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EXPERIMENTAL ASPECTS OF THE THERAPEUTIC ACTION OF UROKINASE IN THROMBOEMBOLIC STATES

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The problem of thrombosis and embolism occupies an important position in modern medicine and has recently become one of considerable urgency because of the increased incidence of cardiovascular diseases and the development of thoracic and vascular surgery, which are attended by a high risk of development of thromboembolic complications. Much evidence has recently been gathered on the surgical and combined (together with fibrinolytic agents) methods of treatment of thrombosis and embolism [2-7]. However, besides the use of fibrinolysin, an approach that is beginning to be more intensively pursued is lytic therapy by means of urokinase, an activator of fibrinolysis which is so far the most promising of the thrombolytic agents [8-10].

The object of this investigation was to study the effect of urokinase on the fibrinolysis system and on the thrombolytic process under experimental conditions and to assess thrombolytic treatment during induction of endogenous fibrinolysis with urokinase.

A highly active preparation of urokinase with specific activity of 30,000-40,000 i.u./mg protein was isolated from healthy human urine by methods of adsorption, ion-exchange chromatography and gel filtration.

To study the fibrinolytic and thrombolytic action of urokinase experiments were carried out on rabbits weighing 2-2.5 kg. Local thrombosis was produced in 8 rabbits by injection of thrombin into a segment of the marginal vein of the ear. The presence of a thrombus in the vein was detected by phlebography, in the form of a filling defect in the vessel (Fig. 1a). Under the influence of urokinase, lysis of the clot took place (Fig. 1b). In 15 rabbits disseminated microthrombosis was induced by heterologous blood transfusion. In this group of animals, after production of thrombosis and after injection of urokinase, the following indices of the fibrinolytic system of the blood was studied: the time of lysis of a clot of euglobulin fractions, fibrinogen content, plasminogen level, degree of activity of antiplasmins, and thrombin time, indicating the combined action of fibrinogen degradation products.

Experiments also were carried out on 72 albino rats in which massive microthrombosis was induced by injection of heterologous blood (2.5 ml human blood/100 g body weight). These

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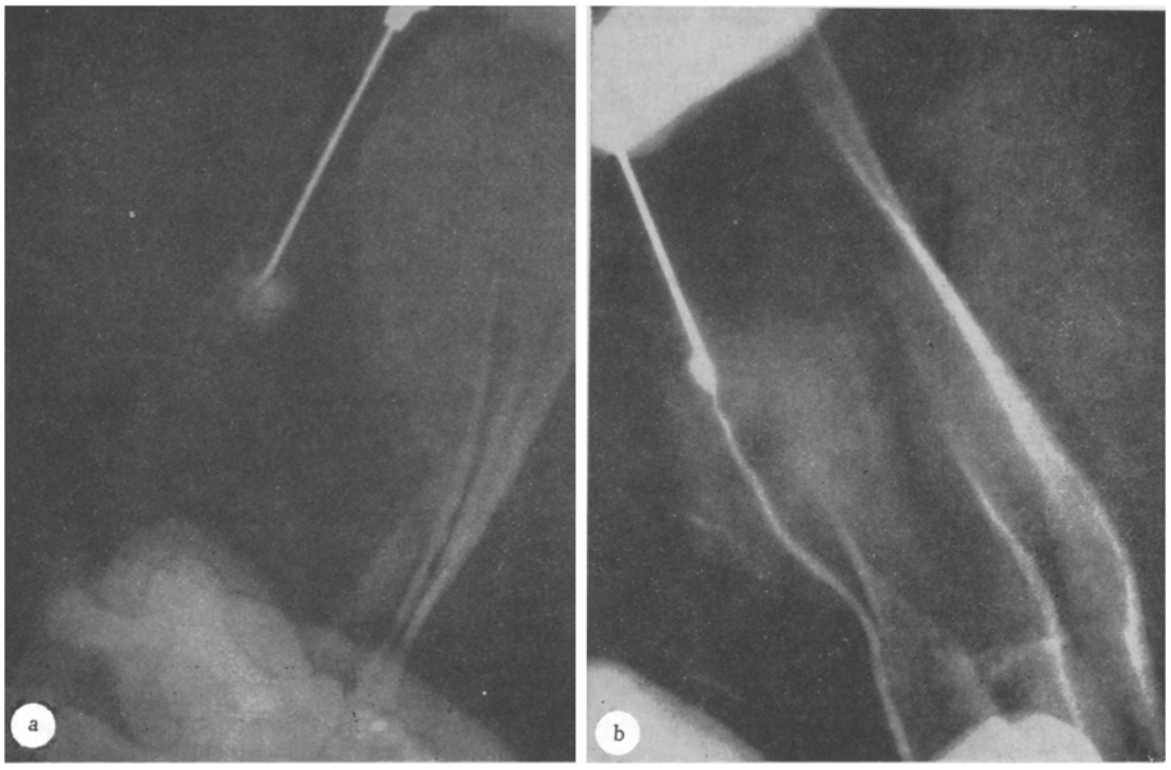

Fig. 1. Phlebogram: a) thrombosis in marginal vein of rabbit's ear; b) destruction of thrombus by urokinase, lumen of vessels free.

animals were divided into two groups: group 1 — control, group 2 — rats receiving urokinase by intravenous injection at different time intervals after induction of disseminated microthrombosis (in a dose of 100 i.u./100g body weight), together with heparin. The animals were killed 1 h after injection of the preparation and material taken (kidney, liver, heart, lungs, spleen) for histological examination. Sections were stained with hematoxylin-eosin.

