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THE EFFECTS OF EXOGENOUS GLUTATHIONE ON REDUCED GLUTATHIONE LEVEL, GLUTATHIONE PEROXIDASE AND GLUTATHIONE REDUCTASE ACTIVITIES OF RATS WITH DIFFERENT AGES AND GENDER AFTER WHOLE-BODY Γ-IRRADIATION

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ABSTRACT

Age- and gender-related changes on reduced glutathione (GSH) level, glutathione peroxidase (GPx) and glutathione reductase (GR) activities in the liver of rat exposed to different dose of whole-body g-ray irradiation were determined. In addition, the effect of administration of exogenous GSH on endogenous GSH levels, GPx and GR activities was investigated. For this aim, male and female rats aged 1 and 5 moths were divided into two groups as g-ray and g-ray+GSH. Both groups were again divided into four groups as irradiated with 2, 4, 6 and 8 Gy doses. GSH level and GPx activity did not change with age while GR activity was decreased with age. Gender-dependent changes in GPx and GR activities were observed, but GSH values were not affect by sex. GSH levels, GPx and GR activities were not observed dose-associated changes of g-irradiation. GSH level and GPx activity in the 8Gy group were increased by GSH. GR activities of old male rats were found to be increased by glutathione in the 6 and 8Gy groups. These results indicate that radiation and administration of exogenous GSH affect gender- and age-dependent GSH level, GPx and GR activities in the rats.

INTRODUCTION

Ionizing radiation is a highly efficient cytotoxic agent (1). Exposure to ionizing radiation produced oxygen-derived free radicals in the tissue environment. The reactive oxygen species (ROS) include the hydroxyl radical (·OH) and superoxide radical anion ($O_2^{\cdot-}$), as well as other oxidants such as hydrogen peroxide (H_2O_2). Damage caused by the same ROS produced metabolically has been implicated in the aging process (2). Senecence is the progressive accumulation of changes with time associated with or responsible for the increasing susceptibility to diseases, toxic effects of xenobiotics, radiation and death which accompanies advancing age (3-5).

According to free radical theory the single basic cause of aging, modified by genetic and environmental factor, involves free radical reactions (4). These reactions could arise upon exposure to ionizing radiation. Age-related changes resulting from the presence of reactive oxygen species include increasing levels of lipid peroxides and alterations in enzyme activities (6).

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It is well known that endogenous glutathione (GSH) contributed to the maintenance of cell homeostasis by scavenging free radicals resulting from physical or chemical injuries such as irradiation and drug administration. Exogenous administration of GSH has been reported to improve the survival time and survival rate of irradiated mice (Arima)

The aim of this investigation was to determine age-dependent sensibilities of rats of different gender to γ-irradiation and to investigate the radioprotective effects of exogenous GSH on rat liver.

MATERIALS AND METHODS

Chemicals

All chemicals for the assays were of reagent grade and obtained from Sigma Chemical Co. (St Louis, MO).

Animals

Male and female Sprague-Dawley rats of different ages (1 and 5 months: 50-100 g and 350-400 g, respectively) were housed in wire mesh cages at a costant temperature (22±2 °C) in a room with a 12 h light-dark cycle and fed with a standard rat chow (Oguzlar Yem, Eskisehir-Turkey) and water ad libitum. They were subjected to 16 h abstention from food immediately before radiation. None of the rats were found to have gross pathological lesions. The six rats per cage were allowed to move freely.

Male and female rats aged 1 and 5 mouths were divided into two groups as γ-ray (control) and γ-ray+GSH. Both groups were again divided into four groups as irradiated rats with 2, 4, 6 and 8 Gy doses (Table 1). GSH was dissolved in sterilized saline and was administered intraperitoneally at a single dose of 50 mg/kg-wt into rats in the γ-ray+GSH group in the first day. The same volume of saline was injected to rats into the γ-ray group. γ-ray irradiation to the whole-body was performed using a γ-ray generator (^{137}Cs source; JBL 437 C; CIS Bio International), (2 Gy/36.4 sec.).

Rats were sacrificed under ether anathesia 30 minutes after irradiation. Their livers were excised, gently washed in ice-cold saline and quickly stored at –70 °C until analyzed.

The liver tissues were homogenized in a ratio of wet tissue to 9 ml of 0.02 M phosphate buffer (pH 7.0) for reduced glutathione (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) with a Ultra Turrax homogenizer (T25, Janke & Kunkel). Homogenates were centrifuged at 5000 rpm for 10 min and Supernatant fractions were then removed and the remaining supernatants used as specimens.

The Biüret Method determined protein levels. GSH levels (Glutathion: hydrogen peroxide oxidoreductase,

E.C.1.11.1.9) activities were measured by the method of Beutler (7). GPx (Glutathion: hydrogen peroxide oxidoreductase, E.C.1.11.1.9) activities were measured by the method of Paglia and Valentine (8). GR (NAD: Oxide glutathione oxidoreductase, E.C.1.6.4.2) activities were determinated by the method of Racker (9). Results are expressed as μg/mg protein for GSH, and U/mg protein for GPx and GR activities, and presented as mean ± SEM.

