Original article:

Effects on thrombocytic hemostasis of a new derivate indolinone

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Abstract:

Objective. Specific activity of an antiplatelet drug of indolinone series (codenamed DI) was studied in vitro in a model of ADP-induced platelet aggregation in vitro and in vivo in a model of streptozotocin-induced diabetes mellitus in rats. ***Material and Methods.*** Acetylsalicylic acid and dipyridamole were used as reference drugs. In vitro tests have demonstrated that DI exhibits antiplatelet activity in a wide range of concentrations ($0{,}75\times10^{-6}$ – 1.5×10^{-5} M, $p<0{,}05$), being comparable to acetylsalicylic acid and dipyridamole. *In vivo* tests have demonstrated dose-dependent antiplatelet activity of DI in doses of 2,5 – 20 mg/kg (21-14 %). ***Results and Discussion.***Increasing the dose of DI above 10 mg/kg doesn't increase its antiplatelet activity. After multiple oral administration to rats with streptozotocin-induced diabetes mellitus in 10 mg/kg dose, DI has exhibited antiplatelet activity, reducing the platelet aggregation rate to that of the control group ($p<0{,}05$).***Conclusion***. Thus, DI isapromisingcompound for furtherdevelopmentof an antiplatelet drug with new mechanism of action

Keywords: antiplatelet, indolinone derivative, acetylsalicylic acid, dipyridamole, platelet aggregation.

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Introduction

Antiplatelet therapy is very important in preventing cardiovascular diseases[1]. However, in addition to the well-known adverse effects of the most popular antiplatelet drugs acetylsalicylic acid (ASA) and clopidogrel a new problem arose in recent years: antiplatelet drug resistance[2]. According to modern guidelines, double antiplatelet therapy uses the combination of aspirin and clopidogrel, which often provoke resistance. According to different authors, the percentage of patients who may be resistant to ASA and clopidogrel varies from 15 to 40 %[3,4]. The underlying mechanisms of drug resistance to ASA and clopidogrel combination are the following: bioavailability changes; changes in platelet sensitivity to ADP and collagen; increase in prostaglandin H^2 intake from endothelial cells and monocytes by platelets; increase in the amount of immature reactive platelets (for ASA) and changes in their functional state in certain diseases (e.g. diabetes mellitus); differences in liver function (since clopidogrel is a prodrug); genetic polymorphism of cyclooxygenase and monooxygenase system enzymes[5-8]. Solving the problem of antiplatelet drug resistance can be achieved, among other means, by developing drugs with new mechanism ofaction. A new promising compound is an indolinone derivative codenamed DI. Compounds of this class are inducers of cytosol guanylate cyclase[9].

Study goal – Assess the action of the new compound DI on platelet aggregation *invitro* and *invivo* in intact animals with experimental diabetes mellitus[10].

Materials and methods

Test compound: DI pharmaceutical substance(2-

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[2-[(5RS)-5-(hydroxymethyl)-3-methyl-1,3-oxazolidine-2-yliden]-2-cyanoethylidene]-1H-indol-3(2H)-one).

Experiments were performed using 67 out bred male Wistar rats with body weight 300–350 g. The animals were obtained from Experimental Biological Model Department of Goldberg Research Institute of Pharmacology and Regenerative Medicine. The rats were kept in standard plastic cages (VELAZ) on fine wood chip bedding, 5 animals per cage. Air temperature in the animal facility was 20–23 °C, humidity – maximum 50 %, air circulation (outtake: intake) – 8:10, lighting (day: night) – 1:1. Animal feeding was performed in accordance with regulatory documents[11].

The first battery of *in vivo* tests was performed in 40 healthy rats (5 animals per group). Five groups have received the test articles once orally as a suspension in 1 ml of 1% carboxymethyl cellulose: one group -DI in 1, 2.5, 5, 10 and 20 mg/kg dose, two other groups –ASA anddipyridamole in 10 mg/kg dose[12]. Control group animals received an equal volume of carboxymethyl cellulose. Blood was sampled 3 hours after drug administration.

