# Zinc as an antiperoxidative agent following iodine-131 induced changes on the antioxidant system and on the morphology of red blood cells in rats


## Abstract

Iodine-131 ($^{131}$I) irradiation is the first line treatment for Graves' disease and thyroid carcinoma. In such cases, $^{131}$I gets accumulated in the thyroid, and is released in the form of radioiodinated triiodothyronine (T3) and tetraiodothronine (T4). Various reports describe changes in the blood picture after radioiodine treatment. Zinc, on the other hand, has been reported to maintain the integrity of red blood cells (RBC) under certain toxic conditions. The present study was conducted to evaluate the adverse effects of $^{131}$I on the antioxidant defense system and morphology of RBC and also to assess the possible protection by zinc under irradiation by $^{131}$I. Thirty two female Wistar rats were equally segregated into four main groups. Animals with Group I served as normal controls; Group II animals were administered a dose of 3.7 MBq of $^{131}$I (carrier free) intraperitoneally, Group III rats were supplemented with zinc (227 mg/L drinking water) and Group IV rats were given a combined treatment of $^{131}$I and zinc, in a similar way as in Group II and IV rats. After seven days of $^{131}$I treatment, RBC lysate was prepared and its antioxidant status assessed. The activity of superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) in the lysate of RBC was increased. On the contrary, the activity of catalase was found to be significantly decreased. The activity of glutathione reductase (GR) remained unchanged. Marked changes in the shape of RBC from normal discocytes to echinocytes, spherocytes, stomatocytes and acanthocytes were also observed in the blood of the rats treated with $^{131}$I. Zinc supplementation to $^{131}$I treated rats, significantly attenuated the adverse effects caused by $^{131}$I on the levels of MDA, GSH, SOD and catalase. In conclusion, the study revealed significant oxidant/antioxidant changes in RBC following $^{131}$I administration in rats, while zinc was shown to act as a radioprotector agent.


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## Introduction

Radiation injury to living cells is to a large extent, due to oxidative stress [1-5]. Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen ($O_2$) react with cellular macromolecules [6] and induce adverse effects on them. The damage to the thyroid gland from deposition of radioiodine-131 ($^{131}$I) has often been described in the literature [7, 8]. $^{131}$I is being used in many nuclear medicine centers for the treatment of Graves' disease (GD) and differentiated thyroid carcinoma [9]. In spite of a safety record unmatched by alternate methods of therapy, the use of $^{131}$I has met with several objections, the most significant of which is the question of oxidative stress and carcinogenicity [10,11]. The irradiation dose in blood depends on the administered amount of $^{131}$I and the fraction passing into the plasma as protein bound $^{131}$I [12].

Changes in the blood picture after radioiodine treatment to GD has been stated in a few reports [13,14]. Ionizing radiations do have a profound effect on the red blood cells (RBC) ghost membranes, followed by the changes in membrane –SH groups and activities of membrane bound enzymes [15]. It has been reported that $^{131}$I treatment led to the intensification of lipid peroxidation (LPO) expressed by a significant increase in malondialdehyde values [16]. Therefore, the antioxidant defense system is an important area, which needs to be considered for exploring the effect of $^{131}$I on RBC.

Certain radioprotectors such as amifostine, constitute the most important means of protection against radiation exposure. Amifostine (WR-2721), a phosphoaminothioate, is capable of providing marked protection from both radiation and selected chemotherapy-induced damage for a wide variety of tissues in both rodents and humans [17,18]. However, the possible protective roles of safer compounds are warranted to be explored. Zinc salts may be


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considered as a new class of radioprotectors. Zinc salts, especially zinc aspartate, have been shown to provide radioprotection against whole body irradiation [19-21]. A number of studies have suggested that zinc acts as a beneficial agent during peroxidative damage [22,23]. Moreover, recent studies from our laboratory have demonstrated the protective potential of zinc in the regulation of antioxidant status and in ameliorating the altered hepatic histoarchitecture in nickel and lead intoxicated animals [24,25]. The present study for the first time explores the possible role of zinc in eliminating changes in the antioxidant enzyme system and in the morphology of RBC following $^{131}I$ administration in rats.

