

# Zinc administration modulates radiation-induced oxidative injury in lens of rat

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## ABSTRACT

**Background:** The aim of this study was to evaluate the antioxidant role of zinc (Zn) against radiation-induced cataract in the rat lens after total cranial irradiation with a single 5 Gray (Gy) dose of gamma irradiation. **Materials and Methods:** Twenty-one Sprague-Dawley rats were used for the experiment. The control group did not receive Zn or irradiation but received 1-ml saline orally plus sham-irradiation. The irradiation (IR) group received 5 Gy gamma irradiation to the total cranium as a single dose plus 0.1 ml physiological saline intraperitoneally. The IR plus Zn group received irradiation to total cranium plus 10 mg/kg/day Zn intraperitoneally. Biochemical parameters measured in rat lenses were carried out using spectrophotometric techniques. **Results:** Lens total (enzymatic plus non-enzymatic) superoxide scavenger activity (TSSA), glutathione reductase (GRD), and glutathione-S-transferase (GST) activities significantly increased in the IR plus Zn groups when compared with the IR group. However, TSSA, GRD and GST activities were significantly lower in the IR group when compared with the control group. Lens non-enzymatic superoxide scavenger activity (NSSA) in the IR plus Zn group was significantly increased compared to that of the IR group. Lens xanthine oxidase (XO) activity in the IR group significantly increased compared to that of both the control and IR plus Zn groups. **Conclusion:** Zn has clear antioxidant properties and prevented oxidative stress by scavenging free radicals generated by ionizing radiation in rat lenses.

**Key words:** Antioxidant enzymes, irradiation, lens, oxidative stress, zinc

## INTRODUCTION

Radiation therapy is a common and important tool for cancer treatment. Eighty percent of cancer patients need radiotherapy at some time or other, either for curative or palliative purpose. The radio sensitivity of the normal tissues adjacent to the tumor limits therapeutic gain. The responses of the normal tissues to the therapeutic radiation exposure range from those that cause mild discomfort to others that is life threatening. The speed at which a response develops varies widely from one tissue to another and often depends on the dose of radiation that the tissue received.<sup>[1,2]</sup> Ionizing radiation is known to generate reactive oxygen species (ROS) in irradiated tissue. Because human body contains 80% water, the major radiation damage is

due to the aqueous free radicals, generated by the action of radiation on water. These free radicals react with cellular macromolecules, such as DNA, RNA, proteins, membrane, etc., and cause cell dysfunction and mortality. These reactions take place in tumor as well as normal cells when exposed to radiation.<sup>[3]</sup>

Cataract is the opacity of eye lens that interferes with vision. Cataracts are formed in response to a variety of different agents and environmental stresses, and this damage seems in almost all cases to have an oxidative damage component. Although cataract of the eye lens is a known late effect of ionizing radiation exposure, most of the experimental work to date has concentrated on single, acute high doses or multiple, fractionated, and chronic exposure of radiation.<sup>[4,5]</sup> Radiation cataracts are expressed after latency. The duration of the latency depends inversely on the dose: the higher the dose, the more rapidly the cataract develops. For a single treatment, the lowest cataractogenic dose was reported to be 2 Gy.<sup>[4-7]</sup>

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Zinc (Zn) has been shown to be essential to the structure and the function of a large number of macromolecules and over 300 enzymic reactions. Total human body Zn content has been estimated to be 30 mmol and its turnover appears to be under very close homeostatic control. Deficiency of this micronutrient in the diet has been widely studied and can lead to growth failure, neuropathy, diarrhea, dermatitis, hypotension, and hyperthermia.<sup>[8,9]</sup>

Cells have developed different antioxidant systems and various antioxidant enzymes to defend themselves against free radical attacks. Superoxide dismutase (SOD) catalyses the dismutation of the superoxide anion radical ( $O_2^{\cdot-}$ ) into hydrogen peroxide ( $H_2O_2$ ).<sup>[6,7,10]</sup>

