EFFECTIVENESS OF TERRILYTIN IN THE TREATMENT OF EXPERIMENTAL PULMONARY THROMBOSIS

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The thrombolytic action of terrilytin, a proteinase from Aspergillus terricola, was studied in rabbits with experimental pulmonary thrombosis of 24 hours duration. The preparation was dissolved in polyvinylpyrrolidone and injected into the blood stream in doses of 175 and 220 proteolytic units (PU)/kg body weight by two methods: locally (into the region of the thrombosed vessel), and systemically. Irrespective of the method of administration and dose of the preparation it was found to have high thrombolytic activity, which was more marked, however, after local infusion of terrilytin in a dose of 220 PU/kg. No side effects of the preparation were observed.

KEY WORDS: terrilytin; thrombosis; lung; pulmonary vessels.

The possibility of using terrilytin for the treatment of thromboembolic diseases of the pulmonary circulation was mentioned previously [1, 2]. The idea was based on the results of experiments carried out to study the accumulation and distribution of radioactive terrilytin-^{125}I in experimental animals [1]. In particular, it was shown that 2 h after administration of the preparation maximal levels were found in the heart muscle and lungs; in conjunction with the active uptake of terrilytin into the substance of the thrombus, this suggested that the preparation might well be used with advantage in the treatment of coronary and pulmonary thrombosis.

The first results of an investigation of the effect of terrilytin on the course of experimental pulmonary thrombosis of immune genesis are described below.

EXPERIMENTAL METHOD

Experiments were carried out on 70 Chinchilla rabbits, 10 of which formed the control group.

To produce immunogenic pulmonary thrombosis, the rabbits were treated for several days with microdoses of thrombin together with atropine [3], and when the fibrinolytic activity of the blood was reduced on average by 25-30% (as verified by thromboelastography and in vitro methods [12]), anti-lung immunoglobulins were injected intravenously into the animals. Diffuse aggregation of blood platelets was observed 2-3 h after the injection in the lumen of the pulmonary capillaries and this was followed by the development of thrombosis in the small, medium-sized, and large branches of the pulmonary arteries.

To monitor the development of thrombosis the pulmonary hemodynamics and contractile power of the right ventricle were investigated by recording the intraventricular pressure and by the use of rheo- and polycardiographic methods [5].

Commercial terrilytin was injected into the experimental rabbits on the 2nd day after the development of thrombosis in doses of 175 and 220 proteolytic units (PU) kg/body weight, dissolved in polyvinylpyrrolidone, as a single dose of 5 ml systemically (into the marginal vein of the ear), or locally (into the right ventricle) through a polyethylene catheter. The catheter was introduced under superficial hexobarbital anesthesia (1.5-2.0 ml of 1% hexobarbital solution, intravenously) through the right jugular vein. Control animals received the same volume of polyvinylpyrrolidone. The rabbits were killed 24 h after the injection of terrilytin. The pulmonary vessels were investigated macro- and microscopically. The degree of thrombolysis was calculated as the thrombotic index, in percent [10].

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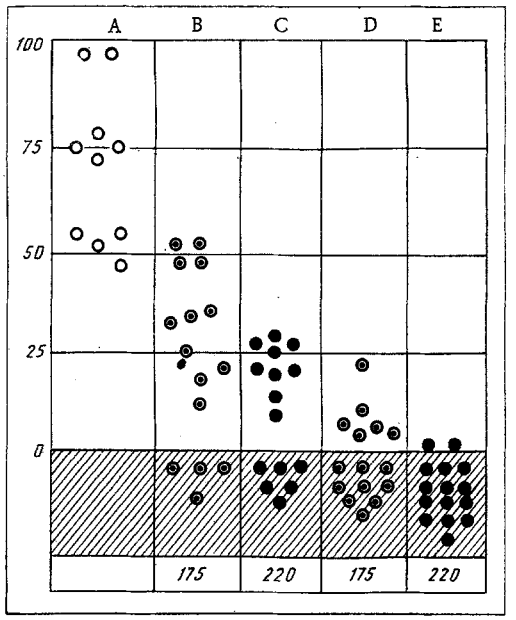

Fig. 1. Thrombolytic activity of terrilytin when administered by different methods. Abscissa, dose of terrilytin (in PU/kg); ordinate, % of thrombosis. A) Control; B and C) systemic infusion; D and E) local infusion. Control: 175 PU/kg. Zone of 100% lysis is shaded.

