FIBRINASE ACTIVITY IN RATS IRRADIATED WITH X-RAYS

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The arrest of bleeding from an injured vessel depends not only on the speed of clotting of the blood, but also on the quality of the blood clot. The formation of a blood clot insoluble in urea during the process of coagulation of the blood takes place with the participation of the fibrin-stabilizing factor of Laki and Lorand (fibrinase) [1]. Changes in the fibrinase activity lead to disturbances of the process of formation of a fully effective blood clot, and this may be the cause of an increased bleeding tendency [2].

The fibrinase activity has not been studied in acute radiation sickness. Because the pathogenesis of the bleeding tendency in acute radiation sickness has not been explained, the study of the influence of ionizing radiation on the fibrinase activity is interesting.

EXPERIMENTAL METHOD

Experiments were conducted on 88 rats of the "August" line, of both sexes, 3 months old, and weighing 125-140 g. Acute radiation sickness was evoked by a single whole-body irradiation with γ-rays (CO^{60}) in a dose of 600 and 3000 R (dose rate 88 R/sec). The severity of the radiation sickness was assessed from the clinical picture and the blood morphology.

Fibrinase was determined by the method of Sigg and Duckert [2]. The principle is based on the property of monoiodoacetic acid of blocking the transformation of fibrin S into fibrin i by bringing into play the action of fibrinase.

The determinations were made in venous blood taken from the jugular vein of the rats into syringes coated with silicone (dichlorodimethylsilane), mixed with 1.34% sodium oxalate solution in the ratio of 9:1 in polystyrene tubes, and quickly centrifuged for 10 min at 2000 rpm; the plasma was drawn off and kept in a refrigerator at 4° until the investigation began.

Seven test tubes were each filled with 0.2 ml plasma, 0.1 ml of a solution of monoiodoacetic acid of different concentration (from 0.6 to 0.1%), 0.4 ml of a 0.277% solution of calcium chloride, and 0.1 ml of thrombin solution (activity 15 sec). After 20 min, 2 ml of a 5 M solution of urea was added to each tube. The speed and degree of solution of the fibrin were measured. The degree of solution of the fibrin was estimated by a five-point system: 0) no clot was formed after addition of calcium chloride and thrombin to the plasma containing monoiodoacetic acid; 1) complete solution of the clot; 2) small floccules; 3) large floccules; 4) the clot was not dissolved.

EXPERIMENTAL RESULTS

In the period of clinical manifestations of radiation sickness, on the 7th day after irradiation, the rats irradiated in a dose of 600 R were lethargic and their hair was untidy; their leukocyte count was 1900 ± 760 per mm^3, their erythrocyte count 7,160,000 ± 439,000 per mm^3, and their Hb concentration 12.4 ± 2.4%*. Twenty four hours after irradiation, the test for latent blood in most animals was positive, and on the 7th day it was strongly positive.

In the period of clinical manifestations, 52 h after irradiation, the rats irradiated in a dose of 3000 R were lethargic, their hair was untidy, scabs and blood-stained discharges were present in the region of the nose, and the stools were liquid and contained mucus. The leukocyte count was 395 ± 180 per mm^3. At autopsy, hemorrhages were found into the subcutaneous cellular tissue, the pleura, and the internal organs.

In the control healthy rats, the leukocyte count was 5800 ± 1340 per mm^3, the erythrocyte count 7,920,000 ± 500,000 per mm^3, and the Hb concentration 15 ± 0.4 g%.

*M ± σ.

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Solution of a Fibrin Clot Formed in the Presence of Monoiodoacetic Acid in a 5 M Solution of Urea

Animals from which plasma is taken	Concentration of monoiodoacetic acid (in %)						Time taken to dissolve (in min)
	0.6	0.5	0.4	0.3	0.2	0.1	
Healthy	1	1	1	2	3	4	120
Irradiated (600 R, 24 h after irradiation)	0	0	1	1	2	2	20
Irradiated (600 R, 7th day after irradiation)	0	0	1	1	2	2	1
Irradiation (3000 R)	0	0	0	0	1	2	60

Legend: 0) no clots formed; 1) complete solution of fibrin; 2) small floccules; 3) large floccules; 4) clots did not dissolve.

As the experimental results showed, the fibrin clots formed from the fibrinogen of the plasma of the healthy rats in the presence of monoiodoacetic acid of different concentrations (0.6-0.1%) were firm opaque, they did not contract for 20 min, and they occupied the whole lumen of the tube.

The clots formed from the fibrinogen of the plasma of the irradiated animals differed essentially from the control clots. When a solution of calcium chloride and thrombin was added to the plasma of the irradiated rats (dose 3000 R), containing monoiodoacetic acid in a concentration of 0.6-0.3%, no fibrin clots were formed, and if the concentration of monoiodoacetic acid was 0.2-0.1%, the clots were very fragile and transparent. No clots were formed in the plasma of the rats irradiated in a dose of 600 R in the presence of monoiodoacetic acid in the solutions in concentrations of 0.6 and 0.5%, in the tubes in which the concentration of acid was 0.4-0.3% the clots were fragile and transparent, and only if the concentration was lower (0.2-0.1%) did the addition of calcium chloride and thrombin to the plasma lead to the formation of firm clots.

The table shows that the fibrin clots formed from the fibrinogen of the plasma of the healthy rats dissolved in the course of 2 h in a 5 M urea solution only if the concentration of monoiodoacetic acid was 0.6-0.4%, while at a lower concentration solution was incomplete, and in the tube with monoiodiacetic acid in a concentration of 0.1% no signs even of partial solution of the clot were present. It is clear from the table that 24 h after irradiation the process of clot formation was disturbed. In the plasma of the irradiated rats, no fibrin clots were formed in the tubes containing monoiodoacetic acid in concentrations of 0.6 and 0.5% after irradiation in a dose of 600 R and in concentrations of 0.6-0.3% after irradiation in a dose of 3000 R. The fibrin clots obtained from the plasma of the irradiated rats dissolved more completely even when the concentration of the acid was 0.1%, and in these circumstances the rate of solution of these clots was much faster than in the case of the unirradiated rats.

Hence, under the influence of ionizing radiation, the fibrinase activity in the rats fell sharply. A fall in activity was observed only 24 h after irradiation of the animals with γ-rays in a dose of 600 R. The mechanism of the change in fibrinase activity is not clear. It may possibly be due to a disturbance of the synthesis of fibrinase, to a change in the activity of its inhibitors, or to direct injury of the fibrinase by the ionizing radiation through its influence on the SH-groups of the enzyme.

The disturbance of the structure of the blood clot caused by the fall in fibrinase activity may be important in the pathogenesis of the bleeding tendency arising in radiation sickness.

LITERATURE CITED

1. K. Laki and L. Lorand, Science, 108, 280 (1948).
2. P. E. Sigg and F. Duckert, Schweiz. med. Wschr., 93, 1455 (1963).