Whole Body Exposure to Low-dose Gamma Radiation Promotes Kidney Antioxidant Status in Balb/c Mice

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Antioxidant status/Kidney/Low-dose γ-irradiation/Radiation hormesis.

We examined the effect of whole body low-dose γ-irradiation on the status of the antioxidant defense system in the rodent kidneys at different time intervals. Young male Balb/c mice were exposed to whole body radiation from a 60Co source at doses of 10, 25 and 50 cGy (48.78 cGy/min). Antioxidant status and lipid peroxidation were estimated in the kidneys at 4, 12 and 24 h after irradiation. Lipid peroxidation increased between 33% and 49% and reduced glutathione between 12% and 47% at 12 h at different radiation doses. Reduced glutathione level remained significantly (p < 0.05) elevated even at 24 h after irradiation to 25 cGy. Superoxide dismutase activity also increased by 37% at 12 h on exposure of animals to all the doses up to 50 cGy. Catalase activity increased significantly at 12 h on exposure to 10 cGy and 50 cGy. Interestingly, glutathione peroxidase activity increased by 31% at 4 h and subsequently returned to control levels at 24 h after exposure to 50 cGy. Glutathione reductase activity increased by 10–12% at 12 h after exposure to 25 cGy and 50 cGy. The results suggest that the whole body exposure of animals to gamma radiation stimulates the antioxidant defense system in the kidneys within 4 to 24 h after irradiation, at doses of 25 cGy and 50 cGy.

INTRODUCTION

Living organisms are constantly exposed to a shower of ionizing radiations from the natural sources such as cosmic rays, radio nuclides present in the earth’s crust (telluric), artificial/man made medical and industrial radiation sources, nuclear exposures, industrial accidents etc. Ionizing radiations are thus integral part of our life. According to the United Nations Scientific Committee on the Effects of Atomic Radiation (UNSCEAR) report in 1986,1 acute doses above 2.0 Gy, between 2 and 0.2 Gy, and below 0.2 Gy are regarded as high, intermediate and low-doses respectively. The deleterious effects due to low radiation dose are theoretically extrapolated from the high-dose by the “Linear No Threshold” (LNT) model. The confidence in LNT model is based on the biophysical concept that the passage of a single charged particle could cause damage to the DNA that could result in cancer. However, based on the current understanding of the basic molecular mechanisms involved in the biological system, the much relied upon LNT hypothesis does not have convincing experimental evidences regarding the deleterious effects at very low-doses and low-dose rates.2

Over the past two decades, our understanding of radiation biology has undergone a fundamental shift in paradigms away from deterministic “hit-effect” relationships and towards complex ongoing “cellular responses”. However, living organisms do not respond to ionizing radiations in a linear manner in the low-dose range 0.01–0.50 Gy (1 to 50 rad) and rather restore the homeostasis both in vitro and in vivo by the normal repair processes such as, normal cellular and DNA repair processes, immune reactions and antioxidant defense,3–5 adaptive responses,6 activation of immune functions,7–9 stimulation of growth,10 enhancement of resistance to high-dose radiation,11 prevention of brain disorders,12 and an increase in the life span of mice.13 The LNT model thus ignores these fundamental processes. This phenomenon of beneficial biological effects of low-dose radiation has generally been termed as ‘radiation hormesis’, and yet little is known about its mechanism(s). Recent reports have shown differential gene expression pattern both in vitro and in vivo after exposure to low-doses of radiation which regulate the cell cycle and cell proliferation.14,15 Thus, the reported phenomenon of ‘radiation hormesis’ and ‘radio-adaptive response’ cannot be rejected outright. These observations challenge the LNT hypothesis and require further

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Recently, the effects of low-dose irradiation on glutathione and antioxidant defense system in different organs of rodents have been reported. From these reports, it can be postulated that the hormetic/adaptive response results from an improvement in the levels of reduced glutathione (GSH) and antioxidant enzyme activities in the brain, spleen, liver, lungs, bone marrow and thymus, macrophages, and natural killer cells. However, the changes observed were at different radiation doses, dose rates and time intervals using a variety of ionizing radiations in different animal models. Moreover, the information regarding the effective dose range and the time duration during which these hormetic changes persist in kidneys is still lacking. Hence, there is need to evaluate the effect of low-dose whole body radiation on the antioxidant defense system of the kidneys, a major excretory organ. In this study, we have attempted to find: i) the low level of critical radiation dose and the post irradiation period during which the antioxidant defense system in the kidneys remains altered; and ii) to evaluate the degree to which these defense mechanisms remain affected in the kidneys after exposure to low-dose radiation. The study was performed in mice at a dose range from 10 to 50 cGy and at post irradiation intervals ranging from 4 to 24 h.

