INVESTIGATION OF THE EFFECT OF SOME XANTHINE DERIVATIVES ON PLATELET AGGREGATION AND OTHER INDICES OF HEMOSTASIS

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Experiments on rabbits and in vitro showed the antiaggregation action of some new xanthine derivatives. It was shown that they also have anticoagulant activity and prevent thrombus formation.

KEY WORDS: xanthines; platelets; aggregation.

An increase in the agglutinating activity of platelets as a result of various causes in many diseases has been described in a wide range of experimental and clinical investigations [1, 3, 4, 9, 10]. The formation of aggregates of platelets may disturb the microcirculation. Accordingly, in clinical practice it is essential to have substances which can regulate the functional activity of the platelets. Ardlie et al., [5] found that caffeine, theophylline, and euphylline inhibit platelet aggregation induced by ADP.

The object of this investigation was to study the effect of various xanthine derivatives on platelet aggregation and on other indices of hemostasis.

EXPERIMENTAL METHOD

Experiments were carried out on rabbits of both sexes weighing 2-4 kg. Platelet aggregation was determined by Born's method [6] in the modification of O'Brien et al., [9]. ADP was mainly used as the aggregating agent, but serotonin and collagen were used in some series of experiments. Adhesion of platelets to collagen was determined by the method of Zucker and Borelli [11]. The effect of substances on the blood clotting system also was tested by the use of methods for determining thromboplastin time (after Quick), activity of plasma factors V and VII, and the blood recalcification time. Fibrinolysis was determined by Donner's method [8]. The effect of active antiaggregation agents on the serotonin content in the platelets also was studied [7]. An experimental model of microvascular thrombosis was obtained by electrical stimulation of the mesenteric venules in rats with a current of 100 V for 20 msec. Observations continued for 5 min. The effect of substance No. 9 (see below) was determined on intact animals and also after preliminary injection of ADP in a dose of 4 mg/kg.

The following xanthine derivatives were studied: euphylline in concentrations of 0.02 and 0.2 mg/ml, caffeine in concentrations of 0.01 and 0.02 mg/ml, and nine derivatives of xanthine obtained at the S. Ordzhonikidze All-Union Pharmaceutical Chemical Research Institute, which were studied in concentrations of 0.1 and 0.4 mg/ml. The names of the substances are given below:

1) 2,3,7-trimethyl-6-iminopurine hydrochloride; 2) 6-dimethylamino-2-imino-3,7-dimethylpurine hydrochloride; 3) 6-dimethylamino-2-imino-3,7-dimethylpurine hydrochloride; 4) 2-methylmercapto-2-imino-3,7-dimethylpurine hydrochloride; 5) theobromine-8-acetic acid trihydrate, sodium salt; 6) theobromine-8-acetic acid diethylaminoethylamide hydrochloride; 7) 1-hexyl-8-diethylaminomethyl-3,7-dimethylxanthine hydrochloride; 8) 2-diethylaminomethylhypoxanthine hydrochloride; 9) 8-diethylaminomethyl-3,7-dimethylxanthine.

All substances were investigated in vitro, and caffeine, euphylline, and substances Nos. 2, 3, 6, and 9 were investigated in vivo for their effect on platelet aggregation induced by ADP. Euphylline and substance No. 9 were studied in detail by the methods described above.

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EXPERIMENTAL RESULTS

In the experiments in vitro euphylline in a concentration of 0.2 mg/ml depressed aggregation induced by ADP from 29 ± 2 to 7.5 ± 0.8%; in a concentration of 0.02 mg/ml the effect was less marked. In doses of 2.5 and 5 mg/kg, depression of the agglutinating activity of the platelets was considerable. In doses of 1.2 and 8 mg/kg, euphylline did not affect this function of the platelets.

In all experimental situations caffeine depressed platelet aggregation. However, its action was less marked than that of euphylline. Investigation of substances Nos. 1-9 showed that compounds Nos. 1, 4, 5, 7, and 8 did not affect platelet aggregation in vitro. Substances Nos. 2, 3, 6, and 9 depressed platelet aggregation when added directly to platelet-enriched plasma, but when tested in vivo, only substance No. 9 significantly depressed platelet aggregation. Substance No. 9 was shown to prevent thrombus formation during anodal stimulation. Meanwhile this substance did not affect thrombus formation in intact animals during cathodal stimulation. After injection of ADP the effect of this compound was exhibited during both anodal and cathodal stimulation. The thrombus formation time was lengthened, the thrombus detachment time was shortened, and the area of the thrombus reduced.

On the basis of these results euphylline and substance No. 9 were distinguished as the most active antiaggregation agents and were tested by the additional methods described above. For instance, during the study of serotonin-induced aggregation it was found that euphylline reduced it from 41 ± 2.5 to 23 ± 6% and substance No. 9 from 41 ± 2.6 to 22 ± 3.1%. Both these substances depressed both aggregation and adhesion of platelets induced by collagen but did not affect the serotonin content in the platelets. Euphylline also shortened the thromboplastin time (after Quick) and increased factor VII activity. Substance No. 9 increased the time of determination of factor VII from 17 ± 0.7 to 23 ± 1 sec, while lengthening the blood recalcification time from 110 ± 10 to 250 ± 30 sec. These substances did not affect the fibrinolytic activity of the plasma.

A previous pharmacological investigation [2] showed that substance No. 9 increases the coronary blood flow, reduces peripheral vascular resistance, and lowers the systemic arterial pressure.

During the investigation of the effect of 11 xanthine derivatives on platelet aggregation and on certain other indices of the hemostasis system, the most active depression of platelet aggregation was produced by euphylline and substance No. 9. The great advantage of this substance No. 9 now being tested is that, besides its antiaggregation action it also has the property of inhibiting blood coagulation. The search for active antiaggregation agents among the xanthine derivatives has interested investigators for a long time. In platelets they inhibit an enzyme which catalyzes hydrolysis of cyclic AMP [5], which plays an important role in the metabolic activity of platelets.

The substances tested are not a homologous series of xanthines. However, investigation of the connection between structure and action with respect to these compounds may be of some interest. As a result of the investigation a substance possessing considerable antiaggregation activity was discovered.

LITERATURE CITED

1. K. M. Lakin, V. A. Fel'dbaum, V. S. Efimov, et al., in: Abstracts of Proceedings of the 12th International Congress on Blood Transfusion [in Russian], Moscow (1969), p. 251.
2. S. S. Liberman, R. A. Al'tshuler, and L. N. Gerchikov, Farmakol. Toksikol., No. 5, 586 (1970).
3. V. A. Lyusov and Yu. B. Belousov, Kardiologiya, No. 7, 50 (1970).
4. V. A. Lyusov and Yu. B. Belousov, Lab. Delo, No. 8, 459 (1971).
5. M. G. Ardlie, G. Glew, B. G. Schultz, et al., Thrombos, Diathes. Haemorrh. (Stuttgart), 18, 670 (1967).
6. G. V. R. Born, J. Physiol. (London), 162, 67P (1962).
7. P. F. Crosti and P. E. Lucchelli, J. Clin. Path., 15, 191 (1962).
8. L. Donner, Vnitrni Lék., 9, 810 (1963).
9. J. R. A. O'Brien, J. B. Heywood, J. A. Heady, et al., Thrombos Diathes. Haemorrh., (Stuttgart), 16 752 (1966).
10. A. Poplawski, M. Skorulska, and S. Niewiarowski, J. Atheroscl. Res., 8, 721 (1968).
11. M. B. Zucker and J. Borelli, Proc. Soc. Exp. Biol. (New York), 109, 779 (1962).