Data were analysed by one-way analysis of variance and Tukey -W test together.

Table 1: The groups of rats are shown in the table.

GROUP (n)	1 age (young), (n=48)					5 ages (old), (n=48)				
	Day					Day				
	0	2	4	6	8	0	2	4	6	8
γ-ray (control)(24+24) Saline	+					+				
Irradiation (2 Gy/day)		+	+	+	+		+	+	+	+
No. animals killed		6	6	6	6		6	6	6	6
γ-ray+GSH (24+24) GSH (i.p.)	+					+				
Irradiation (2 Gy/day)		+	+	+	+		+	+	+	+
No. animals killed		6	6	6	6		6	6	6	6

RESULTS

As shown in Fig. 1, rats had not sex-associated difference in GSH levels after the same radiation dose. GSH levels of young rats did not also differ from old rats of the same group after the same radiation doses. In the control group of female and male rats, the GSH levels of young and old in the 8Gy group increased significantly when compared with those of the 2Gy group (young: $p<0.05$, old:$p<0.001$).

The GSH values of rats in the γ-ray+GSH group increased significantly in comparison with the control group irradiated 8Gy ($p<0.05$).

According to Fig. 2, the GPx activities in female rats were higher than those of the male rats of the same group after the same radiation dose. In the GPx activities, no statistically significant changes were found between young and old rats of the same group after the same radiation dose.

The GPx activities in the control group gradually rised by slightly increased irradiation dose, but it suddenly droped in the 8Gy group. Both young and old rats given GSH had significantly higher GPx activities than rats given no GSH.

The control group of female rats had the higher GR levels than those of the male rats ($p<0.01$ for 2Gy and 6Gy, $p<0.001$ for 4Gy, $p<0.05$ for 8Gy). The GR activities of young rats in both the control and the γ-ray+GSH group were significantly higher than those of old rats, but not in the γ-ray+GSH group irradiated with 8Gy ($p<0.05$ for the control group irradiated with 8Gy, $p<0.01$ for the control and γ-R+GSH group irradiated with 2Gy, $p<0.001$ for the control and the γ-R+GSH group irradiated with 4 and 6Gy), (Fig.3). The GR values of young and old rats in the control group had peak after 4Gy irradiation and then they droped below the 8Gy group. The GR activities of old male rats in the γ-ray+GSH group do not differ from the control group, but not irradiated with 6Gy and 8Gy. In the 6Gy and 8Gy groups, they were found to be increased significantly when compared with those of the control group ($p<0.01$).

DISCUSSION

In this study, we wanted to investigate that the effects of exogenous GSH against whole body-γ-irradiation in rats of different age and sex. For this aim, we searched glutathione metabolism *in liver of rats*. Glutathione is the main non-protein thiol in many enzymatic reactions which are necessary to preserve thiol homeostasis, redox state of the cells and defense against radiation damage (10). We found that rats did not have an age- and sex-associated differences in the liver GSH (Fig. 1). Although γ-radiation caused a slight decline gradually in the GSH levels, a significant decrease was observed with the 8Gy irradiation. We observed that administration of exogenous GSH protected the GSH levels in the liver cells exposed to different dose of γ-irradiation. Several studies had an age-related GSH decrease (10-15). We also have a similar results in age- and sex-related changes with Rikans et al.(16). According to Rikans et al (16), the effects of aging on antioxidant levels may also depend on the strain of the rats. It was thought that there were no differences in the GSH levels with age-related because age of the rats was not so different each other. Furthermore, in the liver cells, GSH is synthesized from its amino acid precursors (glutamat, cystein, glysin) through a pathway involving two ATP-dependent reactions and it was regulated. Erden Inal and Kahraman (17). have shown that GSH levels in rat liver were not affected also by UVA irradiation An interesting question concerns the disparity between GSH and GR parameters studies: GSH is very slightly reduced while GR is lowered significantly after UVA radiation. This issue becomes even more intriguing when the role of GR, which is to reduce GSSG to GSH, is considered. According to Erden Inal and Kahraman (17), this is explained by two facts. First, until the GR is reduced below 25% of the normal level, glutathione is also synthesized within the cells from amino acids. The well-known reaction is not catalyzed by GR and therefore is not influenced by its reduction, so allowing the GSH to be independent to some extent changes of GR.

The activity of liver GPx did not change significantly with age, but it varied significantly with sex. The GPx level of female rats was significantly higher than those of male rats. These results agree with previous finding within different sex and in aging age (10,16,18). According to Rao (18) the age-related changes in the activities of GPx were paralled by a similar change in the relative level of the mRNAs coding for this enzyme.

The GR enzyme showed reduced activities with age. At the same time, in male rats, it was found to be decreased when compared with those of female rats. Rikans et al. (16) have reported that age-associated changes in the GR activities were characterized by sex-dependent differences that disappeared in the older rats. They suggest that the efects of aging may also depend on the strain of rats. Sanz et al. (10) found to be no changes in the GR levels from 21 days to 6 mounths.

