BIOPHYSICS AND BIOCHEMISTRY

Antithrombotic Activity of N,N-Dichlorotaurine on Mouse Model of Thrombosis *in Vivo*

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Intravenous injection of chloramine derivatives of amino acids and taurine reduced the mortality rate in mice with thrombosis induced by intravenous injection of ADP or collagen-epinephrine mixture. Intravenous injection of N,N-dichlorotaurine caused 50% inhibition of platelet aggregation induced by ADP and measured in the platelet-enriched plasma *in vitro*. The antithrombotic effect of chloramine derivatives of amino acids and taurine is related to their ability to suppress functional activity of platelets.

Key Words: *thrombosis; mouse; biogenic chloramine; N,N-dichlorotaurine; antithrombotic compound*

Platelets play an important role not only in the formation of hemostatic thrombi at the site of vascular wall damages, but also in the pathogenesis of arterial thromboses and atherosclerosis. The release of thromboxane A_2 after intravascular activation of platelets produces vasospasm and cardiac arrhythmias. Antiaggregating preparations that block platelet adhesion and aggregation and release of compounds from these cells hold much promise for the prevention of thromboses, particularly, in the arterial system. Antiaggregants are widely used for the prevention of arterial thromboses and coronary heart disease.

Our previous studies showed that chloramine derivatives of amino acids and taurine inhibit functional activity of platelets [1-4,7], including receptor-dependent aggregation, secretion of dense granules, and cyclooxygenase oxidation of lipids. These compounds block intracellular signal transduction in platelets. The modulatory effect of chloramines on blood cells is probably realized via chemical (covalent) modification of peripheral serum-containing groups in the plasma membrane.

Nor data on the effects produced by chloramine derivatives of biogenic compounds on platelets allowed elaboration of new antithrombotic preparations. N,N-dichlorotaurine (N,N-dichloro-2-amino-ethanesulfonic acid, DT) characterized by high stability and obtained in the solid state is a promising antithrombotic agent. DT effectively inhibits initial and final aggregation of human platelets and release of the contents from dense granules [4]. DT added to human whole blood inhibits platelet aggregation and not damages erythrocytes. It should be emphasized that DT inhibits spontaneous and induced aggregation of human platelets and causes disaggregation of formed aggregates. Published data show that DT, aspirin, and ticlopidine are equally potent in suppressing secondary aggregation of platelets, but DT more effectively inhibits primary aggregation [4].

Here we studied antithrombotic activity of DT in mice with cellular thromboses.

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MATERIALS AND METHODS

Experiments were performed on 130 male albino outbred mice (20-25 g) and 35 male CBA mice (20 g).

Studies were performed on models of cellular thromboses used to assay antithrombotic agents that affect platelet aggregation [5,6]. ADP in relatively high dose (series I) or a collagen-epinephrine mixture (series II) was used as the thrombotic agents, since platelet aggregation inductors in low concentrations do not cause death of experimental animals. The concentration of these agents was selected so that they caused death of 85-95% control mice. In series I, ADP in physiological saline (300 mg/kg in 0.1 ml) was injected intravenously into the caudal vein over 10 sec. The animals died 0.5 min after ADP administration. In series II the mice received 0.1 ml mixture of 15 mg collagen and 8.6 mg epinephrine in physiological saline. Death of animals occurred 2.5-3.0 min after treatment. In some mice paresis of hindlimbs and decrease in locomotor activity for more than 15 min were observed.

Published data show that under these experimental conditions massive occlusion of pulmonary microvessels with platelet aggregates is the main cause of animal's death [6]. Histological assay revealed no thrombotic injuries to microvessels of other organs in experimental mice (similarly to control animals receiving physiological saline) [6].

We evaluated the ability of antithrombotic compounds to prevent death and paralysis in animals. We used preparations of taurine (2-amino-ethanesulfonic acid), ADP, acetylsalicylic acid (aspirin), ticlopidine, collagen, epinephrine (Sigma), sodium hypochlorite (Aldrich), and amino acids (Reanal and Reakhim). Chloramine derivatives were synthesized in the reaction of initial compounds with hypochlorite [1].

Acetylsalicylic acid was dissolved in 0.3 M sodium acetate and injected intraperitoneally (20 mg/kg in 0.4 ml) 1 h before treatment with the thrombotic agent [6]. Ticlopidine was dissolved in physiological saline and injected intravenously in a dose of 10 mg/kg 10 min before ADP administration. The concentration of preparations was selected so that they displayed maximum activity. These doses correspond to the amount of aspirin and ticlopidine administered perorally for 1 week. DT (3.4-6.8 mg/kg) and N-chlorophenylalanine (13.6 mg/kg) were administered 10 min before treatment with the thrombotic agent.

The blood was obtained from the heart in ether-anesthetized mice and stabilized with 3.8% sodium citrate (ratio 9:1). The blood was centrifuged at 270*g* for 5 min to obtain platelet-enriched plasma (PEP). Platelet aggregation in PEP was induced with ADP in a final concentration of 10 mM and recorded by the turbidimetric method of Born on a lumiaggregometer (Chronolog Corporation). Changes in light transmission of the cell suspension served as the measure of aggregation activity.

