USE OF LIPOSOMAL FORMS OF TERRILYTIN TO PRODUCE LYSIS OF EXPERIMENTAL THROMBI

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The preparation terrilytin, which was isolated from the mold *Aspergillus terricola* [7], and whose active principle is a proteolytic enzyme, possesses high thrombolytic activity, when injected intravenously into animals with experimental thrombi [2, 3, 4, 9, 10]. Meanwhile, when given by this method terrilytin was found to possess considerable toxicity, and in order to reduce it, it has been given perorally. However, peroral administration of terrilytin, in the form of a water-lipid emulsion requires high concentrations of the substance in order to obtain a lytic effect, and accordingly, in order to protect it against inactivation by gastric juice, a liposomal form of terrilytin has been used. Liposomes are structures formed by a bilayer membrane, which can act as carriers of drugs, protecting them against destruction by enzymes [6, 12]. It has been shown that drugs can be applied by means of liposomes to the walls of the small intestine, from which the contents enclosed in them can enter the blood stream [13].

In the investigation described below the thrombolytic effect of the liposomal form of terrilytin was investigated.

EXPERIMENTAL METHOD

A model of experimental thrombus was created in an isolated region of the rabbit femoral vein by mechanical trauma and by injection of 0.2 ml of thrombin, in a concentration of 50 units in 1 ml of physiological saline [1]. The presence or absence of thrombin in the femoral artery was verified histologically [14]. The state of hemostasis was evaluated from the data of thromboelastography, by determination of the aggregating activity of the paltelets in response to stimulation by ADP in a final concentration of $1 \cdot 10^{9}$, the fibrinogen concentration, and changes in fibrinolytic activity. Liposomes were made from lecithin (phosphatidylcholine) and cholesterol, for which purpose a preparation of 10% of standard lecithin was used. Cholesterol was first recrystallized by dissolving it in hot ethyl alcohol 2 or 3 times. The lecithin was mixed with cholesterol in the ratio of 7:3 (by molecular weight), dissolved in chloroform, and evaporated on a rotary evaporator. Terrilytin, dissolved in phosphate buffer (pH 7.0-7.2) was then added and the mixture was shaken and treated with ultrasound [13]. The resulting emulsion contained 30 mg of lipids and 10 mg of terrilytin in 1 ml. The liposomes measured approximately 1 μ under the electron microscope. The degree of incorporation of terrilytin in the liposomes was determined by a radioisotope method using ^{125}I-labeled terrilytin. The latter compound was obtained by electrochemical iodination by a potentiostatic method [8]. ^{125}I-terrilytin had the following characterictics: concentration 25 PU/ml.* Volume radioactivity 30 MBq/ml. The radiochemical purity (as labeled protein) was 96.7%. To determine the degree of incorporation of terrilytin into liposomes, according to data of electrophoresis, conducted in an acetylcellulose film (pH 8.6) [8] was 62 ± 9%. The resulting liposomal form of terrilytin, in a volume of 2 ml, was administered perorally through a gastric tube in a dose of 50 to 280 PU/kg body weight. Experiments were carried out on 32 rabbits.

*PU = proteolytic unit.

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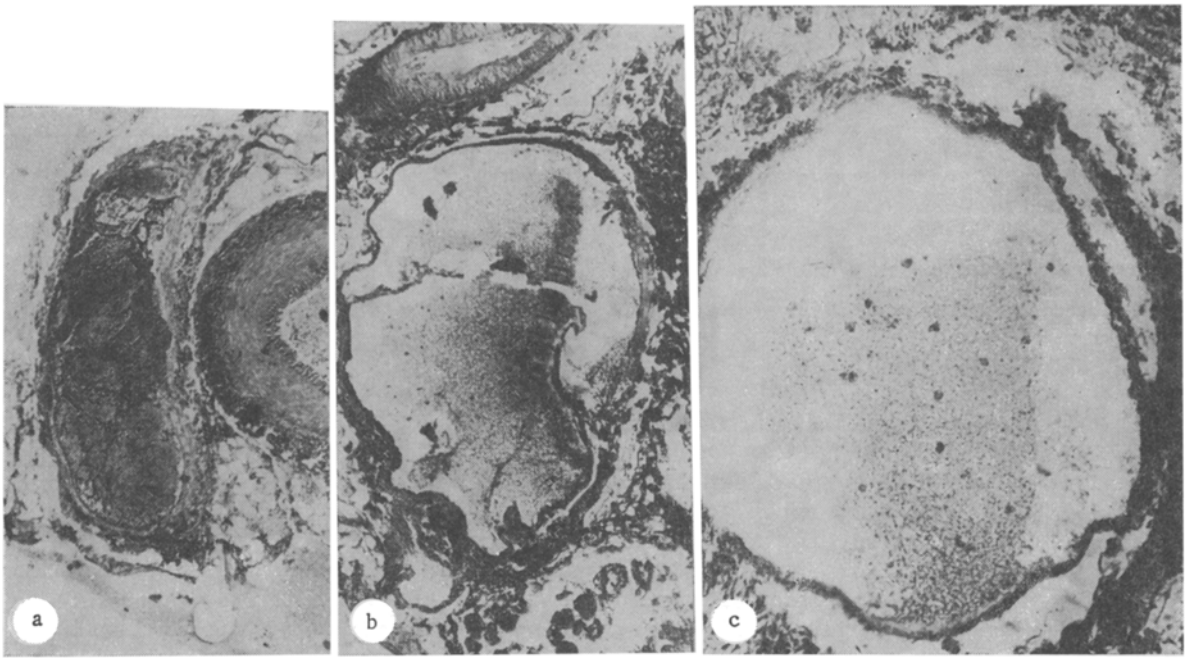