In the second battery of tests diabetes mellitus was induced in animals by single administration of streptozotocin in 40 mg/kg dose as a solution in 1 ml citrate buffer (pH 4.0). Testing was performedin 15 rats, 10 of which were administered streptozotocin and 5 remained intact. Blood glucose level was controlled using test strips with SmartScan glucometer (Lifescan, USA) before and 3 days after streptozotocin administration. Testarticlegroup (n=5) was administered DI in 10 mg/kg dose (preliminary experiments in intact animals have shown this is the minimal effective dose) as a suspension in 1 ml of 1% carboxymethyl cellulose for 7 days. Experiments used animals with blood glucose concentrations above 10 μmol/L. Animals of control (n=5) and intact (n=5) groups have received the same volume of1% carboxymethyl cellulose for the same amount of time. The last administration was 3 hours before blood sampling.

In *invitro* tests DI was administered into the plasma of 12 intact donor rats 10 minutes before the start of aggregation in the following end concentrations: 0.75×10^{-7}M, 0.75×10^{-6}M, 0.75×10^{-5}Mи 1.5×10^{-5}M. Reference drugs ASA and dipyridamole were also administered into the plasma 10 minutes before the start of aggregation in the following end concentrations: 0.75×10^{-5}Mand 1.5×10^{-5}M[12]. DI and dipyridamole were dissolved in dimethyl sulfoxide, ASA was dissolved in water

To assess anti aggregant effects of the test compounds, blood was taken from animals from common carotid artery under ether sedation. 3.8% solution of sodium citrate was used as a stabilizer with blood in volume ratio 1:9. Platelet aggregation was assessed by Born's nephelometric method. Production of platelet-rich plasma (PRP) and platelet-poor plasma (PPP) and platelet counting was done using the standard method in13. To assess platelet count in PRP the platelet count was standardized by diluting the PRP with the required amount of PPP to 400±

Group		Platelet aggregation rate, %
Control		25±1
Dimethyl sulfoxide		23±1
DI	0.75×10^{-7} M	22±1
	0.75×10^{-6} M	20±1*
	0.75×10^{-5} M	17±1* #
	1.5×10^{-5} M	15±1* #
ASA, 0.75×10^{-5} M		12±1*
ASA, 1.5×10^{-5} M		9±1*
Dipyridamole, 0.75×10^{-5} M		16±1*
Dipyridamole, 1.5×10^{-5} M		13±1*

*Note: * – $p<0.05$ compared to control; # – $p<0,05$ compared to ASA in same concentrations.*

DI after single oral administration in 2.5, 5, 10 and 20 mg/kg to intact rats has exhibited significant antiplatelet activity. In dose range 2.5-20 mg/kg platelet aggregation was 19-46 % lower than in control group. DI was as potent as ASA and dipyridamole (Table 2). DI in 1-5 mg/kg range has dose-dependent effect on platelets, increasing the administered dose from 5 to 20 mg/kg didn't change its effect.

Table 2.Effect of single oral administration of different doses of DI,ASA (10 mg/kg) anddipyridamole (10 mg/kg) on ATP-induced platelet aggregation

Group		Platelet aggregation rate, %
Control		26±1
DI, mg/kg	1	25±1
	2.5	21±1*
	5	15±1*
	10	13±1*
	20	14±1*
ASA, 10 mg/kg		10±1*
Dipyridamole, 10 mg/kg		12±1*

*Note: * – p<0.05 compared to control.*Table

3.Effectofmultiple (7 times) oral administration of DI (10 mg/kg) on ADP-induced platelet aggregation in rats with streptozotocin-induced diabetes mellitus (M ± m)

Group	Plateletcount, thousand/ml	Platelet aggregation rate, %
Intact animals (n=5)	658±19	23±2
Control group (n=5)	661±13	36±2*
DI (n=5)	651±13	27±2

*Note: * – p<0.05 compared to intact animals.*

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