## Materials and methods

### Animals

Female Wistar rats weighing 150±20 g were procured from the central animal house, Panjab University, Chandigarh. The registration number for the use of experimental animals obtained from the ministry of social justice and empowerment, Government of India is 45/1999/CPCSEA, dated 15.01.2000. The animals were housed in polypropylene cages in the departmental animal house under hygienic conditions. The animals were maintained on the standard laboratory feed and water, ad libitum, throughout the period of experimentation.

### Chemicals and equipment

All chemicals used for the study were of analytical grade. $^{131}I$ (carrier free) as sodium iodide in dilute sodium thiosulphate solution was obtained from Bhaba Atomic Research Centre (Trombay, India). Zinc sulphate was purchased from E. Merck (Germany). NADPH, GSH and DTNB were purchased from Sigma Chemicals Co. (USA). UV spectrophotometer (Beckman Co, USA) was used for the enzymatic estimations.

### Experimental design

The animals were segregated into four groups. Each group comprised 8 rats and was subjected to different treatments for a period of seven days. Animals in Groups II and IV were administered a dose of 3.7 MBq of $^{131}I$ (carrier free) intraperitoneally. Animals in Group IV also received zinc as $ZnSO_4.7H_2O$ in a dose level of 227 mg/L added to their drinking water [24] for a period of seven days. Animals in Group I and III served as untreated normal and zinc controls, respectively. Animals in Group III received zinc treatment similar to that of Group IV animals.

### Collection of blood samples

For the purpose of studying various hematological and biochemical parameters, blood samples were drawn from all animals, seven days after the administration of $^{131}I$ to Groups II and IV. Blood samples were collected in test tubes containing 8% sodium citrate in order to separate the RBC, after subjecting the animals to light ether anesthesia and then puncturing the ocular vein (retro-orbital plexus) with a fine sterilized glass capillary.

### Preparation of erythrocyte lysate

Two ml of citrated blood was centrifuged for 10 min at 1000 xg and plasma was removed by suction. The RBC were washed twice with phosphate buffered saline (PBS), (0.1 M $Na_2HPO_4/NaH_2PO_4$: normal saline, 1:9) pH 7.4. Distilled water (10 ml) was then added and the erythrocytes were resuspended by agitation and lysed for 2 h at 4AC. A volume of 0.8 ml of a mixture of chloroform-ethanol (3:5 v/v), which was centrifuged at 3000 xg for 10 min at 4°C so as to precipitate the haemoglobin, and 0.3 ml of water was added to the lysate. The enzymes viz: superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and reduced glutathione (GSH) were assayed in the clear supernatant.

The activity of superoxide dismutase was estimated by the method of Kono (1978) [26]. Catalase activity was determined by the method of Luck (1971) [27]. Glutathione reductase (GR) was assayed by the method of Carlberg and Mannervik (1985) [28]. Estimation of GSH was performed in the tissue homogenates of liver by the method of Ellman (1959) [29]. The protein content was measured according to the method of Lowry et al (1951) [30].

### Preparation of packed cell volume (PVC) for the determination of lipid peroxidation in erythrocytes

The blood samples collected in citrated vials were centrifuged at 1000 xg for 10 min at 4°C. The plasma and buffy coat were removed by gentle aspiration. The erythrocytes were washed 3 times with PBS, pH 7.4. After washing, PCV was adjusted to 5% with PBS for the determination of LPO. The LPO in erythrocytes was determined by measuring the MDA produced using thiobarbituric acid (TBA) by the method of Wills (1966) [31].

### Scanning electron microscopic studies of RBC

Fresh blood samples were drawn by puncturing the ocular vein with a fine sterilized glass capillary. A drop of this blood was immediately immersion fixed in 2.5% glutaraldehyde made in 0.2 M phosphate buffer (pH7.4). After 2 h of fixation, cells were gently centrifuged at 1000-1500 rpm. The supernatant was discarded and the pellet was suspended in triple distilled water. The pellet was again reconstituted in water and this process was repeated 2-3 times. Finally the pellet was suspended in triple distilled water and a drop of the suspended pellet was smeared on the metallic scanning electron microscopy (SEM) stubs, which were loaded with a conducting silver tape on its top. These stubs were then coated with gold to a thickness of 100 Å using a sputter ion coater, with a gold source, for 4-5 min. These specimens were finally observed using a scanning electron microscope (JSM 6100, Jeol, Japan) at the Regional Sophisticated Instrumentation Centre (RSIC), Panjab University, Chandigarh, India.

