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EFFECT OF BIORESOLVING MICROSPHERIC PREPARATIONS OF IMMOBILIZED FIBRINOLYSIN ON THE FIBRINOLYSIS SYSTEM

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The effect of fibrinolysin, immobilized on microspherical carriers, on the fibrinolytic system of the blood was studied in dogs. A marked increase in fibrinolytic activity of whole blood was found at the site of deposition of the preparation on account of the enzyme introduced and increased activity of plasminogen activator. Administration of immobilized fibrinolysin leads to a more marked increase in activator activity than of plasmin activity. The results suggest the therapeutic value of this method of administration of the thrombolytic preparation.

KEY WORDS: blood fibrinolysis; plasminogen activator; thrombolytic preparations.

The creation of bioresolving microspheric preparations containing immobilized drugs and, in particular, fibrinolysin, provides a completely fresh approach to the treatment of thrombosis, thromboembolism and possibly, of ischemic heart disease [2-4].

The suggested method of administration of thrombolytic preparations enables the preparation to be deposited (as microgranules with a particle size of 20-40 μ) in the affected blood vessel, a high local concentration of the lytic agent to be maintained near the thrombus, and the degree of contact with natural inactivators of the preparation and the doses used to be reduced, which in turn reduces the immune response of the recipient.

Advances in the method of selective angiography not only enable the location of the thrombosis to be determined accurately, but at the same time, the immobilized preparation can be applied in its vicinity.

The aim of the present investigation was to assess the specific action of fibrinolysin, immobilized on microspheric carriers, on the blood fibrinolysis system under experimental conditions.

EXPERIMENTAL METHOD

Fibrinolysin immobilized on modified Sephadex, with a carrier granule size of 20-40 μ and with a total biological resolving time under physiological conditions of not more than 3 h, was obtained by the method described by Torchilin et al. [3]. In this way preparations of the immobilized enzyme containing active protein in a concentration of 10-80 mg/g carrier were obtained.

Experiments were carried out on nine mongrel dogs weighing 15-20 kg; the femoral artery and vein were isolated under morphine-pentobarbtial anesthesia and the distal and proximal segments of the vein and the abdominal aorta were catheterized. Ten mg of the preparation, containing not less than 1500 units fibrinolysin dissolved in 3 ml rheopolyglucin, was injected into the distal segment of the femoral artery. Blood samples

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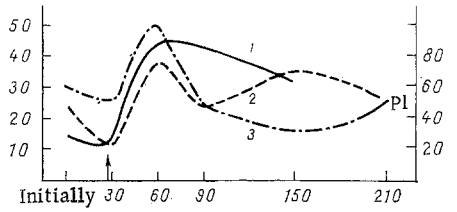

Fig. 1

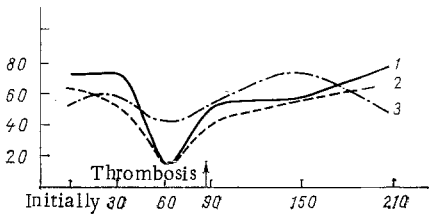

Fig. 2

Fig. 1. Fibrinolytic activity of whole blood flowing from site of deposition of fibrinolysin (1) and in systemic blood flow (2); plasmin activity in arterial blood (3). Arrow indicates injection of preparation. Here and in Fig. 2 mean data of three measurements are given; scatter does not exceed 10%. Abscissa, time (in min); ordinate, left: plasmin activity (in mm^2), right: fibrinolytic activity (in %).

Fig. 2. Injection of immobilized fibrinolysin in experiments with artificial thrombosis of femoral artery. Changes in fibrinolytic activity in blood flowing from (1) and toward (2) site of thrombosis and in systemic blood flow (3). Ordinate, fibrinolytic activity (in %). Remainder of legend as in Fig. 1.

were taken from the aorta, superior vena cava (systemic circulation), and femoral vein (blood flowing from the site of deposition of the preparation).

The next stage of the investigation was to study local and systemic fibrinolysis after deposition of immobilized fibrinolysin on an artificially formed thrombus in the femoral artery. Thrombosis of the femoral artery was induced as follows. A segment of the artery 1.5-2 cm long was ligated distally and proximally, a fixing device having been implanted in it beforehand (a copper spring of size corresponding to the cross-section of the artery, with 6-10 turns). Next, 0.1-0.2 ml of freshly prepared thrombin solution was injected into this segment and the proximal ligature was removed 10 min later, while the distal ligature remained in situ for 1 h. The presence of complete thrombotic occlusion was verified by means of a NASA (USA) ultrasonic flowmeter. An injection of 10 mg of the immobilized fibrinolysin was given directly into the region of the thrombus.

