ANTICOAGULANT AND ANTISCLEROTIC EFFECTS OF ISOPROPOXYGERMATRANE AND METHYLETHYL(SILATRAN-1-YLMETHYL)SULFONIUM IODIDE

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The value of the various antisclerotic agents is determined by their ability to protect the vascular wall [2]. It is in this context that a new class of biologically active compounds, the silatranes, deserves attention [1, 7]. Some silatranes, as preliminary experiments [3, 5] have shown, possess an antiatherosclerotic and anticoagulant action. However, replacement of the silicon atom in the silatrane molecule by a germanium atom leads to the formation of germatranes and often to modification of the biological activity of the metalloatranes [6].

The aim of this investigation was a detailed study of the effect of isopropoxygermatrane (IPG) and methylethyl(silatran-1-ylmethyl)sulfonium iodide (MESI) on the development of experimental atherosclerosis and on the dynamics of the coagulation properties of blood.

EXPERIMENTAL METHOD

Experiments were carried out on chinchilla rabbits weighing 2.5-3 kg. A model of atherosclerosis was produced by the method of Anichkov and Khalatov by feeding animals with cholesterol with their diet in a dose of 250 mg/kg daily. Blood levels of cholesterol, total lipids, triglycerides, and β-lipoproteins were determined 1 h after the beginning of feeding. Experimental and control groups were formed on the basis of the results. All the animals continued to receive cholesterol. Rabbits of the experimental groups received atranes, whereas the control animals received physiological saline. Rabbits in the experiments of series I received IPG intramuscularly in a dose of 40 mg/kg of a freshly prepared aqueous solution daily for 2 months. Rabbits in the experiments of series II received IPG just as those in series I, but in a dose of 200 mg/kg. In the experiments of series III, IPG was added to the animals' diet at the rate of 150 mg/kg daily, also for 2 months. In the experiments of series IV, which followed the general plan, the animals received IPG with their diet in a dose of 500 mg/kg daily. In the experiments of series V a solution of IPG was injected intramuscularly in a dose of 40 mg/kg, starting with the 1st day of the experiment, along with cholesterol. In the experiments of series VI rabbits of the experimental group were given MESI intramuscularly in a dose of 5 mg/kg of the freshly prepared aqueous solution daily for 2 months. The rabbits were given MESI just as those of group VI, but the dose of the compound was increased to 25 mg/kg. In the experiments of series VIII MESI was added to the animals' diet at the rate of 25 mg/kg body weight for 2 months. In the experiments of series IX the animals were given MESI with their diet in a dose of 100 mg/kg. In series X a solution of MESI was injected intramuscularly in a dose of 5 mg/kg, starting on the 1st day of the experiment, along with cholesterol. The experiments continued for 3 months, after which the animals were killed by air embolism. At the end of the experiment, levels of cholesterol, total lipids, triglycerides, and β-lipoproteins were determined in the venous blood of all groups of rabbits by standard methods. Concentrations of lipids and cholesterol also were determined in the thoracic aorta and an index of the severity of the atherosclerotic plaques in the aorta was estimated in accordance with the scheme [4]. The effect of MESI on the clotting and anticlotting systems of the blood was studied in experiments in vitro and in

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vivo, following administration of the compound as a single dose and of its "chronic" administration to the rabbits in the form of repeated daily doses. As in the previous long-term experiments, the following groups of animals were formed: 1) receiving MESI intramuscularly in a dose of 5 mg/kg; 2) receiving MESI intramuscularly in a dose of 25 mg/kg; 3) receiving MESI in a dose of 25 mg/kg with the diet; 4) receiving MESI in a dose of 100 mg/kg with the diet. The rabbits were kept on a standard diet. Parameters of the clotting power of the blood were studied in all groups of animals at intervals of 2 days, i.e., on the 3rd, 5th, and 7th days, and so on, after the beginning of the experiment. In experiments with a single dose of MESI, dose-effect and time-effect relationships were studied 15, 30, 45, 60, 120, and 240 min after intraperitoneal injection of the compound in doses of 5, 25, 100, 250, and 500 mg/kg. Standard methods were used to test: 1) the coagulation hemostasis system – clotting time of whole blood (by the method of Lee and White), thrombin time (Szirmai), prothrombin time (Quick), and plasma recalcification time (Begerhof); 2) the platelet hemostasis system – the platelet count in the peripheral blood (per 1000 erythrocytes, counted in a Goryaev's chamber), platelet aggregation; the anticoagulant system – antithrombin III (AT III); 4) the fibronolytic system – fibrinolytic activity, fibrin degradation products (FDP). The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

Intramuscular injection of IPG in a dose of 40 mg/kg daily for 2 months led to a significant fall of the triglyceride, β-lipoprotein, and total lipid levels in the blood serum. The blood cholesterol was unchanged. Concentrations of total lipids and cholesterol in the aortic tissue were virtually unchanged and the severity of the atherosclerotic plaques in the aorta also remained unchanged. With an increase in the dose of IPG to 200 mg/kg intramuscularly, i.e., in series II of experiments, the levels of the parameters of lipid metabolism in the animals' blood were depressed even more. Meanwhile a fall of the cholesterol and total lipid levels in the aortic tissue was recorded and, a particularly important finding, the index of the severity of the atherosclerotic plaques in the aorta was significantly reduced.