MATERIALS AND METHODS

Materials
Glutathione reductase, thiobarbituric acid (TBA) and tert-Butyl hydroperoxide (BHP) were purchased from Sigma Chemicals, New Delhi, India. Dithionitrobenzoic acid (Ellman’s reagent) (DTNB), nicotinamide adenine dinucleotide phosphate reduced (NADPH), nitroblue tetrazolium (NBT) were purchased from M/s Ranbaxy Fine Chemicals Ltd., Mumbai, India. Ethanol and acetic acid hydroxylamine hydrochloride, sodium azide, reduced glutathione (GSH), potassium chloride, sodium carbonate and hydrogen peroxide were purchased from M/s Sisco Research Laboratories Pvt, Ltd, Mumbai, India. Ethylenediamine tetra acetic acid (EDTA), Tris-buffer, sodium dodecyl sulfate (SDS), ferrous sulfate, hydroxylamine hydrochloride, sodium azide, reduced glutathione (GSH), potassium chloride, sodium carbonate and hydrogen peroxide were purchased from M/s Sisco Research Laboratory Pvt, Ltd, Mumbai, India. Ethanol and acetic acid were purchased from M/s Ranbaxy Fine Chemicals Ltd, New Delhi, India. The pellet diet duly approved by the Institute’s Animal Ethics Committee for animals was obtained from M/s Ashirwad Industries, Punjab, India.

Animals and γ-ray irradiation
The experiments were performed on pathogen free inbred male Balb/c mice of 5–6 weeks age group after the clearance of the study by the Institute’s Animal Ethics Committee. The animals were obtained from the Central Animal House of Postgraduate Institute of Medical Education and Research, Chandigarh, India. Animals were allowed free access to water and normal pellet diet. They were housed in polypropylene cages bedded with sterilized rice husk under 12 h cycles of light and dark. The animals were exposed to whole body radiation with gamma-rays from 60Co source (Theratron 780 from Atomic Energy Canada Limited, Canada) at doses ranging from 10–50 cGy (dose rate 48.78 cGy/min) at 80 cm source skin distance (SSD). Ionization chamber (Thimble) dosimeter (Physikalisch Technische Werkstatten Unidose, Freiburg, Germany) having sensitivity 100 μGy and accuracy ± 0.5% was used for measurement of radiation dose. Control animals were sham irradiated. Five animals were used in each group.

Tissue processing
Animals, after whole body radiation exposure to 10, 25 and 50 cGy, were sacrificed at various time intervals (4, 12, 24 h). Animals were anaesthetized with sodium pentathiol (60 mg/kg b.wt). Kidneys were immediately perfused with ice-cold solution (0.15 M KCl, 2mM EDTA, pH 7.4) in situ, removed and quickly frozen in liquid nitrogen prior to homogenization and preparation of post mitochondrial supernatant (PMS) as described by Litterst et al. Briefly, the tissue was excised, weighed, diced into small pieces and then homogenized in 50mM Tris-HCl, pH 7.4, containing 150mM KCl and 250mM sucrose by using motor-driven glass-Teflon homogenizer at a ratio of 1g wet weight of tissue/3ml of buffer. The homogenate was centrifuged at 10,000xg for 20 min at 4°C. The pellet was discarded and the supernatant was used for further biochemical assays.

Estimation of lipid peroxidation (LPx)
Lipid peroxidation was estimated by the method of Ohkawa et al. Briefly, the reaction mixture contained Tris-HCl buffer (50mM, pH 7.4), BHP (500 mM in ethanol) and 1mM FeSO4. After incubating the tissue sample with reaction mixture at 37°C for 90 min, the reaction was stopped by adding 2 ml of 8% SDS followed by 1.5 ml of 20% acetic acid (pH 3.5). The amount of malondialdehyde (MDA) formed during incubation was estimated by adding 1.5 ml of 0.8% thiobarbituric acid (TBA) and heating the mixture at 95°C for 45 min. After cooling, samples were centrifuged and the TBA-reactive substances (TBARS) were measured in supernatants by spectrophotometer (Spectronic genesis 2, Rochester, New York, USA) at 532 nm using the extinction coefficient value of 1.53 × 105 M–1cm–1. Lipid peroxidation was expressed in terms of nanomoles of TBARS/mg protein/90min.