In this study, although the GPx activities increased significantly by 6Gy irradiation and then they decreased by 8Gy irradiation, GR levels increased by 4Gy irradiation and then they decreased by 8Gy irradiation (Fig. 2 and 3). Kojima et al.(19) have shown that small-dose g-irradiation (25-50 cGy) induced the GPx and GR activities of liver. This probably due to substrate activation and increasing concentration of H_2O_2 within cell, which is known to occur after radiation. The increasing H_2O_2 on the other hand, causes GPx to be oxidized which becomes resistant to radiation. At the dose of 8Gy irradiation, GPx and GR activities decreased. The changes can probably be because of enzyme exhaustion. Korganou et al (20) have decrease in GPx and GR activities in the 8Gy group.

The fact female rats had higher GPx and GR activities than male rats suggest that these enzymes can be regulated by sexual factors. Our finding also indicate that the administration of GSH increased the GPx levels of young male and famele rats irradiated with 8Gy and of old famele rats irradiated with 8Gy and of old male rats irradiated with 4, 6 and 8Gy (Fig. 2). The GR activities of young and old rats irradiated with 8Gy only increased with the administration GSH (Fig. 3).

In conclusion, 1. GSH and GPx levels did not change with age although GR activities were decreased with age. 2. While GSH levels were uneffected by sex, GPx and GR activities of male rats lower than those of famele rats. 3. GSH, GPx and GR levels displayed dose-dependent variations of g-ray irradiation. 4. Our findings suggest that endogenous antioxidant activity increased with the administration of GSH and protected against deleterious effects of g-ray irradiation.

Figure 1. The GSH levels of young and old rats (Female and male).

■ The control group of young rats □ The γ-ray+GSH group of young rats. ▨ The control group of old rats, ▩ The γ-ray+GSH group of old rats are shown. (a) Different from the control group of young and old rats irradiated with 2Gy ($p<0.05$ for young rats. $p<0.001$ for old rats). (b) Different from the control group of rats irradiated with 8Gy ($p<0.05$).

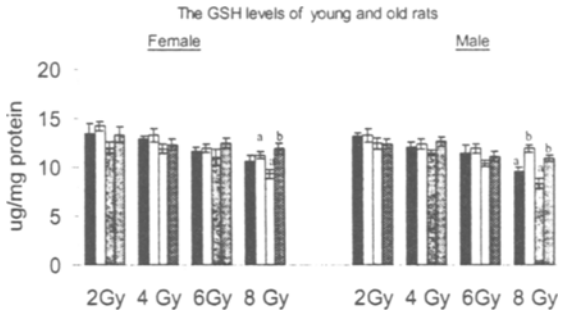


Figure 2. The GPx activities of young and old rats (Female and male).

■ The control group of young rats □ The γ-ray+GSH group of young rats ▨ The control group of old rats, ▩ The γ-ray+GSH group of old rats are shown. (a) Different from female rats. (b) Different from the γ-ray+GSH group of young and old rats irradiated with 8Gy (young female:$p<0.01$, young male:$p<0.05$, old famele and male:$p<0.001$).

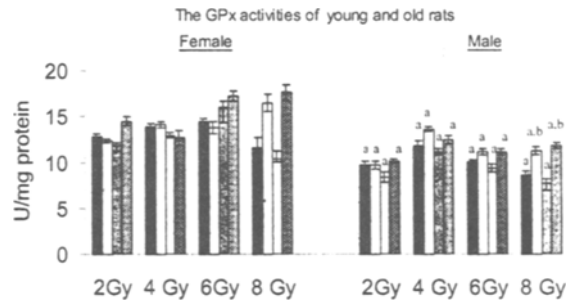
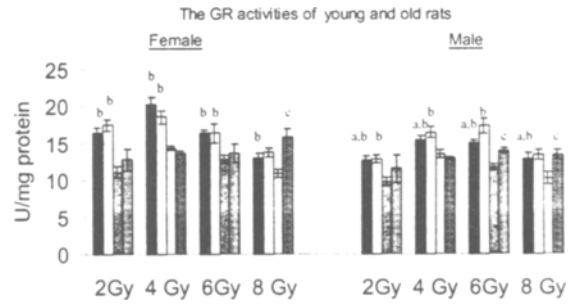


Figure 3. The GR activities of young and old rats (Female and male).

■ The control group of young rats, □ The γ-ray+GSH group of young rats. ▨ The control group of old rats, ▩ The γ-ray+GSH group of old rats are shown. (a) Different from the control group of female rats ($p<0.01$ for 2Gy and 6Gy, $p<0.001$ for 4Gy. $p<0.05$ for 8Gy). b) Different from the control and the γ-ray+GSH group of old rats ($p<0.05$ for the control group, $p<0.01$ for the control and γ-ray+GSH group irradiated with 2Gy. ($p<0.001$ for the control and γ-ray+GSH group irradiated with 4 and 6Gy). (c) Different from young rats in the control group ($p<0.01$).



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