EXPERIMENTAL RESULTS AND DISCUSSION

All ten rabbits of the control group developed pulmonary thrombosis: In four rabbits the thrombi blocked up to 75% of the pulmonary vascular system with extensive lobar infarcts of the lungs, and in two animals total thrombosis of both pulmonary arteries was observed (Fig. 1). The development of thrombosis was expressed clinically as a sharp decrease in motor activity and an increase in the respiration rate.

Spontaneous lysis of the thrombi was not found in any of the rabbits of the control group.

After injection of terrilytin into the systemic blood flow thrombotic masses were absent in the pulmonary vessels of 10 of the 30 rabbits, four of which had received the preparation in a dose of 175 PU/kg and six in a dose of 220 PU/kg.

Histological examination showed that, despite complete patency of the main trunks in the group of animals receiving terrilytin in a dose of 175 PU/kg, thrombotic masses were still present in the small pulmonary vessels (under 800 μ in diameter); in three cases a hemorrhagic microinfarct of the lungs was found. In four of the 20 rabbits with a negative result of treatment the thrombosis was localized to within 50% of the area of one branch of the pulmonary arteries, in three animals the area of occlusion was 30-35%, in four rabbits 10%, and in nine animals 20-25%. Together with thrombosis, in 12 rabbits multiple foci of necrosis of lung tissue were observed (Fig. 1).

Whatever its dose, the preparation was on the whole well tolerated. Only in five rabbits, receiving terrilytin in a dose of 220 PU/kg, were brief excitation (3-5 min) and isolated twitches of the lower limbs observed.

After local infusion of the preparation (30 rabbits) a positive lytic effect was found in 24 animals; most of them (14 animals) received terrilytin in a dose of 220 PU/kg. In 12 rabbits the large branches of the pulmonary arteries were free from thrombotic masses, but in two animals small fragments of thrombi were present in the lumen of the pulmonary arteries although without affecting the blood flow through them.

Lysis of the thrombi took place in nine of the 15 animals treated with terrilytin in a dose of 175 PU/kg. The pulmonary vessels of one rabbit were occluded by 25% and those of five rabbits by 5-10% (Fig. 1). No toxic manifestations were observed. Meanwhile, after administration of terrilytin in a dose of 220 PU/kg, very small perivascular hemorrhages were found histologically, but were less frequent in the experiments with the smaller dose of the preparation.

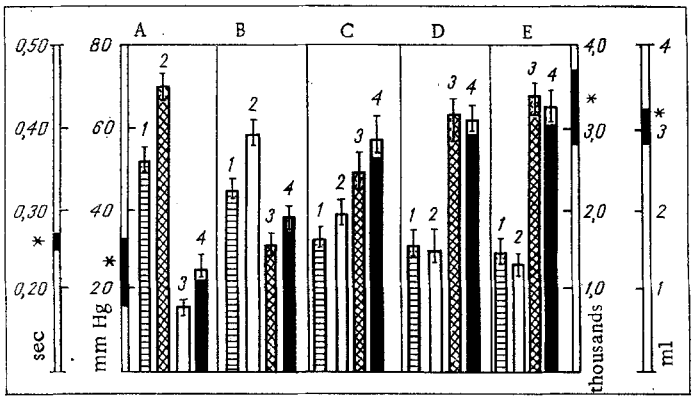

Fig. 2. Changes in indices of myocardial contractility and cardiodynamics in rabbits with pulmonary thrombosis after local injection of terrilytin in a dose of 220 PU/kg. A) Before treatment; B, C, D, and E) 1, 3, 5, and 24 h after treatment. 1) Period of expulsion; 2) systolic pressure in right ventricle; 3) Siegel–Sonnenblick index; 4) stroke volume. Areas shaded black and marked by asterisk indicate normal scatter of values.

The study of the cardiodynamic indices showed that, by contrast with the animals of the control group, in which the systolic and, in particular, the end-diastolic pressure in the right ventricle were high, in the rabbits treated with terrilytin morphological features of thrombolysis were accompanied by distinct reversal of the trend of the above indices. Meanwhile the contractile power of the right ventricular myocardium was restored in these animals, as shown by the results of rheo- and polycardiography (Fig. 2).