The final part of the experiments was as follows. The animal was anesthetized, the femoral artery and vein were isolated, and, under control of an electron-optical converter and the use of 76% verografin as contrast medium, the left coronary artery and the coronary venous sinus were selectively catheterized. The bioresolving microspheres with fibrinolysin were injected by the intracoronary route in a dose of 2-4 mg in 2-3 ml of rheopolyglucin (to prevent aggregation of the microparticles the suspension was treated with ultrasound before injection). Blood samples for testing were taken from the coronary sinus (blood flowing from the site of deposition of fibrinolysin), artery, and superior vena cava (systemic circulation) at the following times: before and 15, 30, 60, 120, and 180 min after injection of immobilized fibrinolysin.

To study the fibrinolysin system the blood levels of fibrinolytic activity [7], activity of plasmin and plasminogen activator [5], and inhibitory activity of antiplasmins [8] were determined.

EXPERIMENTAL RESULTS

Injection of immobilized fibrinolysin into the femoral artery of the dog caused a local increase in plasmin activity in the artery and an increase in fibrinolytic activity in the outflowing blood and the systemic blood flow. The indices calculated later showed a decrease because of the compensatory reaction of the healthy dog (Fig. 1).

In the experiments with artificial thrombosis of the femoral artery the fibrinolytic activity of the whole blood was sharply reduced locally against the background of thrombosis, and it increased after injection of the immobilized fibrinolysin; changes in fibrinolysis in the systemic blood flow were less marked (Fig. 2). The results obtained under these conditions indicate that this method of local deposition of immobilized fibrinolysin brings about the desired change of fibrinolytic activity to a considerable degree at the site of thrombosis. This fact is evidence of the clinical value of this method of administration of the thrombolytic preparation.

After intracoronary deposition of immoblized fibrinolysin there was an increase in the fibrinolytic activity of whole blood (by 16% locally and by 10.5% in the systemic circulation), followed by a decrease both locally and systemically. A decrease also was found in the arteriovenous difference of fibrinolytic activity

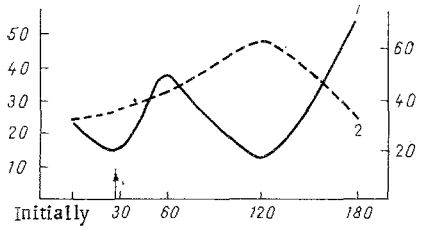

Fig. 3. Reciprocal relations between activities of plasminogen activator (1) and plasmin (2) after intracoronary deposition of immobilized fibrinolysin. Ordinate, right: plasminogen activator (in mm^2). Remainder of legend as in Fig. 1.

of whole blood from −40 to −8.4% after injection of the preparation; a more marked decrease in the fibrinogen concentration was observed in the artery than in the vein (by 212 and 160 mg % respectively).

Measurement of inhibitory activity of antiplasmins showed that initially the high concentration of antiplasmins could inhibit the enzyme as it passed from the carrier into the blood flow, and the increase in fibrinolytic activity took place in this case probably on account of fibrinolysis caused by the release of a considerable quantity of tissue activator into the blood stream. Against the background of low antiplasmin activity in the blood stream reciprocal relations were found between plasminogen activator and plasmin (Fig. 3). The crossing of the curves observed at the 180th minute of the experiment indicates that the changes induced by injection of the immobilized fibrinolysin were directed toward increasing the fibrinolytic potential of the blood. Even if plasmin activity was inhibited because of the compensatory reaction of the recipient animal, plasminogen activator activity was sharply increased.

Injection of immobilized fibrinolysin led to a more marked increase in activator activity than of plasmin activity. For instance, a single or double rise of plasmin activity was observed in the systemic blood flow. Similar results were obtained during intracoronary perfusion of fibrinolysin with heparin in patients with acute myocardial infarction [1]. This fact can be explained by binding of fibrinolysis inhibitors with the injected fibrinolysin, with the result that activity of endogenous plasminogen activator was exhibited [6], and (or) by contamination of the fibrinolysin preparation used with trypsin, added in order to activate the plasminogen during the production of fibrinolysin.

Dependence of activator activity on its initial level is noteworthy: When plasminogen activator activity was zero, injection of immobilized fibrinolysin led to a sharp increase in its activity; if the initial value reached a certain level, activator activity first decreased, and only increased later.

To sum up what has been said it can thus be stated that injection of fibrinolysin immobilized on modified Sephadex leads to a marked increase of fibrinolysis in whole blood at the site of deposition of the preparation because of the enzyme thus introduced and an increase in plasminogen activator activity. The results suggest the therapeutic value of this method of administration of the thrombolytic preparation.

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