Experiments also were carried out on five female dogs in which chronic pyelonephritis accompanied by disseminated microthrombosis of the renal vessels was induced [1]; the development of this condition could be judged from the appearance of pyuria and a sharp fall in the partial kidney function.

EXPERIMENTAL RESULTS

Injection of urokinase into rabbits with disseminated microthrombosis caused an increase in the degree of fibrinolysis and a decrease in fibrinogen concentration 30-60 min after injection of the enzyme. For instance, the fibrinolysis time 30 min after injection of urokinase was shortened from 348 ± 90.2 to 153 ± 40.6 min, after 60 min it was 114 ± 29.0 min, and after 24 h it returned to its initial values. The fibrinogen concentration fell from 5.86 ± 0.82 to 2.24 ± 1.05 g/liter after 30 min, and to 2.66 ± 0.60 g/liter after 60 min. The fibrinogen concentration after 24 h was 3.28 ± 1.12 g/liter. The plasminogen level was reduced almost by half at the time of highest fibrinolytic activity of the blood, evidence of endogenous activation of plasminogen by urokinase when conditions were optimal for the body. During the same period a maximal decline in antiplasmin activity and lengthening of thrombin time were observed.

Histological examination of the rats' organs revealed the presence of microthrombi (Fig. 2a) in the lumen of many small blood vessels of the parenchymatous organs of the control group (Fig. 2a). In animals of the experimental group, sacrificed at different times after injection of urokinase, complete circulatory arrest was found in the vessels of the parenchymatous organs (Fig. 2b), evidence of the good thrombolytic effect of the preparation.

After intravenous injection of urokinase with heparin (500 i.u./kg and 2 mg/kg, respectively), into dogs with experimental pyelonephritis, rapid and complete recovery of the filtration—reabsorption—secretion function of the kidneys and of the renal blood flow took

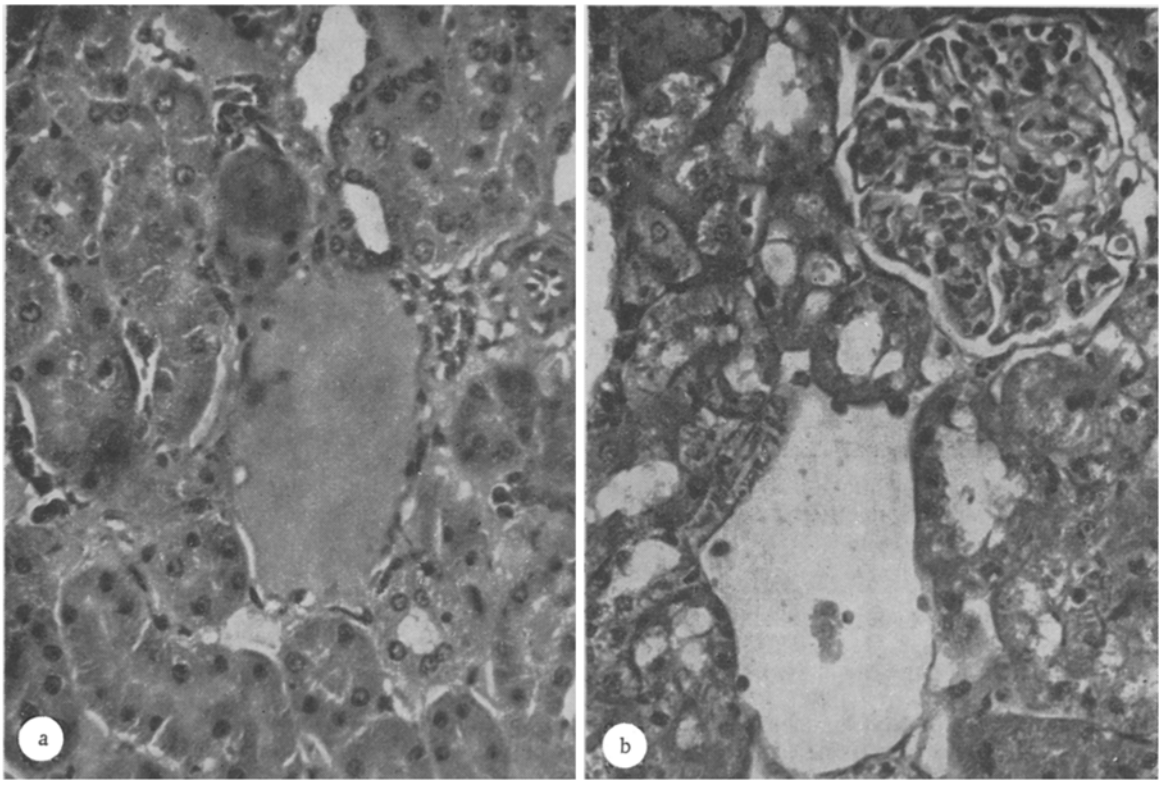

Fig. 2. Histological section through rat kidney: a) thrombosis of blood vessel, fibrinoid swelling; b) lumen of vessel free. Stained with hematoxylin–eosin, 63 ×.

place. Only 2 h after injection of urokinase the glomerular filtration had increased from 32.6 to 63.7 ml/min, the maximal tubular secretion from 13.9 to 21.1 mg iodine/min, tubular reabsorption from 85.6 to 91.3 ml/min, and the renal plasma flow from 108.5 to 195 ml/min. Restoration of partial kidney functions in dogs with pyelonephritis after injection of urokinase is evidence of the thrombolytic action of the preparation.

The experimental data on the thrombolytic action of urokinase thus obtained confirmed that it is a promising agent for the treatment of thromboembolic states.

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