In experiments with the addition of IPG to the animals' diet the effectiveness of the compound was greatly reduced. For instance, in a group of rabbits receiving IPG in a dose of 150 mg/kg daily no changes were found in the parameters of lipid metabolism either in the blood or in the aortic tissues; in the group of animals receiving IPG in a dose of 500 mg/kg daily, only a fall of triglyceride, β-lipoprotein, and total lipid levels in the blood serum was recorded. Thus peroral administration of IPG proved to be ineffective.

Finally, in the experiments in which IPG was given in a dose of 40 mg/kg daily for 3 months, starting from the first day of the experiment, i.e., to some degree prophylatically, parameters of lipid metabolism in both the blood and aortic tissue fell significantly. The severity of the atherosclerotic plaques in the aorta of the rabbits of this group was significantly ($p < 0.001$) less than in the control animals. Thus IPG, when injected parenterally in a dose of 200 mg/kg for 2 months and in a dose of 40 mg/kg for 3 months had an antisclerotic action, expressed as a decrease in the severity of the sclerotic plaques in the aorta. In the modern view, this is the basic criterion for assessment of antiatherosclerotic activity for all such factors [2].

In the next experiments to study the effect of MESI, its intramuscular injection in a dose of 5 mg/kg for 2 months was found to lead to a significant fall in serum triglyceride, β-lipoprotein, and total lipid levels. The blood cholesterol was unchanged. Concentrations of total lipids and cholesterol in the aortic tissue were virtually unchanged, as also was the severity of the clerotic plaques in the aorta. With an increase in the dose of MESI to 25 mg/kg intramuscularly, blood levels of the parameters of lipid metabolism were repressed even more. Meanwhile the cholesterol and total lipid levels in the aortic tissue were lowered, and the index of severity of the sclerotic plaques in the aorta was reduced almost by half. In experiments in which MESI was added to the diet, the efficacy of the compound was sharply reduced. Thus, in the group of animals that received MESI in a dose of 25 mg/kg, no changes in the indices of lipid metabolism were observed, either in the blood or in the aortic tissue, while in the group of rabbits that received MESI in a dose of 100 mg/kg, only a decrease in the level of triglycerides, β-lipoproteins, and total serum lipids was recorded. The reason for these series of experiments was the recommendation that long-acting preparations in a solid form be created. The fact that peroral administration of MESI is ineffective is most proably due to the fact that it undergoes rapid hydrolysis in an acid medium. Determination

TABLE 1. Coagulating Properties of Blood following Single Dose of MESI

Parameter	Control (physiological saline)	Dose, mg/kg			
		5	p	25	p
Clotting time of whole blood, min	4,1±0,3	5,4±0,6	>0,1	6,3±0,58	<0,05
Thrombin time, sec	27,0±3,1	40,6±5,8	<0,05	42,8±5,4	<0,05
Plasma recalcification time, sec	40,5±3,9	52,9±8,1	>0,1	54,0±11,3	>0,1
Prothrombin time, sec	10,4±0,7	11,2±0,8	>0,1	10,8±0,9	>0,1

Legend. Blood for testing was taken 20 min after injection; criterion of significance (p) in all columns determined relative to control (n = 10).

TABLE 2. Coagulating Properties of Blood during Repeated Administration of MESI

Parameter	Control	Intravenous injection, mg/kg				Peroral administration, mg/kg			
		5	p	25	p	25	p	100	p
Clotting time of whole blood, min	6,4±0,8	10,3±1,1	<0,05	16,7±1,4	<0,05	8,2±0,6	<0,05	11,6±0,9	<0,05
Thrombin time, sec	30,6±0,4	45,0±3,8	<0,05	104,8±9,6	<0,05	54,3±4,9	<0,05	74,6±8,7	<0,05
Plasma recalcification time, sec	42,5±4,3	43,8±4,1	>0,1	58,3±6,4	<0,05	35,2±3,6	>0,1	40,2±3,6	>0,1
Platelet count	136±17,8	94±10,1	>0,1	78±8,1	<0,05	126±15,8	>0,1	109±10,1	>0,1
Platelet aggregation, %	42±0,7	31,9±4,1	>0,1	28±3,1	<0,05	33,5±2,6	>0,1	29,4±2,7	<0,05
AT III level, %	106±7,3	104±8,6	>0,1	111±9,6	>0,1	98±8,6	>0,1	101±10,3	>0,1
Fibrinolytic activity, min	254±26,4	228±30,4	>0,1	201±22,3	<0,05	297±35	>0,1	211±25,4	<0,05
FDP, mg %	0,56±0,07	1,4±0,12	<0,05	1,6±0,01	<0,05	0,89±0,07	>0,1	1,56±0,12	<0,05