$H_2O_2$  is generally considered a major oxidant in cataractogenesis. Addition of  $H_2O_2$  to an *in vitro* system can cause oxidative damage to the lens resembling that seen in photochemical stress. In *in vitro* models, the relative roles of different reactive oxygen species (ROS), such as  $H_2O_2$ , the  $O_2^{\cdot-}$ , hydroxyl radical ( $OH^{\cdot}$ ), as well as the relative roles of the different defense mechanisms against these ROS can be studied. The major systems degrading  $H_2O_2$  in the lens involve glutathione peroxidase (GSH-Px), glutathione reductase (GRD), and glutathione (GSH).<sup>[11,12]</sup> The glutathione-dependent antioxidant system consisting of reduced glutathione and an array of functionally related enzymes plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Of these enzymes, glutathione peroxidase (GSH-Px) is a selenoprotein that reduces hydroperoxides as well as  $H_2O_2$  while oxidizing glutathione. A number of potentially toxic electrophilic xenobiotics are conjugated to the nucleophilic glutathione by glutathione-S-transferases (GSTs) present in high amounts in cell cytosol. GST can also catalyze reactions reducing peroxides like GSH-Px. Reduction of oxidized glutathione (GSSG) to glutathione (GSH) is mediated by the widely distributed enzyme glutathione reductase (GRD) that uses NADPH as the reductant.<sup>[10,13,14]</sup> Xanthine oxidase (XO) functions in purine and free radical metabolism. It also catalyses the conversion of xanthine and hypoxanthine to uric acid and the production of  $O_2^{\cdot-}$ , which is potentially toxic to cellular structures.<sup>[15]</sup>

To our knowledge, there is no experimental study that simultaneously investigates the effect of Zn supplementation on total (enzymatic plus non-enzymatic) superoxide scavenger activity (TSSA), non-enzymatic superoxide scavenger activity (NSSA), GRD, GST and XO activities in the lens of rats with ionization-induced cataracts. Therefore, in the present study, we aimed to investigate the effect of Zn supplementation on antioxidant (TSSA, NSSA, GRD, GST) and oxidant

parameters (XO) in the lens of rats with or without exposure to total cranium irradiation with a single dose of 5 Gy gamma rays.

## MATERIAL AND METHODS

### Chemicals

Nicotinamide adenine dinucleotide (NADH), reduced glutathione (GSH), glutathione disulfide reductase, hydrogen peroxide ( $H_2O_2$ ), xanthine, xanthine oxidase (XO), nitrobluetetrazolium (NBT) and trichloroacetic acid (TCA) were purchased from Sigma Chemical Co (St Louis, MO, USA). Zinc Sulphate (containing 50 mg zinc, Zinco 220 capsule) was purchased from Berko Pharmaceuticals (Istanbul, Turkey).

### Rats and experiments

Twenty-one Sprague-Dawley rats, 12-14 weeks old, weighing  $190 \pm 20$ g at the time of radiation, were used for the experiment. All procedures involving the Sprague-Dawley rats adhered to the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Research. The rats were quarantined for at least 3 days before gamma irradiation, housed seven to a cage in a windowless laboratory room with automatic temperature ( $22 \pm 1^\circ C$ ) and lighting controls (12h light/12h dark), and fed standard laboratory chow and water ad libitum.

The rats were randomly divided into three equal groups. Group 1 (control group) did not receive Zn or irradiation but received 1-ml physiological saline orally plus sham-irradiation. Group 2 received total cranium 5 Gy of gamma irradiation as a single dose (IR group) plus 1-ml physiological orally. Group 3 received the same total cranium irradiation plus 10 mg/kg/day Zn (IR plus Zn group). Zinc Sulphate (containing 50 mg zinc, Zinco 220 capsule, Berko Pharmaceuticals, Istanbul, Turkey) was administered to the rats in the IR plus Zn group as diluted in 1-ml physiological saline through orogastric tube by starting from 3 days before irradiation and during 10 days after irradiation (total 13 days). One ml saline was administered daily orally through orogastric tube by starting from 3 days before irradiation and until 10 days after irradiation (total 13 days) to both the control group and the IR group.

Prior to total cranium irradiation, the all rats were anesthetized with 80 mg/kg ketamine HCl (Pfizer Ilac, Istanbul, Turkey) and placed on a plexiglas tray in the prone position. While the rats in the control group received sham irradiation, the rats in the IR, and the IR plus Zn groups were received irradiation using a Cobalt-60 teletherapy unit (Picker, C 9, Maryland, NY, USA) from a source-to-surface distance of 80 cm by  $5 \times 5$  cm anterior fields with the total cranium gamma irradiation as

a single dose of 5 Gy. The dose rate was 0.49 Gy/min. To insure the lens received a maximal dose, a wax bolus material 0.5 cm thick, was placed over the rat eyes. The central axis dose was calculated at a depth of 0.5 cm. The maximum dose was normalized to 95% on the lens.

