

## EFFECTS OF TWO DIFFERENT HIGH DOSES OF IRRADIATION ON ANTIOXIDANT SYSTEM IN THE LIVER OF GUINEA PIGS

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**Aim:** To examine the state of the oxidant-antioxidant system in the liver of guinea pig caused by high doses of ionizing radiation in the early period. **Methods:** the research was carried out on guinea pigs irradiated with the doses of 8 Gy (group 2) or 15 Gy (group 3) (single dose/whole body) in comparison with control group (group 1). The levels of thiobarbituric acid reactive substances (TBARS) and glutathione (GSH), the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and the levels of selenium in the liver were measured. **Results:** TBARS levels in the irradiated animals were markedly higher than those in controls. In group 3, GSH levels and GSH-Px activity were significantly increased while activity of SOD and CAT were significantly decreased compared to groups 1 and 2. Liver selenium levels were not influenced by irradiation. **Conclusion:** The data have shown that  $\gamma$ -irradiation at the doses of 8 Gy or 15 Gy results in significant increase in free radical formation while antioxidant enzymes were affected only at a dose of 15 Gy.

**Key Words:** radiation therapy, guinea pig liver, antioxidant enzymes.

Ionizing radiation provokes the decomposition reaction of water producing a variety of reactive oxygen species (ROS) [1]. ROS such as hydroxyl radicals ( $\text{OH}^\cdot$ ), superoxide anion radicals ( $\text{O}_2^{\cdot-}$ ), and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) are extremely reactive and react with the molecules of cell membranes that are composed of a double layer of lipids with proteins dispersed throughout [2]. Under normal conditions, there is a balance between the generation of ROS and the cellular antioxidant systems [3]. Exposure to ionizing radiation produces significant alterations in the oxidant activity in different tissues, and causes overproduction of ROS leading to oxidative damage of the lipids, proteins and DNA. The oxidation of polyunsaturated fatty acids in membranes induced by ROS is called lipid peroxidation (LPO) which has been shown to increase in irradiated tissues [1, 4]. However, organisms have protective systems against ROS, like endogenous antioxidant enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) constitute primary enzymatic defense system [5, 6]. Reduced glutathione (GSH) is a major antioxidant that provides reducing equivalents for the GSH-Px [7, 8]. Selenium is an important trace element and as a part of the active site of GSH-Px, plays major protective roles against oxidative stress [9].

In the present study, we examined the effects of two different high doses of ionizing radiation on the radiation-induced oxidant-antioxidant changes in the liver tissue of guinea pigs in early period.

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**Abbreviations used:** CAT — catalase; GSH — glutathione; GSH-PX — glutathione peroxidase; ROS — reactive oxygen species; SOD — superoxide dismutase; TBARS — thiobarbituric acid reactive substances.

### MATERIALS AND METHODS

Guinea pigs weighing approximately 350 g, were used in this study. The guinea pigs were divided into three groups each consisting of 10 animals. Group 1: control; group 2: irradiated with a dose of 8 Gy (single dose, whole body); group 3: irradiated with a dose of 15 Gy (single dose, whole body). Irradiation was carried out using a  $^{60}\text{Co}$  source at Ankara Oncology Hospital, Department of Radiation Oncology (Ankara, Turkey). All animal procedures were carried out according to the rules of local Ethic Committee.

The animals in group 2 were exposed to a dose of 8 Gy ( $^{60}\text{Co}$ , source axis distance (SAD) 80 cm) to whole body following ketamine hydrochloride anesthesia. The guinea pigs in group 3 were applied to a dose of 15 Gy ( $^{60}\text{Co}$  SAD 80 cm) to the whole body following anesthesia. 24 h after irradiation, all animals were euthanized using ketamine hydrochloride (ketalar<sup>®</sup>, Eczacibasi, Turkey). The tissues were briefly washed in ice-cold 0.9 % saline (w/v), and frozen in liquid nitrogen. The tissues were stored at  $-70^\circ\text{C}$  until the subsequent protein and enzyme assays.

