CHANGES IN FRACTIONAL COMPOSITION OF PLATELET PROTEINS IN EXPERIMENTAL ACUTE RADIATION SICKNESS

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Platelet proteins of healthy and irradiated rats were divided into 13 fractions by anodic electrophoresis in polyacrylamide gel. In rats with acute radiation sickness the content of platelet proteins with electrophoretic mobility corresponding to prealbumin, albumin, γ-globulin, and fibrinogen was reduced whereas content of proteins with mobility corresponding to α- and β-globulins was increased.

KEY WORDS: rat platelets; electrophoresis of proteins; ionizing radiation.

Acute radiation sickness is accompanied not only by thrombocytopenia, but also by disturbance of the morphology and function of circulating platelets [2, 3]. In some hemorrhagic diatheses (afibrinogenemia, thrombasthenia) simultaneously with a disturbance of platelet function, changes in the protein composition of the platelets also are observed [5, 8, 10].

This paper gives data on the fractional composition of platelet proteins in animals with a postirradiation hemorrhagic syndrome.

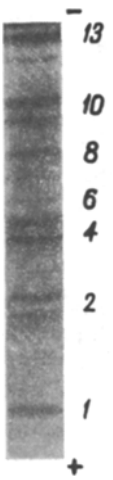

Fig. 1. Electrophoresis of platelet proteins of healthy rats. Fractions numbered in order of diminishing electrophoretic mobility.

EXPERIMENTAL METHOD

Experiments were carried out on 90 Wistar rats weighing 250-300 g. Of this total number of rats, 60 were irradiated with Co^{60} γ-rays in a dose of 600 R (dose rate 23 R/sec). Tests were carried out 7 days after irradiation when, according to the clinical and hematologic evidence, moderately severe acute radiation sickness had developed. Blood was taken from the jugular vein of the healthy and irradiated rats and mixed with anticoagulant (3.8% sodium citrate) in the ratio of 9 : 1. Isolation of the platelets from the blood was carried out by differential centrifugation at 300 and 750 g. The residue of platelets after three washes in 0.1 M tris-HCl buffer, pH 7.5, was resuspended in the same buffer and treated by freezing and thawing three times (−20° C). The undestroyed platelets and their fragments were sedimented at 23,000 g for 30 min. The resulting supernatant was used for electrophoresis, after preliminary determination of its total content of platelet proteins [7]. Disc electrophoresis of the platelet proteins was carried out in 7% polyacrylamide gel (pH 8.9), using tris-glycine buffer [9]. To determine the comparative characteristics of the various fractions, rat blood–plasma proteins and preparations of human albumin (Schuchardt, West Germany), fibrinogen, and γ-globulin, obtained by chromatography on DÉAÉ-cellulose [1], were fractionated parallel with the platelet proteins. Densitometry of the stained gels was carried out on the MF-4 microphotometer.

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TABLE 1. Relative Percentages of Fractions of Platelet Proteins from Healthy and Irradiated Rats (M ± m)

Group of animals studied	Fraction No. 1	2	3	4	5	6	7
Healthy	0,5±0,1	2,3±0,3	3,4±0,3	6,4±1,0	10,3±1,1	5,8±0,6	11,3±1,1
Irradiated	0,5±0,1	0,7±0,2*	0,6±0,1*	4,1±0,2*	14,5±1,8	9,1±0,8*	8,8±0,6

Group of animals studied	Fraction No. 8	9	10	11	12	13
Healthy	11,0±1,1	6,0±0,7	11,7±0,7	8,3±0,6	7,1±0,5	15,9±1,1
Irradiated	8,9±0,8	13,4±1,2*	9,7±0,7	11,0±0,7*	6,4±0,5*	12,3±1,0*

Legend. Fractions numbered in order of decreasing electrophoretic mobility. * $P < 0.05$ (comparison with intact rats).

EXPERIMENTAL RESULTS

The mean quantity of protein isolated from 10^9 healthy rat platelets was 259 ± 28 μg. On electrophoresis the platelet proteins were separated into 13 fractions (Fig. 1, Table 1). In their electrophoretic mobility fractions Nos. 1-3 of the platelet proteins corresponded to blood plasma prealbumin, No. 4 to albumin, No. 5 to α_1-globulin, Nos. 6-11 to α_2- and β-globulins, No. 12 to γ-globulin, and No. 13 to fibrinogen. If before fractionation the platelet proteins were incubated with thrombin (5 units thrombin to 1 mg platelet proteins) for 1 h at 37° C, no fraction No. 13 appeared thereafter; this fact confirms the location of platelet fibrinogen on electrophoresis. The results for the relative percentages of the platelet proteins and the mobilities of albumin, γ-globulin, and fibrinogen are in agreement with the results of immunochemical and chemical investigations of platelet proteins described in the literature [4, 6].

The quantity of protein isolated from 10^9 platelets from irradiated rats was the same as from healthy animals (mean 270 ± 36 μg). On fractionation the platelet proteins of the irradiated rats also were divided into 13 fractions, but their relative percentages were different from those of the proteins of healthy animals (Table 1): in acute radiation sickness the content of platelet proteins with the mobility of prealbumins, albumin, γ-globulin, and fibrinogen was reduced, and the content of proteins with the mobility of α- and β-globulins was increased. Changes in the fractional composition of the platelet proteins in acute radiation may be connected with a change in the synthesis of the platelet proteins and also with changes in the composition of the blood–plasma proteins, (albumin, γ-globulin, fibrinogen) and their ability to be adsorbed on the surface of the platelets.

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