Effect of Inhibitors of Arachidonic Acid Conversion in Platelets on Thrombin—Fibrinogen Interaction and Thrombin Tolerance

E. M. Shapovalova and A. J. Rudzevich

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 10, pp. 408-409, October, 2007
Original article submitted March 14, 2007

Experiments on 200 rats showed that inhibitors of arachidonic acid conversion capable of reducing coagulation activity of platelets decrease the content of markers for thrombin--fibrinogen interaction in the plasma and, therefore, decelerate intravascular blood coagulation and increase thrombin tolerance.

Key Words: *arachidonic acid; intravascular blood coagulation*

Variations in the rate of thrombin—fibrinogen interaction (TFI) reflect the degree of continuous intravascular blood coagulation (CIBC) and determine the predisposition to thrombosis or hypocoagulation [2,3,11,12]. The rate of TFI and thrombin tolerance depend on coagulation activity of platelets [3,10], which is determined by the rate of arachidonic acid conversion [8,9].

Here we studied the effect of inhibitors of arachidonic acid conversion mepacrine, aspirin, and dazoxiben (inhibitors of phospholipase, cyclooxygenase, and thromboxane synthase, respectively [9,10]) on TFI.

MATERIALS AND METHODS

Experiments were conducted on male outbred albino rats weighing 145±10 g. Mepacrine, aspirin, or dazoxiben was introduced into semisolid food. In preliminary experiments, the inhibitors were administered at increasing doses. Blood samples were taken at 6-h intervals to evaluate the half-inhibition concentration (IC_{50}) by inhibition of ADP-induced aggregation [4]. The blood was sampled from the jugular vein of diethyl ether-narcotized rats as described elsewhere [1]. We measured plasma content of TFI markers including fibrin degradation products (FDP), soluble fibrin monomer complexes (SFMC) [7], and D-dimers (D-dimer test kit, Rosche), and thrombin tolerance. The samples were taken 24 h after administration of the inhibitor in IC_{50}. Thrombin tolerance [6] was directly evaluated by animal mortality (or survival) rate after intravenous injection of thrombin in LD_{50} (1 ml/kg, thrombin activity 17 sec by the rate of clotting of 2% fibrinogen). The study was performed on intact animals and treated rats (24 h after administration of mepacrine, aspirin, or dazoxiben in LD_{50}).

The results were analyzed using statistical tests for small samples. The differences were significant at $p<0.05$. Intensive parameters were compared by the method of alternative variation.

RESULTS

IC_{50} for mepacrine, aspirin, and dazoxiben were 12.0±0.6, 150±4.6, and 30.0±1.1 mg/kg, respectively. ADP-induced aggregation decreased 24 h after administration of these compounds (by 49.2±1.3, 50.6±1.4, and 53.7±1.6%, respectively).

The contents of platelet factor 3, platelet factor 4, FDP, SFMC, and D-dimers decreased by 13.6,

Department of Biochemistry, Tyumen Medical Academy, Russian Ministry of Health. ***Address for correspondence:*** biochem@tgma.info. E. M. Shapovalova

0007-4888/07/1444-0527

TABLE 1. Content of TFI Markers and Thrombin Tolerance in Rats Receiving Mepacrine, Aspirin, and Dazoxiben

Parameter	Control (*n*=15)	Mepacrine, 12 mg/kg (*n*=10)	Aspirin, 150 mg/kg (*n*=10)	Dazoxiben, 30 mg/kg (*n*=10)
Platelet factor 3, %	82.5±1.0	71.3±0.9*	68.2±0.9*	63.4±0.9*
Platelet factor 4, sec	2.30±0.01	1.90±0.01*	1.60±0.02*	1.50±0.01*
FDP, mg/ml	15.3±1.1	12.1±0.8*	11.1±1.1*	10.2±0.6*
SFMC, μg/ml	22.0±0.9	18.3±0.7*	16.8±0.7*	15.3±0.8*
D-dimers, ng/ml	0.18±0.03	0.15±0.02	0.14±0.01*	0.120±0.007*
Thrombin resistance, %	100	118.0±3.1	127.0±3.2*	136.0±3.1*

Note. *$p<0.05$ compared to the control.