### Statistical analysis

The statistical significance of the data has been determined using one-way analysis of variance (ANOVA), followed by a multiple post-hoc test (Student Newman Keuls). The results are represented as Means ± S.D.

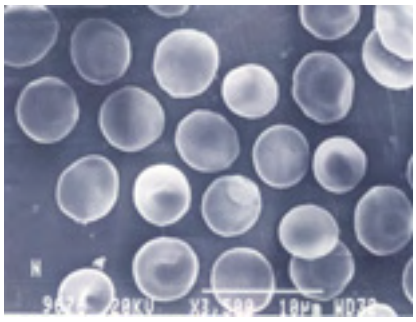

**Figure 1.** Scanning electron micrograph of the red blood cells from normal control rats

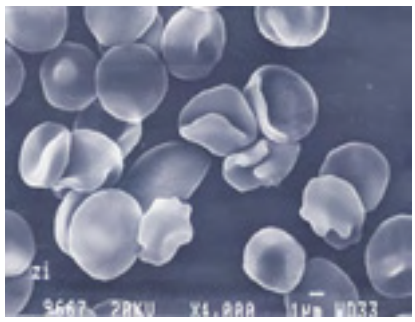

**Figure 2.** Scanning electron micrograph of the red blood cells from $^{131}$I treated rats

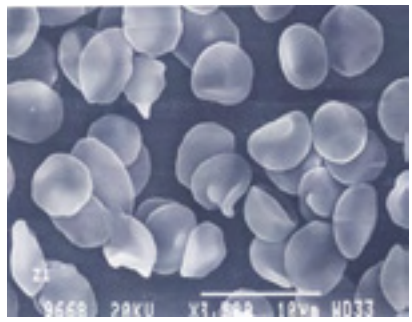

**Figure 3.** Scanning electron micrograph of the red blood cells from zinc plus $^{131}$I treated rats

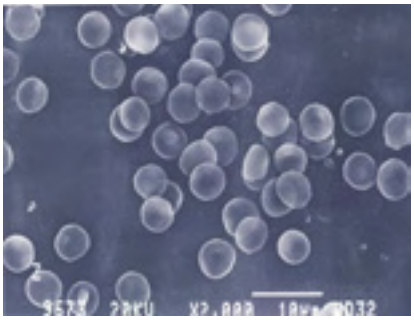

**Figure 4.** Scanning electron micrograph of the red blood cells from zinc treated rats

## Results

Our results and their statistical significance are shown in Table 1. The results of scanning electron microscopy of RBC before and after the administration of zinc are depicted in Figures 1-4. The protective effect of zinc was evident in the combined group whereby marked alterations of the blood cells were reverted close to the normal appearance of the cells. Despite all these preventive effects of zinc, moderate population of echinocytes, acanthocytes and spherocytes were still present.

## Discussion

The study was designed with a broad theme to elucidate the possible protective role of zinc on the key antioxidative enzymes and the morphology of RBC following exposure from $^{131}$I radiations. An analogous dose is given to humans for the treatment of differentiated thyroid carcinoma. Furthermore, zinc is an essential trace element for the function of many key enzymes and a daily dietary intake of around 9-11mg in humans is required to carry out various body functions in humans whereas in rats, the daily intake of zinc is around 200 mg. The homeostatic mechanism that regulates its entry, distribution and excretion from cells and tissues is so efficient, that no disorders are associated with its excessive accumulation [32].

RBC are considered as an early model for studies on oxidative stress. This model should be highly prone to oxidative reactions because it has a relatively high oxygen tension, because of the presence of haemoglobin, and a plasma membrane rich in polyunsaturated lipids [33]. Therefore, we have examined RBC for the determination of oxidative stress caused upon them by $^{131}$I and for the possible protection provided by zinc.

