EXPERIMENTAL BASIS OF THE DIAGNOSIS OF THROMBOSIS BY MEANS OF I^{131}-LABELED FIBRINOLYSIN

(UDC 616-005.6-073.916)

E. I. Chazov, V. M. Bogolyubov, E. I. Denisov and M. Ya. Ruda

Institute of Therapy (Director, Active Member AMN SSSR Professor A. L. Myasnikov) of the AMN SSSR and Institute of Medical Radiology (Director, Active Member AMN SSSR Professor G. A. Zedgenidze) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR A. L. Myasnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 7, pp. 28-31, July 1965
Original article submitted May 15, 1964

It is unnecessary to mention the importance of the diagnosis of thrombosis in general and of coronary thrombosis in particular, for a correct and early diagnosis makes it possible to solve a problem of fundamental importance—the choice of a method of effective treatment.

The basis of the thrombus is formed by threads of fibrin which are deposited as a result of the enzymic conversion of fibrinogen by the action of thrombin. Besides an increase in the concentration of the blood clotting factors, an important role in this process is played by a lowering of the fibrinolytic activity of the blood. The depression of fibrinolysis, diminishing the possibility of destruction of fresh fibrin threads, favor the development of thrombosis. Accordingly, in recent years the intravenous injection of fibrinolysin, activated in vitro, has been used successfully for the treatment of thrombosis. The specific substrates of the action of fibrinolysin are fibrinogen and fibrin. Some workers consider that fibrinolysin exerts its greatest action on fibrinogen [1, 4], while others are of the opinion that it acts mainly on fibrin [5]. A third group believes that fibrinogen and fibrin are equally subjected to the splitting action of fibrinolysin [6]. Despite the conflicting nature of the data concerning the action of fibrinolysin on the components of the thrombus, it is considered that the point of its action is in fact the thrombus: it is here that extrinsic fibrinolysin is concentrated after its administration [3]. If this is true, then by labeling fibrinolysin with isotopes, the concentration of the radioactive isotope can be obtained in the region of the thrombus.

The object of the present investigation was to discover whether in fact the concentration of fibrinolysin increases at the point of its action, and also to study the diagnostic possibilities of the method of injection of I^{131}-labeled fibrinolysin for the detection of thrombosis.

EXPERIMENTAL METHOD

Thrombi were produced in different parts of the vascular system by the method described previously [2]. I^{131}-fibrinolysin was obtained by iodation of commercial fibrinolysin with iodine monochloride after isotope exchange with NaI^{131}. After iodation, the mixture was passed through an ion-exchange column containing the anion exchange resin AB-17 in the Cl-form.

EXPERIMENTAL RESULTS

Control observations on dogs with thromboses in different parts of the vascular system, receiving injections of I^{131} alone in a dose of 20-30 μCi, revealed no regular changes in radioactivity in the region of the thrombus or in the peripheral blood.

The following investigations were carried out to study the concentration of radioactive fibrinolysin in the thrombus.

In five dogs thrombosis of the anterior coronary artery was produced by injecting thrombin, warmed to 37°, into an isolated segment (2 cm long) of the vessel. After 30-40 min the ligatures were removed and 20-30 μCi

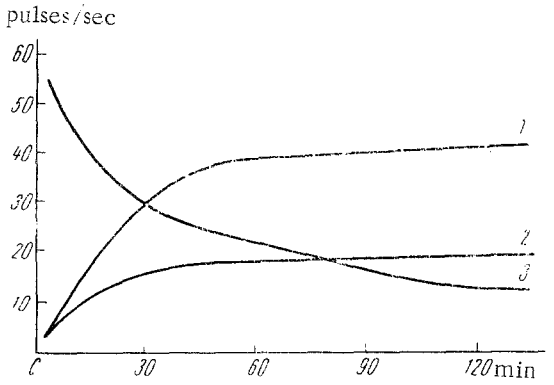

Fig. 1. Changes in radioactivity in the region of the thrombosed (1) and intact (2) femoral vein and in the region of the heart (3) after intravenous injection of 20 μCi of I^{131}-fibrinolysin into a dog.

of I^{131}-fibrinolysin was injected into the femoral vein. The dose of fibrinolysin injected with radioactive iodine, amounting in these experiments to 40-50 units, was insufficient to cause destruction of thrombus. One hour later three pieces of myocardium of equal weight, containing vessels of approximately the same caliber, were excised. One piece was excised together with the thrombus, another near to the site of the thrombus, and the third from the opposite wall of the myocardium. The resected areas of myocardium were weighed on analytical scales. The activity of I^{131}-fibrinolysin, determined with a well-type scintillation counter, was expressed as the number of pulses per minute per gram weight of excised myocardial tissue.

The experimental results showed that 1 h after the injection of I^{131}-fibrinolysin the mean activity of 1 g myocardial tissue at the site of the thrombus, in the adjacent area without thrombus, and in the opposite wall of the heart amounted to 2672, 1692, and 1228 pulses/min respectively, i.e., the activity was highest in the thrombosed segment of the vessel.

In four dogs thrombosis of the femoral vein was produced by the application of ligatures and injection of thrombin. The length of the thrombus varied from 6 to 7 cm. One hour after formation of the thrombus and removal of the ligatures, 20-30 μCi of I^{131}-fibrinolysin was injected into the vein in a segment free from thrombus. After an interval of 24 h the portion of the vein with the thrombus and the corresponding portion of the vein from the opposite limb were excised and placed, together with the blood, in penicillin flasks. The results of these experiments showed that the activity of I^{131}-fibrinolysin per gram of vessel with and without thrombus was on the average 724 and 380 pulses/min respectively.