17.3, 20.2, 16.8, and 16.6%, respectively, 24 h after administration of mepacrine. Thrombin tolerance increased by 18% (Table 1).

The contents of platelet factor 3, platelet factor 4, FDP, SFMC, and D-dimers decreased by 18.2, 30.4, 27.4, 23.6, and 22.2%, respectively, after administration of aspirin in LD_{50}. Thrombin tolerance increased by 27%.

Dazoxiben in IC_{50} produced a more potent effect than mepacrine and aspirin. The contents of platelet factor 3, platelet factor 4, FDP, SFMC, and D-dimers decreased by 23.1, 34.8, 33.3, 30.4, and 33.3%, respectively. Thrombin tolerance increased by 36%.

The effect of arachidonic acid conversion inhibitors in IC_{50} on CIBC and thrombin tolerance decreased in the following order: dazoxiben>aspirin>mepacrine.

Our results are consistent with published data on the role of thromboxane synthase in arachidonic acid conversion: inhibition of thromboxane synthase-catalyzed reactions in platelets prevents the formation of platelet products that stimulate aggregation of these cells [5,9,10].

Since thromboxane synthase inhibitor dazoxiben suppresses the formation of lipid peroxides in platelets [2] and since acumulation of conjugated dienes and malonic dialdehyde increases platelet activity during oxidative stress of different genesis [3,7], dazoxiben is more potent in preventing CIBC compared to other inhibitors of arachidonic acid conversion.

A negative correlation was found between the content of markers for TFI (CIBC) in blood plasma and thrombin tolerance. The higher is the degree of CIBC, the greater is the tolerance to thrombin.

We found that 24 of 50 intact rats (48%) survived 24 h after intravenous injection of thrombin. Experiments with injection of thrombin 24 h after administration of mepacrine, aspirin, and dazoxiben in IC_{50} showed that 26 (52%, $p>0.05$), 28 (56%, $p<0.05$), and 33 of 50 animals (66%, $p<0.02$) survived under these conditions.

We conclude that inhibitors of arachidonic acid conversion in platelets (particularly inhibitor of the final stage of conversion) *in vivo* decelerate TFI and, therefore, prevent CIBC. These changes are accompanied by a proportional increase in thrombin tolerance. Experimental animals gain the increased ability to respond adequately to hyperthrombinemia and risk of thrombosis.

REFERENCES

1. Z. S. Barkagan and A. P. Momot, *Main Methods of Laboratory Diagnostics for Damage to the Hemostatic System* [in Russian], Barnaul (1998), pp. 83-84.
2. A. Sh. Byshevskii, S. L. Galyan, R. G. Alborov, *et al*., *Tromboz Gemostaz Reologiya*, **1**, No. 5, Suppl., 77-78 (2001).
3. A. Sh. Byshevskii, S. L. Galyan, R. G. Alborov, *et al*., *Khirurgiya*, **10**, 38-41 (2004).
4. Z. A. Gabbasov and E. G. Popov, *Byull. Eksp. Biol. Med*., **108**, No. 10, 437-439 (1989).
5. D. M. Zubairov, *Molecular Bases of Blood Coagulation and Thrombus Formation* [in Russian], Kazan (2000).
6. B. A. Kudryashov, *Biological Problems of Regulation of Blood Fluidity and Coagulation* [in Russian], Moscow (1975).
7. A. P. Momot, V. A. Elykomov, and Z. S. Barkagan, *Klin. Lab. Diagn*., No. 4, 17-20 (1999).
8. A. A. Nelaeva, E. A. Aleksandrova, Yu. V. Strelina, *et al*., *Nauchn. Vestn. Tyumensk. Gos. Med. Akad*., Nos. 3-4, 70-71 (1999).
9. A. S. Shitikova, *Platelet Hemostasis* [in Russian], St. Petersburg (2000).
10. M. P. Gawaz, *Blood Platelets*, Stuttgart, New York (2001).
11. M. Levi, E. Jonge, and T. van der Poll, *Ann. Med*., **36**, No. 1, 41-49 (2004).
12. H. M. Spronk, J. W. Govers-Riemslag, and H. Cate, *Bioessays*, **25**, No. 12, 1220-1228 (2003).