During the course of the present study, MDA levels in animals that had received $^{131}$I treatment, showed a significant increase when compared to their respective normal controls as shown in Table 1. Various studies have shown that free radicals induced by ionizing radiation have a damaging effect on lipids [34,35]. Since ionizing radiation abstracts hydrogen from a molecule to form a radical [36], it is likely that erythrocyte MDA levels may be elevated due to enhanced radical generation. It is also likely that the peroxidation of phos-

**Table 1.** *Effect of zinc on the antioxidant status of red blood cells in the control and experimental rats (Values are expressed as mean ± SD of eight animals)*

| | Group | LPO | GSH | GR | SOD | CAT |
|---|---|---|---|---|---|---|
| I | Normal control | 1.67 ± 0.048 | 0.141 ± 0.046 | 10.69 ± 0.126 | 26.10 ± 1.65 | 748.4 ± 12.82 |
| II | $^{131}$I (3.7 MBq) | 3.17 ± 0.294$^{c}$ | 0.303 ± 0.023$^{c}$ | 10.95 ± 0.129 | 30.32 ± 1.61$^{a}$ | 712.4 ± 8.264$^{a}$ |
| III | zinc (227 mg/L) | 1.65 ± 0.072 | 0.133 ± 0.003 | 10.71 ± 0.402 | 25.85 ± 2.54 | 766.0 ± 27.16 |
| IV | zinc + $^{131}$I | 2.47 ± 0.269$^{c, z}$ | 0.251 ± 0.021$^{b, z}$ | 10.87 ± 0.953 | 27.29 ±0.66$^{x}$ | 732.6 ± 11.69$^{x}$ |
| F-value | | 63.489 | 134.76 | 1.534 | 3.671 | 4.426 |
| | | P<0.001 | P<0.001 | NS | P<0.05 | P<0.01 |

Lipid peroxidation (nmoles of MDA min$^{-1}$ mg$^{-1}$ protein), Glutathione reduced (mmoles of GSH mg$^{-1}$ protein), Glutathione reductase (nmoles of NADPH oxidised min$^{-1}$ mg$^{-1}$ protein), Superoxide dismutase (I.U i.e. Inverse of the amount of protein required to inhibit the reduction rate of NBT by 50%), Catalase (mmoles of $H_2O_2$ decomposed min$^{-1}$ mg$^{-1}$ protein)

$^{a}$<0.05, $^{b}$<0.01, $^{c}$<0.001 by Newman – Keuls test when the values of Groups II, III and IV are compared with those of Group I,
$^{x}$<0.05, $^{y}$<0.01 by Newman – Keuls test when the values of Group IV are compared with those of Group II

pholipids takes place on the RBC membranes as a result of formation of active loci either due to the direct interaction of radiations or because of low zinc concentrations as a consequence of indirect effect of radiations on zinc binding ligands. Furthermore, the increased levels of MDA as observed in our study, following $^{131}I$ exposure, are in accordance with Bartoc et al. (1993) [37] who reported that radioactive iodine treatment led to the intensification of lipid peroxidation expressed by a significant increase in MDA values. However, in our study, zinc has been found to be a protective antioxidant as it was able to bring the MDA levels to near normal levels when it was supplemented to the $^{131}I$ treated rats. It has been hypothesized that the increase in zinc turnover in concert with the synthesis of metallothionin (MT) might relate to radioprotection as shown by various studies [38,39]. Radioprotection could also be due to the induction of GSH as a result of zinc treatment.

In the present study, the levels of GSH were increased following the exposure of RBC to $^{131}I$ and a statistically insignificant increase in the levels of GR was noticed following $^{131}I$ treatment. The increase in GSH is understandable since GSH plays an important role in providing defense against oxidative attacks [40]. Of course increased GSH levels may result from several factors. Oxidizing radiations stimulate the synthesis of GSH in response to elevated free radicals and may lead to a paradoxical increase in erythrocyte glutathione levels [41] or to enhanced antioxidant response. Similarly in an earlier study conducted by Konukoglu et al. (1998) [15], GSH as well as GSH related enzyme activities were significantly increased in patients seven days after radioiodine treatment, when compared to their own initial levels. On the other hand, zinc was able to attenuate the levels of GSH when supplemented to the $^{131}I$ treated rats. The observed normalization of GSH levels following zinc treatment could be due to its property to induce metallothionein (S-rich protein) as a free radical scavenger, or to its indirect action in reducing the levels of oxygen reactive species. However its mechanism for these actions remains to be elucidated, with regard to protecting the important thiols in toxic conditions [42].