Fig. 1. Time course of lysis of thrombi after peroral administration of liposomal forms of terrilytin: a) fibrinous-endothelial thrombus 24 h after mechanical trauma; b) intial stage of lysis; c) restoration of lumen of vessel. Transverse section through rabbit femoral vein. 100 ×. Stained by Biondi's method.

EXPERIMENTAL RESULTS

The formation of a typical thrombus of mixed type, consisting of interwoven fibrin threads, blood cells, and groups of desquamated endothelial cells, was observed 24 h after the beginning of the experiment (Fig. 1a). After peroral administration of liposomal forms of terrilytin, in 43.75% of cases total lysis of the 24-h thrombi was observed in 43.75% of cases, and juxtamural thrombi in different stages of lysis were observed in 37.5%; the thrombi remained intact in 18.75% of cases. In the control group preservation of the thrombi was observed in 75% of cases, and spontaneous lysis (at various stages) was observed in 25%. Lysis of the thrombi occurred in two stages: in the first stage the fibrinous part of the thrombus underwent lysis and the destroyed blood cells disappeared (Fig. 1b), and in the second stage destruction of the endothelial zones of the thrombogenic masses and enlargement of the lumen of the vessel were observed (Fig. 1c). Swelling of the vessel walls and of the nuclei of the endothelial cells could be classed among these effects. The atypical arrangement of the smooth-muscle cells of the vessel and the number of capillaries feeding the vessel wall did not return to normal.

Statistically significant changes in the parameters of hemostasis were evidence of the appearance of a hypocoagulation background after peroral administration of liposomal forms of terrilytin. It will be clear from Table 1 that 1 h after administration of terrilytin the clotting activity of the blood was reduced, on account of lengthening of the prothrombin and thrombin formation time (according to results of thromboelastography), which correlated with lowering of the fibrinogen concentration. The greatest decrease in the clotting potential of the blood, as shown by thromboelastography, was observed after 2-3 h, when the fibrinogen concentration also remained low. Investigation of aggregating activity of the platelets showed that 1 h after administration of terrilytin, incorporated into liposomes, the intensity of aggregation decreased, and a fall was observed after 2-3 h also. The aggregating activity of the platelets was low. Meanwhile increased fibrinolytic activity of the plasma was recorded, and was greatest 2-3 h after administration of terrilytin (the level of fibrinolytic activity remained high even after 24 h).

As the writers showed previously [5], the thrombolytic effect of terrilytin, unprotected by liposomes and administered in the form of a water-lipid emulsion, was exhibited only when high doses were given (2000-3000 PU/kg). The use of liposomal forms of terrilytin for peroral administration, which led to a reduction of the clotting activity of the blood, enables the dosage of terrilytin required to produce lysis of experimental thrombi to be considerably reduced.

TABLE 1. Changes in Parameters of Hemostasis after Peroral Administration of Liposomal Forms of Terrilytin (M ± m)

Parameter	Time of determination			
	background data	hours after administration		
		1	2—3	24
Number of measurements	23	15	12	19
R of thromboelastogram, min	4,89±0,41	6,30±0,93**	7,00±0,82*	5,70±0,76
Number of measurements	20	15	14	17
Platelet aggregation, %	56,9±1,97	47,1±2,69**	43,9±2,67**	49,7±3,27
Number of measurements	17	14	10	12
Fibrinogen concentration, g/liter	4,79±0,29	3,97±0,59*	3,77±0,33*	4,77±0,51*
Number of measurements	11	8	8	6
Fibrinolytic activity, mm^2	30,0±1,38	35,5±1,86**	40,12±3,85**	34,0±4,25

Legend. *P < 0.05, **P < 0.01 compared with background data.

The positive effect of peroral administration of liposomes containing drugs was first obtained in 1976 [13] on rats, in which a state of hyperglycemia was induced artificially. Administration of insulin, incorporated into liposomes, lowered the blood sugar level of the animals. Later similar experiments were conducted both with insulin and with other substances. Although in most cases this method of administration of drugs proved to be unsuccessful, nevertheless, there were some investigations which yielded positive results [11, 12]. The mechanism of this phenomenon has received little study, but there is no doubt about the results. The results of the present experiments can evidently be explained, on the one hand, by the ability of liposomes to screen the terrilytin contained in them against the action of active compounds contained in the digestive tract, and on the other hand, by their ability to introduce substances incorporated in them intracellularly.

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