Antioxidant defense system
Reduced glutathione: Reduced glutathione (GSH) in the tissue homogenate was measured by the method of Moron et al. Required amounts of the homogenate were taken and 0.6mM DTNB was added. After 10 min the optical density

of the yellow colored complex formed by the reaction of GSH with DTNB was measured at 405 nm. GSH was expressed in terms of μg/mg protein.

**Superoxide dismutase activity:** Superoxide dismutase (SOD) determinations were performed according to the method of Kono. The rate of nitroblue tetrazolium (NBT) dye reduction by O$_2$– anion generated from photoactivation of hydroxylamine hydrochloride was recorded at 560 nm in the absence of PMS (post mitochondrial supernatant). In brief, small aliquots of PMS were added to the reaction mixture and the inhibition in the rate of NBT reduction by SOD present in the enzyme source was recorded. One unit of enzyme activity was defined by the 50% inhibition of NBT and expressed in terms of U/mg protein.

**Catalase activity:** Catalase (CAT) activity was measured in the PMS by the method of Luck. The final reaction volume of 3 ml included 0.05 M Tris-buffer, pH 7 and 10mM H$_2$O$_2$ (in 0.1 M KH$_2$PO$_4$ buffer, pH 7.0). Fifty microlitres or 100 μl aliquots of the cellular supernatant was added to the mixture. The rate of change per min in absorbance at 240 nm was recorded. Catalase activity was calculated using the molar extinction coefficient of 43.6 M$^{-1}$cm$^{-1}$ for H$_2$O$_2$ and expressed in terms of μmoles H$_2$O$_2$ consumed/mg protein/min.

**Glutathione peroxidase activity:** Glutathione peroxidase (GPx) activity was measured by the method of Lawrence and Burk. The reaction mixture contained 50mM potassium phosphate buffer at pH 7.0, 1mM EDTA, 1mM sodium azide, 0.2mM NADPH, 1 enzyme unit of glutathione reductase and 1mM glutathione. The aliquot of the sample was added and allowed to equilibrate for 5min at 25°C. The reaction was initiated by adding 0.1 ml of 2.5mM H$_2$O$_2$. Absorbance at 340 nm was recorded for 5min. The data was expressed as nanomoles of NADPH oxidized to NADP by using the extinction coefficient of 6.26 $\times$ 10$^3$ M$^{-1}$cm$^{-1}$ at 340 nm and expressed in terms of nanomoles NADPH consumed/mg protein/min.

**Glutathione reductase activity:** Glutathione reductase (GR) was estimated by the method of Carlberg and Mannervik. Briefly, the reaction mixture contained 0.067M sodium phosphate buffer (pH 6.6), NADPH in 1% sodium carbonate and 7mM oxidized glutathione. The reactions were incubated at 37°C for 5min and the reaction was initiated by adding PMS. The decrease in absorbance per min was recorded at 340 nm. GR activity was calculated by using an extinction coefficient of 6.22 $\times$ 10$^3$ M$^{-1}$cm$^{-1}$ and expressed in terms of nanomoles of NADPH consumed/mg protein/min.

**Protein estimation**
Protein content in the samples was measured by the method of Lowry et al.

**Statistical analysis**
The statistical significance of differences between various groups was determined by two way analysis of variance (ANOVA) for multiple comparison using Students-Newmann-Keul’s (SNK) procedure and Dunnet’s procedure was used for data analysis with respect to control. P values less than 0.05 were considered significant.

**RESULTS**
The animals were exposed to whole body radiation at different doses of γ-rays (10, 25, 50 cGy). After each radiation exposure the animals were sacrificed at 4, 12 and 24 h for the analysis of lipid peroxidation and antioxidant defense status in kidneys.

**Lipid peroxidation**
Lipid peroxidation levels in the kidneys were enhanced by 60%, 41% and 32.8% respectively at 4, 12 and 24 h after whole body irradiation of animals with 10 cGy (Fig. 1). The
LPx levels increased by 33% and 49% at 12 h on exposure of animals to 25 cGy and 50 cGy doses respectively. However, these enhanced LPx levels returned to the normal by 24 h.

**Reduced glutathione**

The whole body irradiation of animals to 10 cGy and 50 cGy significantly increased the kidneys GSH levels by 21% at 12 h and returned to the control levels by 24 h (Fig. 2). Exposure to 25 cGy increased the kidney GSH level from 12% at 4 h to 50% at 24 h post irradiation. At 50 cGy dose, the elevated GSH level at 12 h decreased by 17% as compared to 25 cGy at the same post irradiation period.