For SOD assay, tissue samples were homogenized in the ratio of 1/10 (w/v) in phosphate buffer (pH 7.4) and centrifuged at 5000g for 30 min. The supernatant was carefully separated, the 3/5 (v/v) chloroform and ethanol were added. This mixture was centrifuged at 5000g for 2 h. The supernatant was used for the determination of SOD. This assay involves xanthine oxidase used as superoxide generator [10]. The protein concentration of the same supernatant was measured by the method of Lowry [11] and the results were expressed as unit per mg protein tissue. One unit of SOD is defined as the amount of protein that inhibits the rate of nitro blue tetrazolium (NBT) reduction by 50%.

For the determination of GSH-Px activity, tissue samples were homogenized at the ratio of 1/10 (w/v) in

phosphate buffer (pH 7.0) containing 0.5 mM EDTA and then centrifugated at 3500 rpm for 15 min. Protein concentration of the supernatant was measured by the method of Lowry [11] and GSH-Px activity was measured by a modification of the coupled assay procedure of Paglia and Valentine [12]. The results were expressed as nmoles NADPH oxidized per min per mg protein.

Tissue CAT activity was measured by the method of Aebi [13]. Tissue samples were homogenized at the ratio of 1/10 (w/v) in phosphate buffer (pH 7.0) and then centrifugated at 3500 rpm for 15 min.  $H_2O_2$  was added to the supernatant and the decrease absorbance was measured at 240 nm for 3 min. The protein concentration of the supernatant was measured by the method of Lowry [11]. The results were expressed as K/mg protein.

The levels of TBARS were determined in tissue samples homogenized in the ratio of 1/10 (w/v) in 1.5 % (w/v) cold KCl solution, by thiobarbituric acid method [14] and the results were obtained in nmol/g tissue weight.

The GSH contents of tissue samples were determined by the method of Ellman [15]. Tissue samples were homogenized in the metaphosphoric acid solution and colored by DTNB. The results were expressed as micromoles per mg protein.

Selenium levels were measured with atomic absorption spectrophotometry (Unicam-AAS 939). The mixture of  $HNO_3/HClO_4$  is used to mineralize the samples. The inclusion of  $HClO_4$  in the digestive process is essential for complete decomposition of the organic matrix and the conversion of organoselenium to selenium. After digesting process, selenium determination has been carried out using the hydride generation atomic absorption spectrometry (HG-AAS) technique [16]. The results have been expressed as ng/g of liver tissue.

Kruskal-Wallis (nonparametric ANOVA) test was used for the statistical analysis and Dunn's multiple comparison test was performed as post-hoc test. A  $p$  value  $< 0.05$  was considered significant.

## RESULTS

The values of liver TBARS, SOD, GSH-Px, CAT activities, GSH and selenium in three experimental groups are presented at Table.

TBARS contents of liver after 8 Gy and 15 Gy  $\gamma$ -irradiation were markedly elevated when compared with group 1 ( $p < 0.05$  and  $p < 0.001$ , respectively), but there was no significant difference between group 2 and group 3 ( $p > 0.05$ ).

Liver SOD activity decreased after exposure to 15 Gy radiation, there was a significant difference between groups 1 and 3 ( $p < 0.05$ ). Also significant decrease in the liver SOD activity was observed after

15 Gy irradiation when compared with 8 Gy irradiation ( $p < 0.001$ ). The difference between group 1 and group 2 was non-significant ( $p > 0.05$ ).

GSH-Px activity in group 3 was higher than group 1 and 2 ( $p < 0.01$  and  $p < 0.001$ , respectively). The difference between groups 1 and 2 was non-significant.

CAT activity decreased significantly in group 3 when compared to control group ( $p < 0.05$ ). Also CAT activity in group 3 lower than that in group 2 ( $p < 0.05$ ). There was no statistically significant difference between groups 1 and 2 ( $p > 0.05$ ).