### Biochemical analysis

Ten days after irradiation, all animals were killed by decapitation, their eyes were enucleated, and the lenses were dissected immediately. Lenses were homogenized in physiological saline solution (Omni Accessory Pack International Homogenizer, Warrenton, VA, USA). The homogenate was centrifuged at 10,000g for 1hr to remove debris. The clear upper supernatant was collected and all assays were carried out on this fraction. All the procedures were performed at + 4°C.

TSSA and NSSA assays, as indicators of tissue antioxidant capacity, were performed in the samples before and after adding trichloroacetic acid (TCA, 20%), as described.<sup>[16]</sup> First, TSSA is measured. In this method, xanthine-xanthine oxidase complex produces superoxide radicals that react with nitroblue tetrazolium (NBT) to form a farmazone compound. TSSA activity is measured at 560 nm by detecting inhibition of this reaction. By using blank reaction in which all reagents are present except the supernatant sample and by determining the absorbance of the sample and blank, TSSA activity is calculated. Second, NSSA activity is measured in TCA-treated fractions prepared by treating part of the sample with final concentration of 20% (w/v) TCA solution (to remove all enzymes and proteins), and centrifuging at 5000×g for 30 min. After the elimination of proteins by this procedure, NSSA activity assay is performed in the supernatant fraction. GRD activity was determined by coupled spectrophotometric registration at 340 nm, using GSSG as substrate and NADPH at 37°C.<sup>[17]</sup> GST activity of the supernatant was measured by using 1-chloro-2,4-dinitrobenzene (CDNB) and GSH as described.<sup>[18]</sup> XO activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbance at 293nm.<sup>[19]</sup> The protein content was determined by using the Bradford method.<sup>[20]</sup>

Results were expressed in U/mg protein for TSSA, NSSA; mU/mg protein for GRD, GST and XO activities. One unit of TSSA, NSSA was defined as the amount of enzyme protein causing 50% inhibition in nitrobluetetrazolium reduction rate. Biochemical measurements were carried out using a spectrophotometer (CECIL CE 3041, Cambridge, UK).

### Statistical analyses

Statistical and correlation analyses were undertaken using a one-way variance analysis and Spearman's rank correlation

test, respectively. Least significant difference (LSD) multiple range test was used to compare the mean values. Acceptable significance was recorded when *P* values were <0.05. Statistical analysis was performed with Statistical Package for the Social Sciences for Windows (SPSS, version 11.5, Chicago, IL, USA).

## RESULTS

All parameters are shown in Table 1. Lens TSSA, GRD, and GST activities significantly increased in the IR plus Zn groups when compared with the IR group. However, TSSA, GRD and GST activities were significantly lower in the IR group when compared with the control group. Lens NSSA activity in the IR plus Zn group was significantly increased compared to those of the IR group. Lens XO activity in the IR group was significantly increased compared to that of both the control and IR plus Zn groups.

Correlation analysis revealed a significant negative correlation between lens tissue XO and TSSA ( $r=-0.89$ ,  $P<.01$ ), and XO and GRD ( $r=-0.82$ ,  $P<0.05$ ) in IR plus Zn group. There was a significant positive correlation between lens tissue TSSA and NSSA ( $r=0.89$ ,  $P<0.01$ ), GRD and NSSA ( $r=0.96$ ,  $P<0.001$ ), GST and NSSA ( $r=0.86$ ,  $P<0.05$ ), and TSSA and GRD ( $r=0.96$ ,  $P<0.001$ ) in IR plus Zn group. However, no correlation could be found among the parameters in other groups.

## DISCUSSION

As the world's population ages, cataract-induced visual dysfunction and blindness are on the increase. Cataract is a major cause of blindness and of severe visual impairment leading to bilateral blindness in an estimated 20 million people worldwide. In developing countries, 50-90% of all

**Table 1: Mean ± SD of total (enzymatic plus non-enzymatic) superoxide scavenger activity, non-enzymatic superoxide scavenger activity, glutathione reductase, glutathione-S-transferase and xanthine oxidase activities in the rat lenses**

	Control group	IR group	IR plus Zn group
TSSA U/mg protein	53.8 ± 12.7 <sup>a</sup>	38.1 ± 8.6	61.8 ± 12.5 <sup>d</sup>
NSSA U/mg protein	10.3 ± 0.9	9.6 ± 1.3	11.2 ± 0.6 <sup>a</sup>
GRD mU/mg protein	35.6 ± 6.9 <sup>c</sup>	25.3 ± 4.5	37.4 ± 6.6 <sup>c</sup>
GST mU/mg protein	36.8 ± 7.7 <sup>a</sup>	25.5 ± 8.6	38.4 ± 6.1 <sup>c</sup>
XO mU/mg protein	1.98 ± 0.24 <sup>c</sup>	2.55 ± 0.28	2.04 ± 0.37 <sup>b</sup>

