STATE OF THE ANTIOXIDATIVE ENZYMES OF RAT BONE MARROW CELLS AFTER IRRADIATION, FRACTURES, AND A COMBINATION OF BOTH

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KEY WORDS: irradiation; fractures of bones; bone marrow; antioxidative enzymes.

Damage to bone marrow is a characteristic result of general exposure of the body to ionizing radiation and, as a rule, it determines the severity and outcome of radiation sickness. Data in the literature indicate that the use of antioxidants is an effective measure in radiation damage [1, 13], and that they stimulate the proliferative activity of hematopoietic cells [14]. Disturbances of the hematopoietic function of bone marrow, depending on the intensity of exposure, have been found in animals with various mechanical injuries to the limb tissues [7]. The use of antioxidants has been shown to stimulate regeneration of bone tissue in fractures of the limbs [4].

Meanwhile information on the state of enzymes belonging to the antioxidative (antiradical) defensive systems (ADS) of bone marrow cells [2] after irradiation, fractures, or, more especially, a combination of both, is extremely limited [6, 10]. The aim of this investigation was to study bone marrow levels of ADS enzymes, namely superoxide dismutase (SOD), glutathione peroxidase (GP), glutathione reductase (GR), and glutathione: dehydroascorbate oxidoreductase (GDAR), in rats and changes in their activity in the bone marrow at various times after irradiation, mechanical trauma, and a combination of both.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 180-200 g were kept on the standard animal house diet. Irradiation in a dose of 206 mCi/kg body weight was given and the bones fractured by the method described previously [5]. A suspension of bone marrow cells was obtained by flushing out the marrow from two femors with 2 ml of cold, buffered physiological saline (0.15 M NaCl in 0.05 M potassium-phosphate buffer, pH 7.4), and homogenized in a homogenizer of Potter type for 3 min at 2000 rpm. The resulting homogenate was treated with 0.1% Triton X-100 (final concentration) and centrifuged for 15 min at 600g. Activity of GP, GR and GDAR was determined as described previously [3] and calculated in nanokatals of reduced glutathione oxidized (GP, GDAR) or formed (GR) per milligram protein and per milligram DNA. SOD activity was determined as the degree of inhibition of cytochrome C reduction in the xanthine oxidase system after treatment of bone marrow homogenates with a mixture of chloroform and ethanol [12]. The results were expressed in conventional units of SOD activity per milligram protein and per milligram DNA. The protein concentration was determined by the method in [11] after treatment of the homogenates with 0.1 N NaOH and a 0.5% solution of sodium deoxycholate. The DNA concentration was determined as in [8]. All determinations were done on a Beckman Model 34 spectrophotometer (Austria).

EXPERIMENTAL RESULTS

Table 1 gives the results of determination of ADS enzymes in the bone marrow of intact rats. They show that the bone marrow contained all the enzymes tested, but the level of their activity (calculated per milligram portein) was only 25 to 50% of that found in the parenchymatous organs. This result confirms data in the literature on SOD activity in rat bone marrow and liver cells [10].

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TABLE 1. Activity of ADS Enzymes in Rat Bone Marrow (M ± m)

Enzyme	Activity, units	
	per milligram protein	per milligram DNA
GP	0,93±0,03 (35)	31,8±3,9 (42)
GR	0,53±0,01 (48)	16,4±1,1 (41)
GDAR	0,19±0,01 (36)	5,70±0,38 (42)
SOD	1,62±0,06 (23)	55,6±2,8 (33)

Legend. Number of determinations given in parentheses.

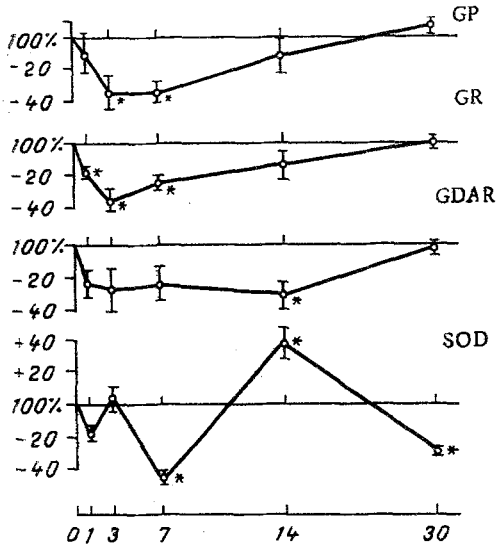

Fig. 1. Effect of single whole-body x-ray irradiation in a dose of 206 mCi/kg body weight on activity of ADS enzymes in rat bone marrow cells. Abscissa, time after irradiation (in days); ordinate, change in specific enzyme activity (in % of control). Here and in Figs. 2 and 3: *p < 0.05.

The development of acute radiation sickness (ARS), as a result of a single irradiation, was accompanied by marked changes in the enzymic antioxidative system of the rat bone marrow cells (Fig. 1). During the first week after irradiation the specific activity of GP and GR fell: the greatest decrease was observed on the 3rd day after irradiation, when GP was 34% and GR 35% of the control level. A fall of GDAR activity by 31% was discovered on the 14th day, but at all other times of observation deviations from the control values were not significant. SOD activity on the 1st and 3rd days after irradiation did not differ significantly from the initial value, on the 7th day it fell by 44%, by the 14th day it had risen by 36%, and later it fell again, so that at the end of the time of observation it amounted to 70% of the control. The fall of SOD activity in the rat bone marrow discovered on the 7th day after x-ray irradiation is in agreement with data in [10]; however, the authors cited, using gamma-irradiation of rats in the same dose, found a decrease in enzyme activity at other times of observation also: on the 3rd and 14th days.

