Antiaggregant Activity of a New Benzimidazole Derivative
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Antiaggregant activity of a new tricyclic benzimidazole derivative, RU-891 compound, was studied on the model of ADP-induced platelet aggregation in vitro and intravascular platelet aggregation in vivo. We evaluated the effect of this substance on blood coagulation potential. Antiaggregant agent acetylsalicylic acid was used as the reference drug. RU-891 produced a dose-dependent antiaggregant effect in vivo and in vitro that exceeded the effect of the reference drug. This compound did not modulate blood coagulation potential.

Key Words: antiaggregant effect; platelet aggregation; RU-891 compound; acetylsalicylic acid

Thrombus formation plays a key role in the pathogenesis of ischemic changes in various organs and systems of the body. This state is primarily related to increased aggregation activity of platelets and intravascular thrombus formation [5,7,11]. The involvement of platelets in hemostasis is associated with their ability for adhesion and aggregation; release of the storage granule content; adsorption, deposition, and transport of biologically active substances; and endothelial supporting function [10]. Therefore, the use of antiplatelet drugs for various manifestations of activation of the platelet component of hemostasis can reduce the risk of thrombotic complications.

Benzimidazole derivatives belong to privileged molecules that can be used to synthesize new substances with various biological properties [1,8]. Previous studies at the Volgograd State Medical University showed that substituted heterocyclic nitrogen-containing systems inhibit platelet aggregation; much attention was paid to studying the compounds with antiaggregant activity [4]. A new active substance RU-891 was revealed.

Here we studied the effect of RU-891 compound (9-[2-(3,4-dioxyphenyl)-2-oxoethyl]-2,3-dihydroimidazo[1,2-a]benzimidazole hydrobromide; Research Institute of Physical and Organic Chemistry, Southern Federal University) [3] on platelet aggregation in vitro and in vivo and coagulation component of hemostasis.

MATERIALS AND METHODS
Experiments were performed on rabbits (n=5, body weight 3-3.5 kg) and male outbred albino rats (n=55, body weight 270-300 g). The animals were maintained in a vivarium (22-24°C, humidity 40-50%) under the natural light/dark cycle and standard diet (GOST R 50258-92). The experiment was conducted in accordance with the GLP Rules for Preclinical Studies in the Russian Federation (State Standard R 51000.3-96 and 1000.4-96) and regulations and international recommendations of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (1997).

The effect of test substances on platelet aggregation in vitro was studied by the method of Born with modifications of V. A. Gabbasov [2]. The study was performed with rabbit platelet-rich plasma. RU-891 compound and reference drug acetylsalicylic acid were examined in a dose range of 10^-3-10^-5 M. ADP (Serva), epinephrine (final concentration 5 μM, Sigma), collagen (20 μg/ml, Renam), thrombin (0.5 U/ml, Sigma), arachidonic acid (50 μM, Sigma), and thromboxane
receptor agonist U46619 (3 μM, Sigma) were used as inducing agents for platelet aggregation.

Modeling and studying the degree of intravascular platelet aggregation were performed as described elsewhere [9]. To evaluate the effect of test compounds on intravascular platelet aggregation, RU-891 in doses of 10, 25, and 50 mg/kg was given perorally to nembutal-anesthetized rabbits (50 mg/kg). The reference drug acetylsalicylic acid was administered in doses of 125, 250, and 350 mg/kg. The aggregation-inducing agent ADP in a dose of 1 mg/kg was injected intravenously.

For evaluation of agent activity, Δ% inhibition of platelet function was measured. The effective concentration or dose, which caused a 50% inhibition of platelet aggregation (EC50 and ED50), was calculated by the regression analysis.

Toxicity of RU-891 compound was studied in accordance with the requirements and instructions of the Federal Service on Surveillance in Healthcare and Social Development [6]. Acute toxicity was evaluated on 75 male outbred albino mice weighing 20-22 g (intraperitoneal treatment). The death of animals was recorded for 2 weeks. The toxicity index (LD50) was calculated by the Litchfield–Wilcoxon method.

The effect of test compounds on rat blood coagulogram was studied chronometrically on a SOLAR turbidimetric blood coagulometer (CGL 2110). The influence of RU-891 compound on coagulation was evaluated in a dose corresponding to ED50 (estimated on the model of intravascular platelet aggregation).

The results were analyzed by Mann–Whitney test (Microsoft Excel 2006 software).

RESULTS

RU-891 compound and reference drug acetylsalicylic acid had a concentration-dependent antiaggregant effect on the in vitro model of ADP-induced aggregation. EC50 of RU-891 compound and acetylsalicylic acid were 1.3×10^-4 and 7.1×10^-4 M, respectively. The effective concentration and therapeutic index of RU-891 compound were higher than those of acetylsalicylic acid (by 5.5 and 10 times, respectively; Table 1).

![Fig. 1. Effects of RU-891 compound (1) and acetylsalicylic acid (2) on intravascular platelet aggregation induced by 1 mg/kg ADP. Ordinate: Δ% antiaggregant activity of substances.](image)

Studying the antiaggregant properties of RU-891 compound on the model of intravascular platelet aggregation showed that this substance has a potent dose-dependent inhibitory effect on platelet aggregation in vivo. The antiaggregant effect of RU-891 compound in doses of 10, 25, and 50 mg/kg was 15.6, 48.8, and 74.6%, respectively. EC50 of this substance was 24.01 mg/kg.

Acetylsalicylic acid in doses of 125, 250, and 350 mg/kg had an inhibitory effect on platelet aggregation (by 28.6, 57.8, and 89.2%, respectively). EC50 of acetylsalicylic acid was 192 mg/kg. Therefore, ED50 of RU-891 compound was 8-fold higher than that of the reference drug (in vivo study; Fig. 1, Table 2).

The platelet membrane expresses receptors for various aggregation-inducing agents, which hinders a selective pharmacological regulation of platelet activity. These inductors interact with specific receptors on the plasma membrane of platelets, which contributes to further changes. We studied whether RU-891 compound can serve as a competitive antagonist for inducing agents of platelet aggregation.

RU-891 compound more significantly inhibited the aggregation of platelets after treatment with study agonists (as compared to an inhibitory effect of the substance on ADP-induced platelet aggregation; Table 1).

### TABLE 1. Effects of RU-891 and Acetylsalicylic Acid on ADP-Induced (5 μM) Rabbit Platelet Aggregation in Vitro (M±m)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Platelet aggregation, Δ% of the control</th>
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<tbody>
<tr>
<td></td>
<td>10^-3 M</td>
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<tr>
<td>RU-891</td>
<td>84.8±3.1**</td>
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<tr>
<td>Acetylsalicylic acid</td>
<td>53.40±4.23*</td>
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Note. *p<0.05 and **p<0.001 compared to the control.