<sup>a</sup>:  $P<0.05$ , <sup>b</sup>:  $P<0.01$ , <sup>c</sup>:  $P<0.005$ , <sup>d</sup>:  $P<0.001$  vs. irradiation group

blindness is caused by cataracts. Pharmacological treatment to prevent human cataract has so far not been achieved. Therefore, surgery to remove the opacified lens is the only effective treatment for the cataract. The challenges are to prevent or delay cataract formation and also to treat cataracts if they occur.<sup>[21]</sup> Cataracts have many associated risk factors, including age, diabetes, nutrition, genetic factors, cigarette smoking, drug use, steroids and alcohol, obesity, and occupational exposures from a variety of sources.<sup>[22,23]</sup> The exact mechanism of cataract formation is still not very clear.<sup>[21]</sup>

The aim of radiation treatment is to deliver carefully determined doses of ionizing radiation to a defined tumor volume to eliminate tumor cells, to cause minimal injurious effects to surrounding healthy tissue to give by eliminating tumor cells, giving a high quality of life and to prolong survival all at a reasonable cost to cancer patients.<sup>[2,6,12]</sup> But, cataract is an unavoidable complication if radiotherapy includes the orbit of eye in the treated volume, even with very low doses of radiation. Ionizing radiation, such as X and gamma rays and ultraviolet lights, is known to be a cataractogenic factor for rat lenses.<sup>[2,12]</sup>

Zn is well known to induce production of metallothionein, which is very rich in cysteine, and this is an excellent scavenger of OH<sup>-</sup>. Iron and copper ions catalyze the production of OH<sup>-</sup> from H<sub>2</sub>O<sub>2</sub>, and Zn is known to compete with both iron and copper from binding to cell membrane, thus decreasing the production of OH<sup>-</sup>. Thus it is clear that Zn has multiple roles as antioxidant and is therefore, an excellent candidate for clinical chemoprevention trials in humans.<sup>[9,13]</sup> Currently, there are increasing evidences, from human and experimental studies, suggesting that Zn can be a beneficial agent in the protection against radiation-related normal tissue injury.<sup>[11,3,24,25]</sup>

In the study, we showed that the irradiation caused a significant decrease in the activities of antioxidant enzymes and also an increase in oxidant enzymes activities in rat lenses. These results are in agreement with the previous studies.<sup>[2,6,12]</sup> Some studies have reported a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxidation after irradiation. Under normal conditions the inherent defense system protects against oxidative damage.<sup>[4,5]</sup>

In our study, we found a significant reduction in GRD and GST activities in the IR group and also a significant increase in GRD and GST activities in the IR plus Zn group in rat lenses. This reduction in the IR group could be due to an enhanced utilization of GSH redox cycle as an attempt to detoxify the free radicals generated by irradiation. Supplementation of Zn protects the endogenous GRD, GST depletion resulting from

irradiation. The increase in of GRD, GST, TSSA and NSSA activities suggests that protection of Zn may be mediated through the modulation of lens antioxidant system. These results suggest that Zn have a free radical scavenging activity. A study has reported that Zn pretreatment significantly lowered the radiation-induced lipid peroxidation in terms of malondialdehyde and increased antioxidant enzymes activities.<sup>[13]</sup> The inhibition of lipid peroxidation in biomembranes can be caused by antioxidants. Significant increases in the levels of free radicals have been reported in both the lens and the aqueous humor of cataract patients when compared with age-matched controls, emphasizing the role of oxidative damage in the pathogenesis of cataracts. A decrease in the antioxidant system can be responsible for increased lens oxidation and cataract development.<sup>[2,6,12,25-27]</sup>

As a result, it is very clear from this study that there are abnormalities in oxidant/antioxidant defense system in the lens of rats with exposure to total cranium irradiation with a single dose of 5 Gy of gamma rays. Our results confirm the presence of oxidative stress in IR group. By increasing antioxidant enzymes activities and decreasing oxidant enzymes activities, Zn prevented oxidative stress by scavenging free radicals generated by ionizing radiation in rat lenses. A far more comprehensive study of the basic mechanism for alteration of Zn level in erythrocyte, and other biological samples is needed.

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