The results of determination of the activity of enzymes regulating the intensity of peroxidation and free-radical oxidation in the bone marrow of rats with closed fractures are given in Fig. 2. Specific activity of GR was 31% higher 24 h after trauma. No statistically significant changes were found in GP and GDAR activity in the bone marrow cells of the rats after injury. SOD activity on the 7th and 30th days after trauma was reduced by 46 and 16%, respectively; this is interesting in connection with the ability of the combination of substances with antioxidative action, including SOD, to stimulate post-traumatic regeneration of bone [4].

Infliction of mechanical trauma on irradiated animals results in earlier and more severe damage to the enzymic glutathione redox system of rat bone marrow (Fig. 3) compared with that

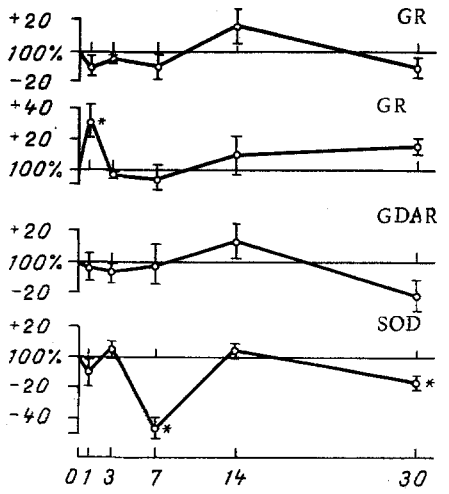

Fig. 2. Activity of GP, GR, GDAR, and SOD in bone marrow of rats with fractures.

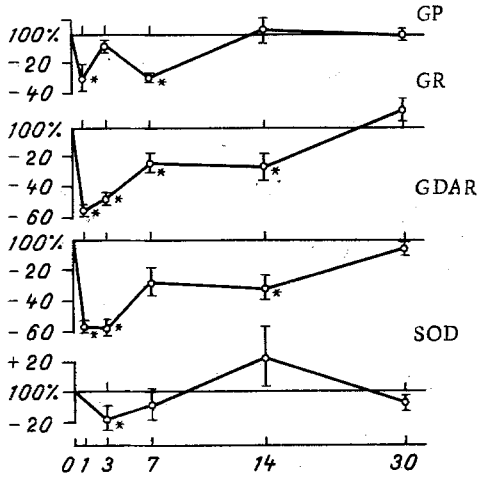

Fig. 3. Effect of combined radiation damage (irradiation in a dose of 206 mCi/kg + mechanical trauma) on activity of ADS enzymes in rat bone marrow cells.

observed in animals with ARS (Fig.1). Specific activity of GP on the 1st and 7th days of combined radiation damage (CRD) was 30% lower than in the control. By the 14th day activity of the enzyme had reached the control level and thereafter it remained unchanged until the 30th day. GR activity on the 1st, 3rd, 7th, and 14th days was reduced by 55, 48, 24, and 26%, respectively. The time course of GDAR activity in CRD was similar in character: on the 1st and 3rd days after trauma it was 58% below the control level, after which it gradually rose to reach the control level again by the end of the period of observation (on the 30th day). The time course of SOD activity in CRD was less marked than that observed as a result of irradiation and mechanical trauma separately: a reduction (by 18%) of activity of the enzyme was not observed until the 3rd day of CRD.

Marked changes in AO activity of the enzymes thus take place in the bone marrow as a result of fracture, irradiation, and CRD, but in different directions; this result probably depends on the direction of hematopoiesis and, consequently, on the cell composition of the tissue at different stages of ARS and CRD. The balance of components of the ADS system of the cells changes in the course of cell division and differentiation [2], and when metabolic intercellular relations are disturbed, this causes additional imbalance of the enzyme systems of the newly formed cells.

In the liver, which is an organ that is relatively stable from the cytokinetic point of view, the time course of activity of enzymes of the glutathione redox system in CRD was found in [5] to differ from that observed in bone marrow. For instance, during the first week, when

GP, GR, and GDAR activity is considerably depressed in the bone marrow, specific activity of GP was found to be reduced in the liver only on the 3rd day of CRD, and GR and GDAR activity was increased on the 7th day after CRD. Exposure to irradiation or mechanical trauma separately does not lead to any significant change in activity of the enzymes studied in the liver, unlike in the bone marrow (data not given).

The comparatively low power of the enzyme system protecting the cell against free-radical damage in the bone marrow and its lability (Figs. 1-3) form the pathochemical basis of selective radiosensitivity of the bone marrow and the radioprotective action of preparations of SOD [13, 14] and exogenous glutathione [9].

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INACTIVATION OF PLASMA α_1-PROTEINASE INHIBITOR BY TWO SPLENIC THIOL PROTEINASES ACTIVE IN A NEUTRAL MEDIUM

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For several years the writers have studied splenic thiol proteinases active in a neutral medium. Two enzymes, identified as cathepsin L and cathepsin H, have been isolated from bovine spleen in a virtually homogeneous form [3]. The study of the properties and specificity of these proteinases has shown that they can take part in the formation and inactivation of several physiologically active proteins and peptides [3-5] and, in experiments in vitro, they can inhibit the transformation of phytohemagglutinin-stimulated lymphocytes [1]. To investigate the biological functions of these proteinases and their role in the development of immunologic and inflammatory reactions further, their action was studied on α_1-proteinase inhibitor

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