**Antioxidant defense enzymes**

The superoxide dismutase activity in kidneys increased by 37% at 12 h after whole body exposure at all the doses up to 50 cGy, and returned to control values at 24 h except at a dose of 10 cGy (Fig. 3). However, when animals were exposed to 10 cGy, the elevated SOD levels decreased by 10% at 24 h as compared to 12 h which were still significantly (p < 0.05) more than the control values.

No significant change in the CAT activity was observed at 4 h after irradiation of animals up to 50 cGy (Fig. 4). However, the CAT activity increased by 18.5% and 12.2% at 12 and 24 h post exposure to a dose of 10 cGy. Interestingly, the CAT activity was elevated significantly (p < 0.05) by 10% and 11.6% at 24 h and 12 h post exposure to 25 cGy and 50 cGy respectively as compared to their respective controls.

Whole body radiation doses of 10 cGy and 25 cGy did not alter the GPx activity significantly in the kidneys (Fig. 5).

![Fig. 2](image_url). Effect of whole body γ-irradiation of Balb/c mice with 60Co at 0 (control), 10, 25 and 50 cGy on reduced glutathione levels in the kidneys. The animals were sacrificed at 4, 12 and 24 h after radiation exposure. Values are Mean ± SEM, n = 5, *p < 0.05 w.r.t control, #p < 0.05 w.r.t 10 cGy, @p < 0.05 w.r.t 25 cGy.

![Fig. 3](image_url). Effect of whole body γ-irradiation of Balb/c mice with 60Co at 0 (control), 10, 25 and 50 cGy on superoxide dismutase activity in the kidneys. The animals were sacrificed at 4, 12 and 24 h after radiation exposure. Values are Mean ± SEM, n = 5, *p < 0.05 w.r.t control.
Fig. 4. Effect of whole body γ-irradiation of Balb/c mice with 60Co at 0 (control), 10, 25 and 50 cGy on catalase activity in the kidneys. The animals were sacrificed at 4, 12 and 24 h after radiation exposure. Values are Mean ± SEM, n = 5, *p < 0.05 w.r.t control, #p < 0.05 w.r.t 10 cGy, @p < 0.05 w.r.t 25 cGy.

Fig. 5. Effect of whole body γ-irradiation of Balb/c mice with 60Co at 0 (control), 10, 25 and 50 cGy on glutathione peroxidase activity in the kidneys. The animals were sacrificed at 4, 12 and 24 h after radiation exposure. Values are Mean ± SEM, n = 5, *p < 0.05 w.r.t control.

Fig. 6. Effect of whole body γ-irradiation of Balb/c mice with 60Co at 0 (control), 10, 25 and 50 cGy on glutathione reductase activity in the kidneys. The animals were sacrificed at 4, 12 and 24 h after radiation exposure. Values are Mean ± SEM, n = 5, *p < 0.05 w.r.t control.
However, after exposure of animals to a dose of 50 cGy the GPx activity increased by 30% at 4 h which subsequently decreased to 14% at 12 h and finally returned to control level at 24 h.

Whole body exposure of animals did not alter the GR activity at a dose of 10 cGy (Fig. 6). However, the activity of this enzyme increased significantly (p < 0.05) by 10.2% and 12.2% at 12 h after exposure to 25 cGy and 50 cGy which subsequently returned to their respective control values at 24 h post irradiation.

**DISCUSSION**

In the biological system, apart from the normal metabolism, one of the active sources of oxidative radicals is the ionizing radiation. Cells can be injured, and even killed under the most serious conditions of radiation exposure, when the content of reactive oxygen species (ROS) gets uncontrolled by the cellular antioxidants. It is believed that the extent of cellular damage by low-radiation dose is proportional to the generation of lipid peroxides at 12 h after exposure to whole body low-dose radiation. The antioxidant enzymes activity and the reduced glutathione (GSH) content increased significantly almost in parallel to the generation of lipid peroxides at 12 h after exposure to whole body low-dose $\gamma$-radiation. The increase in GSH levels may be due to the activation of protective response in the kidneys to counteract the excessive formation of ROS. Moreover, it was interesting that this effect was observed over a very narrow dose range of 25 cGy to 50 cGy.

Under mild oxidative stress, cells are able to regulate a variety of physiological mechanisms in an attempt to cope up with the oxidative stress. One of the mechanisms is through the redox balancing by the GSH. Glutathione, a non-enzymatic antioxidant, is the most abundant endogenous thiol-containing tri-peptide present in millimolar concentrations in eukaryotic cells. It plays a pivotal role in maintenance of the balance of cellular redox status, metabolism, transport, catalysis as coenzymes, maintenance of the thiol moieties etc. It acts as radical scavenger, due to redox-active sulphhydril group directly reacting with oxidant and transforms itself into oxidized glutathione. Recent evidences suggest that patients suffering from oxidative stress-induced pathological diseases have decreased glutathione/antioxidant levels. Thus, decreased glutathione levels have generally been considered as an "index" of increased formation of ROS, and the subsequent glutathione depletion causes oxidative stress-induced cellular damage.