The results of these investigations showed that administration of terrilytin to animals with pulmonary thrombosis led to definite lysis of the thrombotic masses and to restoration of the hemodynamic indices in the pulmonary circulation. This effect was largely dependent both on the dose of terrilytin and on the method of its administration.

Human and animal blood plasma is known to contain a powerful system of inhibitors of proteolytic enzyme, which prevents the manifestation of their biological activity [11]. According to some investigators [8, 11], the ability of plasma to cause inhibition is due to two types of inhibitors – reversible and irreversible. An inhibitor of the first type is α_2-globulin and of the second type α_1-globulin. By binding proteolytic enzymes, which they do at different rates, they form complexes that are without proteolytic activity.

The attempt to depress the inhibitory activity of the blood by administration of terrilytin in large doses in such cases not only does not solve the problem of thrombolysis but, on the contrary, considerably aggravates it. Bergkvist [9] and, later Roschlau [13] showed that after administration of extremely large doses of proteinase from Aspergillus oryzae to animals free proteolytic activity appeared in the blood and had an unfavorable effect not only on thrombolysis but also on the body as a whole. To prevent such complications it is better to use enzyme preparations in doses capable of producing lysis without at the same time depressing the natural inhibitory activity of the blood [11]. Since in practice this cannot always be achieved, recently the local infusion of thrombolytic enzymes into the site of occlusion of the vessels has been widely adopted; this method produces very favorable conditions for direct contact between enzyme and thrombus and, consequently, for overcoming the inhibitory barrier of the blood.

In the present experiments terrilytin was injected into the animals in two ways: either into the systemic blood flow or locally, through a catheter into the right ventricle and pulmonary artery. Comparison showed that both methods of administration of the preparation are sufficiently effective, although better results were obtained with local injections of terrilytin. Of the 34 rabbits in which the outcome of treatment was favorable, 24 received terrilytin locally at the site of the thrombus and only 10 received it systemically.

It is important to note that signs of normalization of the hemodynamic indices of the pulmonary circulation appeared much more rapidly in the group of rabbits receiving terrilytin locally than in those receiving it by systemic infusion, evidence of the earlier development of thrombolysis in the pulmonary vessels.

It is interesting to note that, by contrast with previous investigations [2, 4], in which terrilytin had not only lytic properties, but also certain toxic properties, manifested even within the range of effective therapeutic doses, in the present study no side effects were found from the use of much larger doses of terrilytin. In the writer's view, the reason for this is that the solvent used for the terrilytin was not physiological saline but a solution of polyvinylpyrrolidone, which has a marked detoxicating action, has a beneficial effect on the systemic and regional hemodynamics, and restores the disturbed acid-base balance [6, 7].

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SIMULATION OF DETERMINANT AND DEPENDENT FOCI OF EPILEPTIC ACTIVITY IN THE RAT CEREBRAL CORTEX

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Foci of increased activity with independent firing patterns were created by means of weak solutions of strychnine in acute experiments on rats. A hyperactive focus of excitation created by means of concentrated strychnine solutions played the role of determinant structure. Its role was to determine the character of activity of the other epileptogenic foci, to enhance their paroxysmal activity, to combine them into a single functional complex, and to determine the behavior of the whole complex. This complex could be destroyed by suppressing the activity of the determinant focus. Elimination of any of the dependent foci forming the complex did not disturb the complex itself. These investigations confirm, on a new model, the general concept of the role of determinant structure in the activity of the CNS.

KEY WORDS: determinant focus; strychnine; neocortex; epileptic complex.

It was shown previously [2, 3] that a focus of powerful excitation created with the aid of strychnine in the cat cerebral cortex plays the role of determinant structure [1], which determines the character of activity of other scattered foci of excitation, enchances excitation in them, unites them into a single functional complex, and determines the behavior of the complex as a whole. Such a complex of foci can be destroyed by suppressing the activity of the determinant focus. The next step was to discover whether the relations established between the foci are connected with species-specific properties of the morphological and functional organization of the cat's brain.

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