Antioxidant tolerance of kidney after irradiation

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Different doses of irradiation were performed in which group 1 (non-irradiated), group 2 (8 Gy/single dose/whole body) and group 3 (15 Gy/single dose/whole body) were formed of guinea pigs. After 24 hr of radiation exposure the levels of lipid peroxidation product, malondialdehyde, (MDA), glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) were measured in the whole kidney. The MDA content increased in animals irradiated with 8 and 15 Gy. And group 3 showed an increase the level of MDA. GSH contents of kidney in group 2 and 3 increased. The activity of SOD decreased markedly in group 3 when compared with control group. The activity of GSH-Px decreased significantly in group 2 and group 3 in comparison to controls. It may be concluded that a high dose of ionizing irradiation cause excessive oxidative stress in kidney.

Kidney has been recognized as a radiosensitive organ that limits radiotherapy of tumors in the abdomen. Kidney irradiation leads to a progressive functional reduction associated with concomitant glomerulosclerosis and/or tubulointerstitial fibrosis. However, many reasons involved in the development and progression of radiation nephropathy remain ill defined. It is known that ionizing radiation induces oxygen radical injury, whereas oxidative stress by the radiation can cause cellular responses to defense cellular injury. The main result of radiation is with water molecules that causes the formation of the reactive oxygen species (ROS). Which react with other molecules of cells. It is well known that in healthy conditions at the cellular level, a subtle balance exists between the free radical generation and the antioxidant defense. There is a little information about the kidney damage after irradiation. Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) and non-enzymatic antioxidants such as reduced glutathione (GSH) and selenium play major role in protection of cells against ionizing radiation-induced damages.

Hence, the present study has been undertaken to study the levels of lipid peroxidation and antioxidant enzymes SOD and GSH-Px and the levels of GSH in kidney tissue of guinea pigs which have been irradiated at different dose levels.

Thirty guinea pigs, each weighing approximately 250 g, were used as experimental animals. The guinea pigs were divided into three groups of 10 animals each. Group 1: control; group 2: irradiated (8 Gy, single dose, whole body); group 3: irradiated (15 Gy, single dose, whole body). Irradiation was carried out using a 60Co source at Ankara Oncology Hospital, Department of Radiation Oncology.

The animals in group 2 were exposed to a dose of 8 Gy (60Co, source axe distance (SAD) 80 cm) to whole body following ketamine hydrochloride anaesthesia. The guinea pigs in group 3 were irradiated with a dose of 15 Gy (60Co, SAD 80 cm) to the whole body following the ketamine anaesthesia.

After 24 hr of irradiation, all the animals were euthanized using ketamine hydrochloride (ketalar®, Eczacıbaşı, Türkiye). The tissues were briefly washed in ice-cold 0.9% saline (w/v), then frozen in liquid nitrogen. The tissues were stored at −70°C until the subsequent protein and enzyme assays.

Protein concentration was measured in tissue homogenate by the method of Lowry using bovine serum albumin standard.

For SOD (EC 1.15.1.1) assay, tissue samples were homogenized in the ratio of 1/10 in 50 mM phosphate buffer (pH:7.4) and the supernatant was carefully separated, then 3/5 (v/v) chloroform and ethanol were added. This mixture was centrifugated at 5000 g for 2 hr. The supernatant was used for assay of superoxide dismutase. This assay for superoxide dismutase activity involves xanthine oxidase used as superoxide generator. 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 (EC 1.11.1.9) activity, tissue samples were homogenized in the ratio of 1/10 in 50 mM phosphate buffer (pH 7.0) containing 0.5 mM EDTA and then centrifuged at 3500 rpm for 15 min. Glutathione peroxidase activity was measured by a modification of the coupled assay procedure of Paglia and Valentine. The results were expressed as mmol NADPH oxidized per minute per mg protein.

The GSH contents of tissue samples were determined by the method of Ellman. Tissue samples were homogenized in the metaphosphoric acid solution and colored by DTNB. The results were expressed as mmol/g tissue.

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

Kruskal-Wallis (nonparametric ANOVA) test was used for the statistical analysis and Dunn’s multiple comparison test was performed as post-hoc test.