Legend. Results given are those in the 21st day of MESI administration. Blood for testing was taken 3 h after last injection; criterion of significance (p) in all columns determined relative to control (n = 10).

of the rate of its hydrolysis in a medium with pH close to that of gastric juice showed that about half the total quantity of the compound is hydrolyzed in 1 h, with the formation of SiO_2. Finally, in experiments in which MESI was given in a dose of 5 mg/kg daily for 3 months starting from the first day of the experiment, parameters of lipid metabolism in the blood and aortic tissue were significantly lowered. The severity of the atherosclerotic plaques in the aorta of the rabbits of this group was only half of that in the control animals.

After addition of MESI to the tube containing whole blood in the proportion of 0.01 and 0.1 μg/ml the clotting time of the blood was unchanged (6.0 ± 0.7 min in the experiment compared with 5.4 ± 0.6 min in the control). The thrombin time, determined 30 min after addition of MESI to the plasma in a dose of 0.01 and 0.1 μg/ml also was unchanged (32.4 ± 3.0 sec in the experiment compared with 29.8 ± 2.8 sec in the control). The dynamics of the blood clotting parameters following a single intravenous injection of MESI in doses of 5 and 25 mg/kg is shown in Table 1. It will be clear from Table 1 that injection of MESI led to an increase only of the blood clotting time, and to lengthening of the thrombin time, whereas reducing the dose to 5 mg/kg caused only an increase in the thrombin time. Investigation of the blood clotting parameters in animals receiving MESI by intravenous and peroral administration daily for 3 months showed that a significant incease in the clotting time of whole blood took place in rabbits receiving MESI intravenously in a dose of 25 mg/kg, starting on the 9th day, whereas in rabbits receiving MESI similarly but in a dose of 5 mg/kg, the effect began on the 17th day. When MESI was given in a dose of 100 mg/kg with the diet, the difference between the value of this parameter and the control was significant by the 19th day, but administration of MESI in a dose of 25 mg/kg did not change the clotting time. Data on the state of coagulation hemostasis are given in Table 2. They show that on the 21st day of administration of MESI all parameters of blood clotting of these animals were changed. A characteristic feature was lenthening of the thrombin time in all the animals. The plasma recalcification time was increased only after intravenous injection of MESI in a dose of 25 mg/kg; significant thrombocytopenia was observed only in this group of rabbits. Platelet aggregation was reduced in all the animals. The AT III level had a tendency to rise in animals receiving MESI intravenously, and also perorally in a dose of 100 mg/kg. More marked activation of the fibrinolytic properties of the blood was observed in all groups: the FDP level in the rabbits rose to the upper limits of physiological normality.

Thus MESI, when injected parenterally in certain doses for a certain period of time has an antiatherosclerotic action. It may also be postulated that in all probability MESI has no direct anticoagulant action, but when the changes of hemostasis revealed by this investigation are interpreted, it can be tentatively suggested that multiple administration of MESI stimulates endogenous heparin production.

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IMMUNIZING ALBINO RATS WITH A COVALENT SYDNOPHEN–SERUM ALBUMIN CONJUGATE DEPRESSES CHRONIC ETHANOL CONSUMPTION

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The use of immunologic methods inducing long-term changes in physiological status offers fundamentally new opportunities. Essentials of methods of this type are examined in a review [1] and can be summarized as immunization of an animal with covalent conjugates of carrier antigens with biological regulators, identical or similar to the intrinsic regulatory compounds of the animal itself; the formation of antibodies binding the corresponding endogenous regulator (or group of regulators) long enough for intensive antibody production to take place (usually months) is induced, and changes take place in the level of the regulator, the general balance of the regulatory systems, and, ultimately, several physiological functions.

The long-term effect, resembling in many of its features the action of neuroleptics [4], was described previously during immunization of rats with a covalent conjugate of sydnophen (an antidepressant and neurostimulator, which interferes with the action of catecholamines) with bovine serum albumin (BSA). The possibility of immunologic depression of alcohol motivation has been demonstrated in several publications [3, 5, 6]. The aim of this investigation was to study the effect of immunization with a conjugate of sydnophen on the attitude of albino rats to chronic ethanol consumption.

EXPERIMENTAL METHOD

Sydnophen was conjugated with BSA with the aid of glutaraldehyde [4]. By varying the molar proportions of the components, conjugates containing from 5 to 33 moles of sydnophen to 1 mole of protein were obtained. The experiments were conducted on 82 noninbred male albino rats weighing 150-200 g. Immunization consisted of three injections of conjugate

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