Superoxide dismutase is the first line of defense against oxygen derived free radicals and functions by dismutating two superoxide ($O_2^-$) ions into $H_2O_2$. The present study indicated a marked increase in the activity of SOD after $^{131}I$ exposure to RBC. The increased activity of SOD in erythrocytes can be attributed to the greater tolerance of the animals in mitigating the toxic stress on the body as it plays a vital role in the detoxification of reactive oxygen species. However, simultaneous zinc treatment to $^{131}I$ treated animals showed a reduction in the levels of SOD that could be a consequence of induction of GSH as an indication of indirect protection afforded by zinc.

In the current study, we have also observed decreased levels of catalase activity in RBC as compared to the control animals. The decrease in the catalase activity could possibly be due to the utilization of this enzyme in converting $H_2O_2$ to $H_2O$. The observed significant inhibition in the catalase activity, further stipulates that oxidative stress due to $^{131}I$ was so severe that it could acutely suppress catalase activity. However, the simultaneous zinc treatment to $^{131}I$ treated animals showed an increase in the activity of catalase, thereby further substantiating that production of reactive oxygen species was much more in case of $^{131}I$ treatment and that zinc was able to neutralize to some extent the accumulation of free radicals.

During the course of the present investigation, drastic alterations in RBC morphology were noticed in the blood of the animals seven days after the $^{131}I$ treatment. The prominent features were the transformation of the normal discocytic appearance of the RBC simultaneously to many different forms viz., echinocytes, spherocytes, stomatocytes and acanthocytes. RBC were indicative of the severity of the toxic effect of radiation. The abnormal shape of the RBC could be attributed to the general causes of abnormalities such as abnormal erythropoiesis and inadequate haemoglobin formation, to effects on the RBC membrane lipid bilayer, to accelerated RBC aging, decrease water permeability across the RBC membranes, decreased RBC thermostability, to deformability, the rate of oxygen release by the RBC [43] or to increased erythropoiesis to compensate for the anaemia [44]. Modifications in the shape of the RBC can also be attributed to changes in the lipid composition of the membrane in response to $^{131}I$ treatment [45]. Another factor may be the formation of active loci on the RBC membrane as a result of the interaction with free radicals, thereby resulting in the alteration of the shape of these cells. In the present study, marked LPO in the RBC was observed, which is in tune with this observation.

Coadministration of zinc and $^{131}I$ significantly improved the morphology of the RBC. This is corroborated by the fact that increased LPO, as observed during $^{131}I$ treatment alone, was brought back to lower levels when zinc was administered, thereby. The protective effects of zinc could be attributed to its antiperoxidative potential, which regulates the RBC membrane lipid composition and maintains its integrity.

A relevant correlation has been observed between the administered $^{131}I$ dose and the RBC oxidant and antioxidant status. Our results confirm the involvement of peroxides after internal radiation. $^{131}I$ can also cause significant apoptosis and mitotic cell death in the thyroid tissue. This effect can release cytokines and toxic metabolites, such as hydroxyalkenals from the degradation of the RBC membrane [46]. These metabolites can facilitate or induce significant changes of the oxidant/antioxidant status in RBC indirectly as a result of the ionizing radiation.

*It may be concluded* from this study that zinc supplementation to $^{131}I$ intoxicated rats could substantially stabilize the increased activities of antioxidative enzymes and also exercise an antiperoxidative potential as indicated by alleviating the LP and GT. The precise mechanism of the observed zinc mediated regulation of enzyme activities and lipid peroxidation cannot be ascertained from this study and remains to be explored. It can be conjectured at this point that a zinc induced stimulation of the enzymatic and other antioxidants may be the key process whereby it restores functional and structural integrity of $^{131}I$ irradiated RBC.

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