EFFECT OF COMBINED RADIATION INJURY ON ENZYME ACTIVITY OF THE RAT LIVER GLUTATHIONE REDOX SYSTEM

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The protective and therapeutic effect of exogenous antioxidants in experimental acute radiation injury is well known [2]. It follows that the supply of antioxidants to the body will determine the severity of radiation damage. Yet the state of endogenous enzyme systems for antioxidant protection of animal cells after exposure to radiation has not been adequately investigated, and such reports as have been published are contradictory. Investigation of endogenous bioantioxidants after a combination of radiation injury and fractures of bones is particularly interesting, because regenerative processes after each of these traumatic influences require increased utilization of antioxidants. No investigations on this subject could be found in the literature.

The object of this investigation was to study enzyme activity of the redox system of glutathione, one of the most important water-soluble bioantioxidants, in the rat liver after combined injury including x-ray irradiation and a closed fracture of the tibia and fibula.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing 150-200 g, kept on the standard animal house diet, were used. X-ray irradiation in doses of 155 and 206 mC/kg was given on a RUM-17 apparatus. The conditions of irradiation were: voltage 200 kV, current 15 mA, dose rate 0.215 mA/kg. A closed fracture of the tibia and fibula was produced under ether anesthesia. The control animals also were anesthetized. Irradiation and the fracture took place consecutively in the course of 1 h. The rats were decapitated 1, 3, 7, 14, 21, and 30 days after trauma; before removal the liver was perfused with cold physiological saline (0.9% NaCl, pH 7.4). The tissue removed was homogenized in a Potter homogenizer for 3 min at 2000 rpm in the same solution, with ratio of volume to weight of sample of 9:1. The resulting homogenate was treated with 0.1% Triton X-100 (final concentration) and centrifuged for 15 min at 600 m/sec^2. Glutathione peroxidase (GP), glutathione reductase (GR), and glutathione: dehydroascorbate oxidoreductase (GDAR) activity was determined as described previously [4, 5] and calculated in micromoles oxidized NADPH/min/mg protein. The protein concentration was determined by Lowry's method [9] after treatment of the homogenate with 0.1 N NaOH and 0.5% sodium deoxycholate solution. All determinations were carried out on a model 34 spectrophotometer (Beckman, Austria). The significance of differences between experimental and control values was estimated by Student's t test [6].

EXPERIMENTAL RESULTS

On the 3rd day after irradiation in a dose of 206 mC/kg combined with mechanical trauma, GP activity in the rat liver was reduced by 29% (Fig. 1). No change in GP activity was found on the 1st and 7th days. GP activity exceeded the control level by 20% on the 7th day after combined radiation injury. Irradiation in a dose of 155 mC/kg combined with mechanical trauma did not cause any significant changes in GP activity in the liver. GR activity was depressed (by 18%) on the 30th day after trauma.

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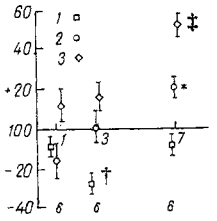

Fig. 1

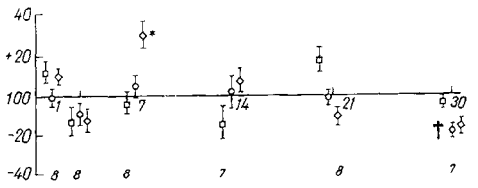

Fig. 2

Fig. 1. Effect of combined radiation injury (irradiation in a dose of 206 mC/kg and mechanical trauma) on enzyme activity of rat liver glutathione redox system. 1) GP, 2) GR, 3) GDAR. *P < 0.05, †P < 0.01, ‡P <0,001. Bottom row of numbers gives number of animals in each of the groups compared; abscissa, time after combined radiation injury (in days); ordinate, change in enzyme activity of glutathione redox system (in percent of control).