Reduced GSH levels were significantly higher in group 3 when compared with group 1 ( $p < 0.001$ ) and group 2 ( $p < 0.001$ ) but the level of GSH after exposure of 8 Gy was found to be unaltered when compared to group 1.

In this study, liver selenium levels were not found to be significantly changed after irradiation.

## DISCUSSION

The use of ionizing radiation to kill tumor cells is a common treatment for cancer. Exposure to ionizing radiation causes radiolysis of water in tissues leading to generation of ROS which are known to affect the antioxidant defense systems and induce LPO [17]. The consequence of this increased free radical generation and imbalances in antioxidant defense is oxidative stress which leads to oxidative damage, resulting in increased lipid peroxide levels. TBARS is used as an indicator of the rate of LPO which is accepted as tissue chain reaction [18]. Also radiation induced increase in ROS cause DNA damage, cell cycle arrest and activation of some transcription factors (e.g. NF- $\kappa$ B) [19, 20].

Whole body  $\gamma$ -irradiation of guinea pigs at 8 and 15 Gy produced a significant increase in the level of liver TBARS. In our experimental conditions, because  $\gamma$ -irradiation caused oxidative stress to the guinea pig, the LPO was induced in tissue. Miura et al [21] reported the increase of TBARS after x-irradiation at 7.5 Gy and 15 Gy after 4 days. Also Ueda et al [22] demonstrated the increase of TBARS in liver after  $\gamma$ -irradiation at 10 Gy.

15 Gy  $\gamma$ -irradiation induces changes in antioxidant activities expressed as a decrease in liver SOD and CAT activities but no markedly difference was found after 8 Gy  $\gamma$ -irradiation when compared with control group. It may be due to the TBARS level as 15 Gy  $\gamma$ -irradiation caused more pronounced LPO. In our observation the significant decrease in both SOD and CAT activities after applying 15 Gy irradiation leads to increase in the formation of  $O_2$  and  $H_2O_2$ . It could suggest that inactivation of SOD [23]. It has been shown that one of the transcription factors as NF- $\kappa$ B was activated by ionizing radiation and activation of NF- $\kappa$ B, so the induction of SOD by NF- $\kappa$ B could be absent in liver tissue [20].

**Table.** Liver thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT) activities and glutathione (GSH), selenium levels of all groups (mean  $\pm$  SD)

Groups	TBARS (nmol/tissue)	SOD (U/mg protein)	GSH-Px (nmol oxidized NADPH/min/mg protein)	CAT (K/mg protein)	GSH ( $\mu$ mol/mg protein)	Selenium (ng/g tissue)
1 (n = 10)	16.5 $\pm$ 3.9	19.35 $\pm$ 3.0	3.57 $\pm$ 1.22	0.35 $\pm$ 0.14	0.72 $\pm$ 0.28	468.45 $\pm$ 167.7
2 (n = 10)	23.6 $\pm$ 2.5 <sup>a</sup>	23.2 $\pm$ 5.19	3.08 $\pm$ 1.63	0.35 $\pm$ 0.16	0.68 $\pm$ 0.24	577.58 $\pm$ 138.4
3 (n = 10)	64.4 $\pm$ 27.5 <sup>b</sup>	11.85 $\pm$ 3.1 <sup>a,d</sup>	14.87 $\pm$ 2.3 <sup>a,c</sup>	0.20 $\pm$ 0.04 <sup>a,e</sup>	1.66 $\pm$ 0.25 <sup>c,f</sup>	396.49 $\pm$ 38.31

<sup>a</sup> $p < 0.05$  compared to group 1; <sup>b</sup> $p < 0.001$  compared to group 1; <sup>c</sup> $p < 0.01$  compared to group 2; <sup>d</sup> $p < 0.001$  compared to group 1; <sup>e</sup> $p < 0.05$  compared to group 2; <sup>f</sup> $p < 0.001$  compared to group 1.