Statistical analysis of changes produced by the antithrombotic preparations (difference between the count of survived control and experimental animals) was performed with Fischer's *F* test.

RESULTS

In series I we observed death of 95% mice receiving 0.1 ml physiological saline before ADP administration. DT in a dose of 4 mg/kg (blood DT concentration 0.25-0.37 mM) prevented death of animals. In these mice locomotor activity returned to normal 3-5 min after treatment (Table 1). Increasing the dose of DT to 6.8 mg/kg (blood DT concentration 0.5 mM) decreased in the mortality rate to 10%. It should be emphasized that locomotor activity of survived mice recovered after 1.0-1.5 min.

Our previous studies showed that antiaggregation activity of N-chloroderivatives of amino acids in PEP increases with the decrease in their molecular weight. However, after infusion of these compounds into the blood they produce the same antiaggregation effect. The only exception is N-chlorophenylalanine, whose efficiency 2-fold surpasses that of other chloramines [1]. This can be explained by its weak interaction with erythrocytes. Taking into account these data, it was interesting to estimate the efficiency of N-chlorophenylalanine and DT administered into the blood in the same molar concentration (1 mM by active chlorine). It should be emphasized that 1 molecule of DT contains 2 atoms of active chlorine. In animals receiving N-chlorophenylalanine the survival rate was lower than in mice treated with DT (45%, Table 1). We compared preclinical data on acute toxicity of DT and N-

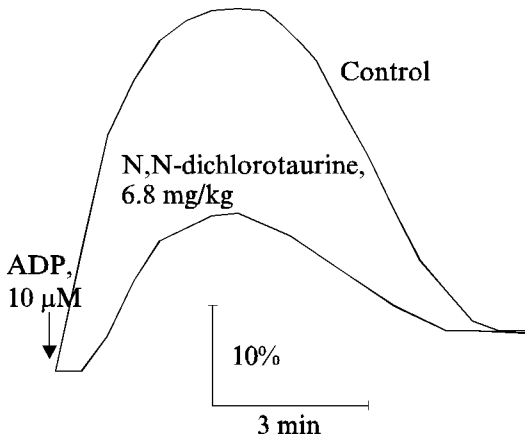

Fig. 1. Kinetic curves reflecting ADP-induced aggregation of mouse platelets in the platelet-enriched plasma.

TABLE 1. Decrease in Mortality of Albino Outbred Mice Induced by Intravenous Injection of ADP after Treatment with Antiaggregation Compounds

Substance, dose, mg/kg (blood concentration is shown in brackets, mM)		Number of experimental animals	Number of dead animals	Survived animals, %
Physiological saline	—	23	22	4
DT	3.4-4.9 (≈0.25-0.37)	6	0	100*
	6.8 (≈0.5)	19	2	90*
N-chlorophenylalanine	13.6 (≈1.0)	9	4	56**
Aspirin	20.0 (≈1.7)	4	3	25
Ticlopidine	10.0 (≈0.5)	6	3	50^{+}

Note. *$p<0.01$ and **$p<0.05$ compared to the control (Fischer's F test); $^{+}p<0.01$ compared to the control (Student's t test).

chlorophenylalanine. Lethal doses of these compounds causing death of 50% mice were 48 and 105 mg/kg, respectively. Our results show that these chloramines produce the antithrombotic effect in doses that are the order of magnitude lower than their toxic concentrations.

The influence of DT was compared with the effects of acetylsalicylic acid and ticlopidine. In our experiments acetylsalicylic acid was ineffective, which agrees with published data [5,6]. Ticlopidine in a dose of 10 mg/kg produced a pronounced protective effect, which did not differ from that observed after administration of N-chlorophenylalanine (Table 1).

In series II we did not observe death of outbred mice. Therefore, this series was performed on CBA mice. The survival rate of control CBA mice was 16%. DT increased this parameter to 64% ($p<0.05$).

To evaluate the mechanism of antithrombotic activity in DT, we studied ADP-induced aggregation of mouse platelets in PEP. Control animals were intravenously injected with physiological saline 10 min before blood sampling. Experimental mice received DT in a dose of 6.8 mg/kg. PEP samples obtained from the blood of experimental and control animals were mixed. DT suppressed platelet aggregation by 50% compared to the control (Fig. 1). Our previous experiments with PEP and blood from humans, rabbits, and rats showed that chloramine derivatives of amino acids and taurine produce a systemic antithrombotic effect, inhibit platelet aggregation and secretion of dense granules, and cause disaggregation of aggregated platelets [1-4]. *In vivo* experiments with rabbits showed that DT markedly inhibits platelet aggregation induced by various clinically important agonists, including collagen, ADP, and epinephrine. These data indicate that the antithrombotic effect of N-chlorophenylalanine and DT in mice is associated with their antiaggregation properties.

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