Radioprotective role of zinc following single dose radioiodine (131I) exposure to red blood cells of rats

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Background & objectives: Irradiation with 131I is used for the treatment of various thyroid disorders. It is likely that radioiodine while in systemic circulation may cause some adverse effects on antioxidative enzymes present in red blood cells (RBCs). Zinc, on the other hand, has been reported to maintain the integrity of cells under certain toxic conditions. The present study was conducted to evaluate the adverse effects of 131I on RBCs and also to assess the protection provided by zinc under these conditions.

Methods: Female Wistar rats (n=32) were divided into four groups. Animals in group I served as normal controls; group II animals were administered a dose of 3.7Mbq of 131I (carrier-free) intraperitoneally, group III animals were supplemented with zinc (227mg/l drinking water) and animals in group IV were given a combined treatment of zinc as well as 131I. Activities of antioxidant enzymes were assessed in erythrocyte lysates after two days of treatments.

Results: An increase in the activity of glutathione reductase (GR), superoxide dismutase (SOD), reduced glutathione (GSH) and malondialdehyde (MDA) in the lysates of erythrocytes was seen after two days of exposure from 131I radiations. However, the activity of catalase was found to be significantly decreased. Interestingly, zinc supplementation to 131I treated rats resulted in attenuating the adverse effects caused by 131I on the levels of antioxidative enzymes.

Interpretation & conclusion: 131I can induce significant oxidant/antioxidant changes in RBCs and zinc may prove to be a candidate with great promise for radioprotection.

Key words 131I - antioxidant status - red blood cells - zinc

The damage to the thyroid gland from deposition of radioiodine has often been described in literature1,2. 131I is used for the treatment of Grave’s disease and thyroid follicular carcinoma3. Inspite of a safety record unmatched by alternate methods of therapy, the use of 131I has met with several objections, the most significant of which is the question of oxidative stress and carcinogenicity4,5. The irradiation dose in blood depends on the administered amount of 131I as well as the fraction of the activity appearing in the systemic circulation in the form of protein bound 131I6. Changes in the blood picture and serum enzymes after radioiodine treatment to thyroid gland have been stated in a few reports7,9. Ionizing radiations have a profound effect on the
erythrocyte ghost membranes, followed by the changes in membrane sulphhydryl (-SH) groups and activities of membrane bound enzymes.

Radiation injury to living cells is to large extent, due to oxidative stress. Reactive oxygen species (ROS) and free radicals induced by partial reduction of oxygen (O₂) react with cellular macromolecules and induce adverse effects in them. Radioactive iodine treatment has been shown to intensify lipid peroxidation (LPO) expressed by a significant increase in malondialdehyde values. Therefore, antioxidant defense system is an important area, which needs to be considered for exploring the effect of radioiodine on red blood cells.

Zinc salts have been shown to provide radioprotection against whole body irradiation. Earlier studies have suggested zinc as a beneficial agent during peroxidative damage. Experimental studies from our laboratory have also demonstrated the efficacy of zinc in regulating the activities of various serum and liver marker enzymes and in ameliorating the altered hepatic histoarchitecture. The present study was undertaken to evaluate the possible role of zinc in eliminating the changes initiated in antioxidant enzyme system in red blood cells following administration of ¹³¹I.

**Materials & Methods**

**Animals**: Female Wistar rats weighing 150 ± 20 g were procured from the central animal house, Panjab University, Chandigarh. The animals were housed in polypropylene cages in the departmental animal house under hygienic conditions, and were maintained on the standard laboratory feed and water, ad libitum, throughout the period of experimentation. The study protocol was approved by the ethics committee of the institution.

**Chemicals and equipment**: ¹³¹I as sodium iodide in dilute sodium thiosulphate solution was obtained from Bhabha Atomic Research Centre (Trombay, India). Zinc sulphate was purchased from E. Merck. NADPH, reduced glutathione (GSH) and 5′5′ - Dithiobis - 2 - Nitrobenzoic acid (DTNB) were purchased from Sigma Chemicals Co., USA. UV spectrophotometer (Beckman company, Germany) was used for enzymatic estimations.