It is clear from the findings described above that in the thrombosed areas the activity and, consequently, the concentration of I^{131}-fibrinolysin was much higher than in the unthrombosed vessels. This provides a basis for a promising method of detection of the thrombosed portion of a blood vessel.

To carry the study of the problem further, the next series of experiments was conducted on 12 dogs, in which both femoral veins were exposed under anesthesia with morphone and sodium amytal, and a thrombus was produced in one of them, measuring 2.5-3.0 cm, by application of ligatures and injection of thrombin; the other vein was used as control. The ligatures were removed 1.5-2 h after formation of the thrombus and 20-30 μCi of I^{131}-fibrinolysin was injected into the control vein. Scintillation counters were placed on both veins—thrombosed and control—and also over the region of the heart, at the same distance from the vessels. Recordings were made on a type EPP-09 apparatus for a period of 1-10 h in the different animals. The results of these experiments showed that the activity in the region of the thrombosed vessel was 20-50% higher than in the control vessel (Fig. 1). This increase in activity was most probably the result of an increased concentration of I^{131}-fibrinolysin in the area of the thrombosed vein.

In the next series of experiments thrombi measuring 2.5-3.0 cm were produced in nine rabbits in a vein of the ear by clamping both ends of the vessel and its collaterals and injecting warmed thrombin into this area. The rabbit's other ear served as the control. I^{131}-fibrinolysin was injected into the vein of the control ear in a dose of 15 μCi 30-40 min after production of the thrombus. The activity was measured with a scintillation pick-up through a lead shield, 5 mm thick, in which holes of equal sizes had been cut for the animal's ears.

During the first 30-60 min after injection of the preparation the activity in the region of the thrombosed ear was actually slightly lower than in the control ear. This fact was evidently caused by incomplete absorption of the preparation following its injection into the vein of the unthrombosed ear. After 1.5-3 h the activity in the region of the thrombosed ear rose considerably and now exceeded the activity in the control ear. After 24 h a further increase in the activity was observed over the region of the thrombus, amounting on the average to 30%.

Blood was taken from the unthrombosed ear of 6 of the 9 rabbits 24 and 48 h after injection of the isotope and compared with blood samples obtained from the 6 control rabbits without thrombi which also received injections of

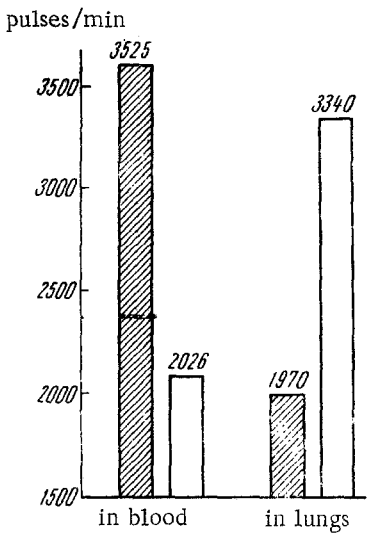

Fig. 2. Mean radioactivity of 1 g peripheral blood and lung tissue from control rabbits (shaded columns) and from rabbits with thrombo-embolism of the lungs (unshaded columns) 2 days after injection of I^{131}-fibrinolysin.

the same dose of I^{131}-fibrinolysin. The results of these experiments showed that the activity of 1 g blood of the rabbits with and without thrombi was 5022 and 6268 pulses/min respectively after 48 h and 2026 and 3525 pulses/min respectively after 48 h. Hence the activity of the blood of the rabbits with thrombi was much lower than that of the control animals: this may indicate that concentration of I^{131}-fibrinolysin takes place at the site of thrombosis.

Investigations were also carried out on 14 rabbits in which thrombo-embolism of the branches of the pulmonary artery was produced by injection of dried human blood clot into the auricular vein of the animal, followed (after 20 min) by injection of 0.3 ml of 1% fibrinogen solution and 0.3 ml of thrombin solution. The results of this investigation showed that after 1.5 h the activity of 1 g of blood from the healthy rabbits and the rabbits with thrombo-embolism was 9830 and 6900 pulses/min respectively. The rabbits were sacrificed after 48 h. The activity of 1 g of lung tissue from the rabbits with thrombo-embolism was 1370 pulses/min and from the control animals—only 780 pulses/min (Fig. 2).

Hence, in areas of tissue containing thrombosed vessels a much higher concentration of radioactive I^{131}-fibrinolysin was observed than in areas of tissue without thrombosis. Meanwhile in the peripheral blood the opposite relationship was found between the activity: it was much lower in the animals with experimental thrombi than in the healthy controls.

The accumulation of radioactive I^{131}-fibrinolysin in the region of the thrombus and its rapid disappearance at the same time from the peripheral blood may be used for the clinical diagnosis of thrombosis.

LITERATURE CITED

1. V. S. Il'in, Uspekhi sovr. biol. 26, 3, (1948), p. 853.
2. E. I. Chazov, Byull. éksper. biol., 8, (1961), p. 22.
3. N. Back, J. L. Ambrus, and I. B. Mink, Circulat. Res., 9, (1961), p. 1208.
4. E. H. Kaplan, Proc. Soc. exp. Biol. (N. Y.), 85, (1954), p. 142.
5. A. J. Quick, Ann. Rev. Physiol., 12, (1950), p. 237.
6. O. D. Ratnoff, J. clin. Invest., 32, (1953), p. 473.

All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.