The results obtained by non-irradiated and irradiated (8 Gy-15 Gy) kidney of guinea pigs have been presented in the table indicate that; in the kidney of 8 Gy irradiated and control guinea pigs the following changes were found, kidney MDA levels increased significantly after 8 Gy irradiation exposure (P<0.05). Kidney GSH levels of 8 Gy irradiated guinea pigs were increased significantly as compared to controls (P<0.05). In 8 Gy irradiated animals, kidney GSH-Px activity dropped significantly (P<0.001). The activity of SOD decreased but the difference was non-significant.

In the kidney of 15 Gy irradiated and control guinea pigs, the following changes were found. MDA levels markedly increased after 15 Gy irradiation (P<0.05) and 8 Gy irradiation. GSH level of 15 Gy irradiated guinea pigs was also increased significantly when compared with control group (P<0.05). SOD activity was found to be significantly decreased after 15 Gy irradiation (P<0.05). GSH-Px activity was also decreased significantly (P<0.001).

In the kidney of 8 Gy irradiated guinea pigs compared with 15 Gy irradiated ones, the following changes were found. GSH level in 15 Gy irradiated guinea pigs was found to be decreased but this difference was non-significant when compared with 8 Gy irradiated ones. The kidney GSH-Px activity in 15 Gy irradiated animals was found to be decreased, but it was non-significant. The activity of SOD in the kidney was unchanged between groups 2 and 3.

There are many factors that effect the radiation nephropathy. Ionizing radiation generates ROS through an excitation of water, resulting in inducing the oxidative stress. ROS damage DNA, lipid and enzymes and are highly toxic, cells can be injured or killed when the ROS level exceeds the cellular antioxidant capacity.

Whole body γ-irradiation of guinea pigs at 8 Gy and 15 Gy produced a significant increase in the level of lipid peroxides. Recent studies have also reported the similar results. 8 Gy γ-irradiation induces a decrease in antioxidant activity expressed by a decrease in kidney GSH-Px activity. It has been reported that radiation exposure decreased glutathione-related enzyme activities such as GSH-Px.

After applying 15 Gy γ-irradiation the activities of SOD and GSH-Px dropped significantly as compared with controls. Hardmeier et al. have reported that SOD activities were significantly higher in radioreistant animals and this finding suggests a role for SOD in radioreistance mechanism. As kidney is a radioreistant organ, declining of the activity of SOD is resembling.

| Table 1 — MDA, SOD, GSH-Px and GSH levels of guinea pigs forming in group 1 (control), group 2 (8 Gy/single dose/whole body) and group 3 (15 Gy/single dose/whole body) of 10 animals individually. Values are expressed as mean ± SD. |
|----------------|----------------|----------------|----------------|
|                | MDA (nmol/g tissue) | SOD (U/mg protein) | GSH-Px (nmol oxidized NADPH/mg protein) |
| Group 1 (control) | 39.7 ± 12.6 | 18.88 ± 4.9 | 32.91 ± 13.4 |
| Group 2 | 64.7 ± 15.7* | 17.27 ± 2.4 | 4.04 ± 3.1* |
| Group 3 | 78.6 ± 27.9* | 13.91 ± 3.3* | 3.85 ± 1.4* |
| P values: *<0.05; **<0.001 as compared to group 1. |
Reduced glutathione (GSH) participates non-enzymatically in protection against toxic compounds. The increasing levels of GSH after 8 and 15 Gy might be effective for the prevention of ROS related damage caused by irradiation. In previous reports, low dose γ-irradiation induced elevation of GSH level we applied high doses of γ-irradiation so our results were different from those observed in studies with low dose irradiation. However, Kojima et al. applied 50 cGy γ-rays and the cellular total glutathione levels in HepG2 cells increased soon after the irradiation, picked between 6 and 12 hr and returned almost to the time 0 value by 48 hr post-irradiation. In our study we sacrificed the animals 24 hr after irradiation these different results could be related to the time. Also Iwanaga et al. have reported that GSH synthesis was induced by ionizing radiation at the transcriptional level.

As we applied high doses of γ-irradiation the synthesis of GSH in responsive to high doses may be occurring. Shimizu et al. applied ionizing radiation to the hemicerebrum and similar results were found.

In conclusion, the activities of GSH-Px, SOD and level of GSH are altered after application of high doses of irradiation. The resultant antioxidative defense mechanisms against ROS, inactivated, might be not efficient enough and high doses cause excessive oxidative stress so this may be related to the radiation-induced nephropathy.

References