Fig. 2. Effect of combined radiation injury (irradiation in a dose of 155 mC/kg and mechanical trauma) on enzyme activity of rat liver glutathione redox system. *P < 0.02. †P < 0.01. Remainder of legend as to Fig. 1.

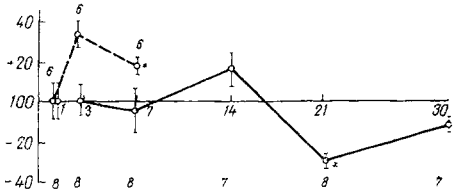

Fig. 3. Changes in GR/(GP + GDAR) ratio in rat liver after combined radiation injury. Continuous line — 155 mC/kg + mechanical trauma, broken line — 206 mC/kg + mechanical trauma. Abscissa, time after combined radiation injury (in days); ordinate, change in GR/(GP + GDAR) ratio (in percent of control). *P < 0.01. Bottom row of figures gives number of animals in each of the groups compared.

The results differ from those obtained as a result of the action of irradiation alone [2] or irradiation combined with thermal burns [1] on animals. For instance, GP activity in the liver of irradiated (181 mC/kg) rats was increased on the first day and returned to its initial level on the second day [2]. After combined irradiation and burns (114 mC/kg) GR activity showed phasic changes whereas GP activity declined steadily until the 15th day [1]. It is perfectly possible that the differences discovered reflect not only differences in the processes concerned, but also differences in the method of investigation. In particular, in the investigation cited above there is no information about so important a stage of the method as perfusion of the liver; activity was determined by the interval method.

Glutathione and ascorbic acid are the most important water-soluble bioantioxidants. The time course of changes in GDAR activity — the enzyme responsible for oxidation-reduction relations between them, — is particularly interesting. However, the activity of this enzyme has not been investigated after either trauma or irradiation. Our experiments showed (Figs. 1 and 2) that the level of GDAR activity in the liver was raised on the 7th day after combined injury: by 30% after irradiation in a dose of 155 mC/kg and by 51% after irradiation in a dose of 206 mC/kg. At other stages of the experiments no differences from the control were

found. In acute radiation injury, toward the 7th day the content of reduced glutathione and ascorbic acid in the liver decreases but there is no corresponding increase in the dehydroascorbic acid level [6]. The time course of changes in GDAR activity revealed by these experiments is in full agreement with the trend of these changes.

The ratio between GR activity and total GP + GDAR activity indicates the relations between the power of enzymic mechanisms of regeneration and oxidation of glutathione. It will be clear from Fig. 3 that after irradiation of the rats in a dose of 206 mC/kg combined with mechanical trauma the GR/(GP + GDAR) ratio was increased by 33% on the 3rd day and by 17% on the 7th day, due to a fall in GP activity (3rd day) and an increase in GR activity (7th day). After irradiation in a dose of 155 mC/kg this ratio was reduced on the 21st day (by 33%), evidently on account of an increase in GP activity. At other times of the investigation this coefficient was unchanged.

It is generally accepted that the fate of any substance in the cell is determined by the ratio between activities of enzyme processes which form and metabolize it. The parameter which we propose indicates that the conditions for intensive reduction of glutathione are created in the rat liver during the first week after combined radiation injury; later the conditions favor more rapid oxidation of the tripeptide. However, the realization of these possibilities depends on the concentration, on the one hand, of NADPH and, on the other hand, of oxidizing agents of glutathione and ascorbate. It must be emphasized that the functional role of glutathione is not confined to antioxidant protection. The involvement of this thiol in metabolism of xenobiotics, natural epoxides, and ketoaldehydes, transport of amino acids through biological membranes, prostaglandin synthesis, and control of the thioldisulfide state of proteins [3, 7, 8] demands that the above-mentioned changes in the glutathione redox enzyme system be taken into account when the state of these processes is examined in irradiated animals with bone trauma.

On the whole the results are evidence of relative stability of the glutathione redox system in the liver of rats with radiation injury combined with fractures, an important condition if glutathione is used for therapeutic purposes.

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