then, for calculation of ED_{50}, Angipur was also administered in doses of 0.5 and 1.5 mg/kg, and tirofiban in doses of 0.75 and 1 mg/kg (6 animals per dose). The rats were anesthetized with chloral hydrate (400 mg/kg intraperitoneally) 20 min before the administration of the drugs, then the carotid artery was dissected as described previously [5].

In addition, two control groups were formed: rats with intravenous administration of saline solution followed by modelling of carotid artery thrombosis and rats with combined pathology (myocardial infarction and administration of saline solution 5 min before induction of thrombosis).

The process of thrombus formation was evaluated using a Minimax-Doppler-K with an ultrasonic sensor (Minimax). The blood flow was recorded until complete occlusion of the vessel with the thrombus. The antithrombotic activity of the tested drugs was evaluated by their influence on the time of blood clot formation.

Statistical processing of the obtained results was carried out by one-way ANOVA using the GraphPad Prism 8.0 and Microsoft Excel 2020 software. The data are presented as $M \pm m$. The differences were considered significant at $p \leq 0.05$.

RESULTS

Application of solution of ferric chloride (III) on the artery induced thrombus formation assessed by the time of complete occlusion of the vessel (blood flow arrest). In the control group, the time of blood clot formation was 19.6 min (Table 1). Angipur and the reference drug tirofiban in pharmacologically active doses prolonged the time of blood clot formation to 25.8 and 22.3 min, respectively. When studying the dose-dependent antithrombotic activity, Angipur in a dose of 0.5 mg/kg increased the time to complete occlusion of the carotid artery to 20.1 min, and in a dose of 1.5 mg/kg — to 30.8 min. The ED_{50} of the antithrombotic activity of Angipur was 1.33 mg/kg.

Analysis of the dose-dependent activity of tirofiban showed that this drug in doses of 0.75 and 1 mg/kg increased the time of thrombus formation to 25.3 and 31.6 min, respectively. The ED_{50} of the antithrombotic activity of tirofiban was 0.93 mg/kg.

Thus, Angipur after single intravenous administration in a pharmacologically active dose to animals without comorbidities was 2.2-fold more effective than the reference drug tirofiban, but in terms of ED_{50} it was

TABLE 1. Antithrombotic Activity of Angipur and Reference Drug Tirofiban after Intravenous Administration on the Model of Carotid Artery Thrombosis in Rats ($M \pm m$)

Drug	Dose, mg/kg	Δ% increase of time to occlusion relative to the control	ED_{50}, mg/kg
Control 1 (model of thrombosis)	—	—	—
Angipur	0.5	3.5±1.3	1.33
	0.9	33.2±2.1*	
	1.5	58.5±2.6*	
Tirofiban	0.5	15.1±1.6*	0.93
	0.75	30.6±1.7*	
	1.0	62.8±3.9*	

Note. *$p<0.005$ in comparison with the control (one-way ANOVA). Six rats were used for each dose.

TABLE 2. Antithrombotic Activity of Angipur and Reference Drug Tirofiban after Intravenous Administration on the Model of Carotid Artery Thrombosis in Rats with Isoproterenol-Induced Myocardial Infarction (*M*±*m*)

Experimental condition	Dose, mg/kg	Time of thrombus formation, min
Control 1 (model of thrombosis)	—	19.6±0.3
Control 2 (myocardial infarction+thrombosis)	—	14.7±0.5*
Angipur (rats with comorbid pathology)	1.33	26.7±2.0^{+o}
Tirofiban (rats with comorbid pathology)	0.93	19.7±0.9^{+}

Note. $p<0.005$ in comparison with *control 1, $^{+}$control 2, ^{o}tirofiban (one-way ANOVA). Six rats were used for each dose.

inferior to the known inhibitor of platelet GPIIb/IIIa receptors (by 1.2 times).

At the next stage, the antithrombotic effect of Angipur was studied in rats with isoproterenol-induced myocardial infarction.

Isoproterenol is a synthetic catecholamine and a β-adrenergic receptor agonist that causes severe stress in the myocardium and can induce myocardial infarction when administered in supramaximal doses [13]. In the rat model, isoproterenol causes myocardial necrosis, which leads to cardiac dysfunction, LPO activation, accumulation of lipoperoxides, and changes in activities of cardiac enzymes and antioxidants [12]. The development of isoproterenol-induced myocardial infarction can be mediated by generation of highly cytotoxic free radicals due to autoxidation of catecholamines. These free radicals can attack polyunsaturated fatty acids in the membranes with the formation of peroxyl radicals. As a result of these processes, the thrombogenic potential of the blood increases. The pathophysiological and morphological aberrations that occur in the hearts under conditions of modeled myocardial infarction in rats are comparable to those that occur with human myocardial infarction [8,9]. Due to the fact that platelet-dependent thrombosis plays an important role in the pathophysiology of myocardial infarction, we used a model of platelet-initiated arterial thrombosis.

Analysis of the antithrombotic activity of Angipur under conditions of isoproterenol-induced myocardial infarction showed that complete occlusion of the carotid artery caused by application of the thrombotic agent ferric chloride (III) solution in control animals with combined pathology occurred significantly faster than in rats without comorbidity. Thus, the mean time of carotid artery occlusion in the control group of animals that were simulated carotid artery thrombosis was 19.4 min, and the time of thrombus formation in the group of animals with experimental myocardial infarction decreased to 14.7 min, which indicates an increased thrombogenic potential of the blood (Table 2). Angipur after single intravenous administration to rats with comorbid pathology showed high antithrombotic activity: it significantly prolonged the time of thrombus formation by 81.6% relative to the control group with isoproterenol-induced myocardial infarction and its activity exceeded that of the reference drug tirofiban by 1.4 times (Table 2).

Since platelets play a key role in the pathogenesis of arterial thrombosis, the revealed high antithrombotic activity of Angipur on the model of ferric chloride (III)-induced thrombosis, both without and with isoproterenol-induced myocardial infarction, is associated with the blockade of the final pathway of platelet aggregation, inhibition of GPIIb/IIIa receptors on the platelet surface, which bind circulating fibrinogen. The obtained data indicate a significant antithrombotic potential of Angipur in the treatment of arterial blood clots, in particular during the acute period of myocardial infarction.

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