24 h after 15 Gy  $\gamma$ -irradiation, the activity of CAT significantly decrease in agreement with previous observations [24, 25], although there was no marked difference after 8 Gy  $\gamma$ -irradiation when compared with control.

Another antioxidant enzyme, GSH-Px, significantly increased after applying 15 Gy  $\gamma$ -irradiation when compared with control and 8 Gy  $\gamma$ -irradiated liver tissue. GSH-Px is a defense enzyme against hydrogen peroxides and another hydroperoxides. 24 h after 15 Gy  $\gamma$ -irradiation, the activities of this enzyme significantly increased although it did not markedly change after 8 Gy irradiation, suggesting that it was not induced by 8 Gy irradiation under these experimental conditions. Mutlu-Turkoglu et al [26] reported that following whole abdomen  $\gamma$ -irradiation at 10 Gy, intestinal GSH-Px activity was significantly elevated. Increased GSH-Px activity might protect against oxidative stress-mediated injury to liver tissue. This protection against damage by lipid peroxides offered by GSH-Px appeared to be more relevant to 15 Gy  $\gamma$ -irradiated tissues than 8 Gy irradiated ones. The increase in GSH-Px may be a consequence of induction due to elevated LPO and/or ROS which may be inadequate to lower the LPO [1].

The glutathione related enzyme activities partly depend upon the selenium concentration in the system but in this study after irradiation (8 Gy–15 Gy) the selenium levels did not change significantly. Selenium might be decreased as a consequence of the impaired liver function. But in the current study, the animals were killed 24 h after irradiation thus the period might not be enough to induce damage in the liver.

15 Gy lethal dose irradiation to the whole body caused an increase in the levels of GSH, although 8 Gy irradiation did not affect the levels of GSH. These results suggested that synthesis of GSH was responsive to ionizing radiation. Shimizu et al [27] applied ionizing radiation to the hemiserebrum of rabbit and, similarly to our results, found increased levels of GSH. In conclusion since GSH and GSH-Px can protect from  $\gamma$ -irradiation-mediated injury, their upregulation in response to lipid peroxidation might represent a cellular adaptation to oxidative stress that promotes cell survival.

The present findings suggest that in the early period after  $\gamma$ -irradiation at the doses of 8 and 15 Gy, free radicals formation is reflected by an increase in TBARS levels, while antioxidant enzymes were affected only at a dose of 15 Gy.

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## ВЛИЯНИЕ ДВУХ РАЗЛИЧНЫХ ВЫСОКИХ ДОЗ ОБЛУЧЕНИЯ НА СОСТОЯНИЕ АНТИОКСИДАНТНОЙ СИСТЕМЫ ПЕЧЕНИ МОРСКИХ СВИНОК

**Цель:** изучить состояние оксидантно-антиоксидантной системы печени морских свинок на ранних этапах после облучения в высоких дозах. **Методы:** исследование проведено на морских свинках, однократно облученных в дозе 8 Гр (группа 2) или 15 Гр (группа 3) (облучение всего тела). Контрольную группу (группа 1) составили необлученные животные. В ткани печени было проанализировано количество реактивных соединений тиобарбитуровой кислоты (TBARS), глутатиона (GSH) и селена, определена активность супероксиддисмутазы (SOD), каталазы (CAT) и глутатионпероксидазы (GSH-PX). **Результаты:** у животных групп 2 и 3 по сравнению с контрольной повышен уровень TBARS. В группе 3 по сравнению с группами 1 и 2 значительно повышен уровень GSH, снижена активность SOD и CAT, повышена активность GSH-Px. Облучение не влияло на уровень селена. **Выводы:**  $\gamma$ -облучение в дозах 8 Гр или 15 Гр приводит к значительному увеличению образования свободных радикалов, в то же время на активность антиоксидантных ферментов влияет только облучение в дозе 15 Гр.

**Ключевые слова:** радиационная терапия, печень морских свинок, антиоксидантные ферменты.