In the present study, exposure to $\gamma$-radiation increased the lipid peroxidation levels in the kidneys up to 12 h which returned to normal levels by 24 h at all the doses except at 10 cGy. However, the results obtained contradict the earlier studies, which report decrease in the lipid peroxidation levels in various rat organs and rabbit brain on exposure of the animals to low-dose $\gamma$-irradiation. This variation in the formation of lipid peroxides could be due to species difference of the animal model, the nature of the radiation source, the radiation dose, the dose rate and the time selected for measurement of various biochemical parameters. Like lipid peroxidation the levels of GSH in the kidneys also increased significantly from 20% at 4 h to 50% at 24 h at a dose of 25 cGy; whereas it increased by 20% at 12 h at a dose of 50 cGy. This data reflects that a dose of 25 cGy is the most critical in inducing the GSH levels to counteract the elevated levels of radiation-induced free radicals responsible for the process of lipid peroxidation. These changes are almost similar to what we reported earlier in case of mouse liver. On the other hand, significant changes in lipid peroxide levels in lungs have been observed only at 12 h on exposure to 25 cGy. This might be due to the fact that the lungs remain exposed directly to outer environment and have enhanced antioxidant defense system which is sufficient to overcome the oxidative stress conditions mediated by the low-doses of $\gamma$-radiations. These observations indicate that the whole body exposure of animals to $\gamma$-radiations increases the non-enzymatic antioxidant levels to different extents in different organs. The increased lipid peroxidation in the kidneys at 12 h after whole body low-dose irradiation returned to the control levels with in 24 h with subsequent increase in the redox agent GSH. It is likely that the increase in GSH contents might be due to an increase in the expression of mRNA for $\gamma$-glutamylcysteine synthase, a rate limiting enzyme in GSH synthesis. Immediate enhancement of GSH content in kidneys after whole body radiation of animal at low-dose reflects that GSH acts as the first line of defense to protect the cells against the increased oxidative stress induced by the ionizing radiation.

The major enzymatic antioxidants in kidneys are SOD, CAT, GPx and GR. Superoxide dismutase catalyzes the dismutation of $O_2^-$ to H$_2$O$_2$, and CAT converts the product into H$_2$O. We have found that the kidney SOD activity peaked at 12 h after whole body exposure to 25 cGy and 50 cGy, which returned to control level by 24 h. However, CAT activity reached to maximum at 12 h on exposure of animals to 10 cGy and 50 cGy. Interestingly, like GSH no change in the antioxidant enzymes activity in the lungs of mice were found on irradiation to low-doses. This indicates that SOD and CAT plays more active role in kidneys for detoxification of ROS as compared to liver and lungs after the whole body exposure of animals to $\gamma$-radiations at low-doses. Glutathione directly reacts with ROS; whereas GPx catalyzes the destruction of hydrogen peroxide and hydroperoxide by utilizing GSH and NADPH. An increase in the GPx activity was observed only at a dose of 50 cGy. It is likely that the
increased GPx and GR provide second line of cellular defense in the kidneys from the radiation-induced oxidative stress at relatively high dose i.e. at 50 cGy. The increase in the lipid peroxidation in the kidneys after whole body exposure to low-dose radiation might cause an increase in the antioxidant defense status to regulate the cellular homeostasis. The differential responses of antioxidant defense system in various organs of mice after whole body irradiation to low-dose (< 50 cGy) γ-rays might be due to variable sensitivities in the activation of related genes in different organs.\(^{36,14,15}\) as low-dose ionizing radiations have been reported to regulate the cellular redox status mainly through phosphorylation of various serine/threonine Mitogen Activated Protein Kinases,\(^{37}\) and the cell cycle signaling pathways.\(^{38}\) The signal transduction pathways are believed to get activated differentially in various organs in response to different doses of ionizing radiations.\(^{37}\)

In conclusion, the whole body exposure of mice with γ-radiation in a dose range of 25 cGy and 50 cGy stimulates the enzymatic and non-enzymatic antioxidant defense system within 24 h in the mice kidneys. The enhancement of endogenous antioxidant machinery might be helpful in protecting the organ damage from the other causes of oxidative stress.

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