**Experimental design**: The animals were divided into four groups of 8 rats each. Each group was subjected to different treatments for a period of two days. Animals in groups II and IV were administered a dose of 3.7Mbq of ¹³¹I (carrier free) intraperitoneally. The animals in group IV also received zinc as ZnSO₄ 7H₂O at a dose level of 227mg/l added to their drinking water for a period of two days. Animals in group I served as normal controls and no treatment was given to these animals. Animals in group III served as zinc controls and received zinc treatment similar to that of group IV animals.

**Collection of blood samples**: For the purpose of studying various haematological and biochemical parameters, blood samples were drawn from animals belonging to normal control and all the treatment groups, two days after the administration of ¹³¹I. Blood samples (3ml) were collected in the test tubes containing 8 per cent sodium citrate to collect erythrocytes, after subjecting the animals to light ether anaesthesia 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 1000g and the plasma was removed by suction. The erythrocytes were washed twice with phosphate buffered saline (PBS), pH 7.4. Distilled water (10 ml) was then added and the erythrocytes were resuspended by agitation and lysed for 2 h at 4°C. A mixture of chloroform-ethanol (3:5, v/v, 0.8 ml) and 0.3ml of water was added to the lysate so as to precipitate the haemoglobin, which was centrifuged at 3000xg for 10 min at 4°C. The enzymes viz., superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and GSH were assayed in the clear supernatant.

**Preparation of packed cell volume (for the determination of lipid peroxidation in erythrocytes)**: The blood samples were centrifuged at 1000xg for 10 min at 4°C. The plasma and buffy coats were removed by gentle aspiration. The erythrocytes were washed three times
with PBS, pH 7.4. After washing, packed cell volume (PCV) was adjusted to 5 per cent with PBS. The LPO in erythrocytes was determined by measuring the MDA produced using thiobarbituric acid (TBA) by the method of Wills.26

Statistical analysis: One-way analysis of variance (ANOVA), followed by a multiple post-hoc test (Student Newman Keuls) was used for data analysis.

Results & Discussion

The present study was conducted to elucidate the possible protective role of zinc on the key antioxidative enzymes of red blood cells following exposure from 131I radiations. Zinc is an essential trace element for the function of many key enzymes and a daily dietary intake of around 9-11 mg is required to carry out various body functions. Moreover, erythrocytes are considered as early models for conducting studies on oxidative stress enzymes as these are highly prone to oxidative reactions because of relatively high oxygen tensions, the presence of haemoglobin, and a plasma membrane rich in polyunsaturated lipids.27 In the present study, MDA levels in animals that received 131I treatment showed a significant increase when compared to their respective normal controls (Table). Various studies have shown that free radicals induced by ionizing radiations have a damaging effect on lipids.11,28 Since the ionizing radiation abstracts hydrogen from a molecule to form a radical,29 it is likely that erythrocyte MDA levels may be elevated due to enhanced radical generation. Bartoc et al13 also found that radioactive iodine treatment led to the intensification of lipid peroxidation expressed by a significant increase in MDA values. 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 131I treated rats (group IV). It has been hypothesized that the induction of free radicals as consequence of radiation exposure might lead to increase in zinc turnover in concert with the synthesis of metallothionein (MT) which is associated with radioprotection.30 Moreover, the protection could also possibly be due to the induction of GSH as a result of zinc treatment.

In the present study, the levels of GSH were increased following exposure of red blood cells to 131I. Oxidizing radiations stimulate the synthesis of GSH in response to elevated free radicals and may lead to a paradoxical increase in erythrocyte glutathione levels or enhanced antioxidant response. On the other hand, zinc was able to attenuate the levels of GSH when supplemented to 131I treated rats. The observed attenuation in GSH levels following zinc treatment could be because of its property to induce metallothionein (S-rich protein) as a free radical scavenger, or its indirect action in reducing the levels of oxygen reactive species, however its mechanism for these actions remain to be elucidated, with regard to protecting the important thiols in toxic conditions.32

A statistically significant increase in the levels of GR was noticed following 131I treatment (Table). In an earlier study conducted by Konukoglu et al, GSH as

<table>
<thead>
<tr>
<th>Group</th>
<th>LPO</th>
<th>GSH</th>
<th>GR</th>
<th>SOD</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.61 ± 0.24</td>
<td>0.136 ± 0.02</td>
<td>11.75 ± 0.09</td>
<td>22.72 ± 1.11</td>
<td>733.4 ± 17.4</td>
</tr>
<tr>
<td>II</td>
<td>4.16 ± 0.57</td>
<td>0.394 ± 0.01</td>
<td>12.50 ± 0.55</td>
<td>29.15 ± 3.08</td>
<td>697.2 ± 23.1</td>
</tr>
<tr>
<td>III</td>
<td>1.66 ± 0.13</td>
<td>0.152 ± 0.02</td>
<td>11.64 ± 0.07</td>
<td>22.20 ± 2.10</td>
<td>738.8 ± 20.3</td>
</tr>
<tr>
<td>IV</td>
<td>2.55 ± 0.36</td>
<td>0.359 ± 0.03</td>
<td>12.32 ± 0.57</td>
<td>25.91 ± 4.06</td>
<td>711.2 ± 14.8</td>
</tr>
</tbody>
</table>

Lipid peroxidation (nmoles of MDA/min/g Hb), glutathione reduced (nmoles of GSH/mg protein), glutathione reductase (nmoles of NADPH oxidised/min/mg protein), superoxide dismutase (U. i.e. inverse of the amount of protein required to inhibit the reduction rate of NBT by 50%), catalase (nmoles of H_2O_2 decomposed/min/mg protein)

P ≤ 0.05 * ≤ 0.01 † ≤ 0.001 by Newman – Keuls test when the values of group II, III and IV are compared with those of group I, P ≤ 0.05 * ≤ 0.01 † ≤ 0.001 by Newman – Keuls test when the values of group IV are compared with those of group II

Values are expressed as mean ± SD of 8 animals

LPO, lipid peroxidation; GSH, reduced glutathione; GR, glutathione reductase; SOD, superoxide dismutase; CAT, catalase
well as GSH related enzyme activities were significantly increased in patients two days after radiiodine treatment, when compared to their own initial levels.

Superoxide dismutase is the first line of defense against oxygen derived free radicals and functions by dismutating two superoxides ($O_2^-$) ions into $H_2O_2$. The present study indicated a marked increase in the activity of SOD after $^{131}I$ exposure to red blood cells. 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 as a consequence of induction of GSH as an indication of indirect protection afforded by zinc.

In the current study, decreased levels of catalase activity were observed in erythrocytes of $^{131}I$ treated animals compared to the control animals. The decrease in the catalase activity could possibly be due to utilization of this enzyme in converting $H_2O_2$ to $H_2O$. This 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, simultaneous zinc treatment to $^{131}I$ treated animals showed an increase in the activity of catalase substantiating thereby that production of reactive oxygen species was much more in case of $^{131}I$ treatment and zinc has been able to neutralize the accumulation of free radicals to some extent.

In conclusion, our results indicated alterations in the levels of antioxidative enzymes in the RBCs of animals exposed to radiiodine. Radiiodine can cause significant apoptosis and mitotic cell death in the thyroid tissue. This can release cytokines and toxic metabolites. These agents can facilitate or induce significant changes of oxidant/antioxidant status in erythrocytes indirectly as a result of the ionizing radiation. Zinc supplementation to $^{131}I$ treated rats substantially stabilized the increased activities of antioxidative enzymes and could prove to be potential radioprotective agent.

References


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