Enhanced erythrocytic lipid peroxides level in rabbits after repeated parental administration of iron

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An experiment was conducted in rabbits to evaluate the possible involvement of oxidative stress in iron-overload animals. Ten adult female New Zealand white rabbits were divided into 2 equal groups with 5 animals each. Group II animals received intramuscular iron dextran injections (120 mg/kg body wt/day) on alternate day for 14 days (8 injections), while Group I animals did not receive any iron supplementation to serve as negative controls. The blood samples were collected by cardiac puncture before the start of iron dosing and thereafter, at weekly intervals for 28 days. The samples were processed to measure blood iron concentration, packed cell volume, erythrocytic lipid peroxide (LPO) level, superoxide dismutase (SOD) and catalase (CAT) activities. The blood iron concentration showed a rising trend following repeated iron administration, and the mean level recorded on day 14 was significantly higher than respective day 0 value. LPO level remained significantly higher from day 14 onwards till the end of the observation period of 14 more days after cessation of iron administration. Erythrocytic superoxide dismutase activities showed a transient significant rise on day 7, and thereafter, showed a declining trend, but remained statistically comparable to respective day 0 or corresponding value of the control animals.

Keywords: Antioxidant, Iron overload, Lipid peroxides, Oxidative stress, Rabbit, Superoxide dismutase

Iron is an essential trace element for almost all living organisms and serves as an integral component of haemoglobin, myoglobin, cytochromes enzymes and other iron dependent enzymes. Its deficiency results in anemia, and enrichment of foods with iron supplements has, thus been advocated to reduce the prevalence of iron deficiency associated anemia and cytotoxic effects of heavy metal toxicants including arsenic. However, scientific revelations have raised a question mark over the utility and safety of iron supplementation as its increased intake and storage have been linked to a variety of chronic diseases including liver cirrhosis, neurological, orthopedic and cardiovascular disorders. Excess iron, regardless of the route of entry, accumulates in parenchymal organs and threatens cell viability. However, the exact mechanism of regulation of iron absorption through intestine and the mechanism of its toxicity remains uncertain. Iron storage diseases are believed to cause organ damage through generation of free radicals and resultant oxidative stress. Free iron catalyses conversion of hydrogen peroxide to more reactive free radical ions via the Fenton reaction that attack cellular membranes, proteins and DNA. The present study was, therefore, aimed to investigate iron overload related changes in oxidative stress indices in the erythrocytes after parental administration iron preparation bypassing the poorly explained intestinal iron absorption regulatory feedback mechanism.

Materials and Methods

Animals and experimental design—Experiment was conducted on New Zealand white rabbits after taking due approval from Institute animal ethics committee. Ten female rabbits, seven to eight weeks old, were procured from Indian Veterinary Research Institute, Mukteshwar campus, Uttaranchal, India. Those were maintained in individual rabbit cages with free access to standard rabbit diet and tap water and allowed to acclimatize in the laboratory for a period of 30 days before the start of the experiment. The experimental animals were divided into two equal groups (Gr. I and II) consisting of 5 animals in each. Animals of Gr. I received no treatment and served as negative control. Gr. II rabbits received iron injections [120 mg/kg body wt/day, im as injection, Jectofer, procured from CFL pharmaceutical (P) Ltd., Bangalore, India; containing 50 mg of elemental iron per ml as iron sorbitol citric acid complex] on
alternate day starting from day 0 to 14 to induce iron overload. Blood samples were collected by cardiac puncture from all the experimental animals on day 0 prior to iron administration and, thereafter, at weekly intervals on day 7, 14, 21 and 28. Blood sample was collected into heparinized vials (1ml) for various hemato-biochemical analyses, and into nitric acid washed heparinized vial (1ml) and stored at -20°C till further processing for iron estimation.

**Haematological analysis**

Packed cell volume (PCV) was estimated in blood samples by microhematocrit method. Blood samples were wet digested using double acid mixture (4 parts of conc. nitric acid and 1 part of 70% perchloric acid) on low heat (below 90°C) using micro-digestion bench. Iron content in the digested blood samples was measured colorimetrically.

**Lipid peroxide—Measurement of malondialdehyde (MDA)** is an indirect, but reliable method for assessing lipid peroxidation in different tissues. Lipid peroxide (LPO) level in RBC haemolysate was determined spectrophotometrically. The blood sample was centrifuged at 2000 rpm per 10 min and the supernatant was discarded. The sediment (packed erythrocytes) was washed three times with chilled NaCl solution (0.85%). Washed erythrocytes were hemolysed with 9-fold volume of distilled water to prepare RBC haemolysate (10%). MDA concentration (nmole per ml of haemolysate) was calculated using 1.56 × 10⁵ as extinction coefficient. Haemoglobin in haemolysate was estimated by cyanomethaemoglobin method. Lipid peroxide level in the erythrocytes was expressed as nmole MDA/hg of haemoglobin.

**Antioxidant enzymes in erythrocytes—**Superoxide dismutase (SOD) activity in haemolysate (10%) was measured using nitro blue tetrazolium as substrate with certain modifications. Catalase (CAT) activity was estimated after suitable dilution of 10% RBC haemolysate and the activity of SOD and CAT were expressed in terms of Units/mg of hemoglobin.

**Statistical analysis**—The data were analyzed statistically using two-way analysis of variance to find out significance of difference in mean values.

**Results and Discussion**

Table 1 shows the blood iron concentrations at weekly interval in rabbits with (Gr. II) or without (Gr. I) intramuscular administration of iron. Its level over different periods of observation in group I remained statistically (P>0.05) comparable. However, a significant increase in the mean iron concentration was observed in group II on day 14 (42.71±1.64 mg/dl) as compared to mean value on day 0 (35.70±1.47 mg/dl). The blood iron concentration was 14.19% higher in iron-injected group as compared to 2.23% in control animal by day 28 from the respective day 0 value (Table 2). Mean PCV values remained statistically comparable among periods in either of the groups and between two groups at different periods of observation.

Erythrocyte lipid peroxide (LPO) level on day 0 in animals of group I was 5.30±0.19 nmole of MDA/mg of Hb and the level remained statistically unchanged till the end of observation period on day 28. In rabbits of group II, a significant increase in LPO from the pretreatment level (5.02±0.47 nmole of MDA/mg of Hb) was observed by day 14 (7.41±0.70 nmole of MDA/mg of Hb) and continued to remain significantly higher till day 28 (6.83±0.44 nmole of MDA/mg of Hb; (Fig. 1). The values recorded in group II on day 28 was higher (26.55%) than the pretreatment level (Table 2).

Control rabbits of group I had no significant change in SOD activity in erythrocytes between day 0 (9.94±1.07 U/mg of Hb) and day 28 (10.01±1.05 U/mg of Hb). The rabbits of group II had transient but significant (P<0.05) increase in SOD activity on day 7 (13.16±0.69 U/mg of Hb) as compared to respective value of the control group (9.99±0.10 U/mg of Hb). Thereafter, the mean SOD activity showed a declining trend and the values recorded on day 14 (8.73±0.96

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<th>21</th>
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Different superscripts in rows vary significantly at P<0.05.

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*Table 1—Blood iron concentration and packed cell volume in rabbits during intramuscular administration of iron preparation* [Values are mean±SE of 5 animals]
Table 2—Changes in blood iron, erythrocytic lipid peroxides level and antioxidant enzymes

<table>
<thead>
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<th>After treatment</th>
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<tr>
<td>I</td>
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<td>6.83±0.44</td>
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<td>SOD (U/mg of Hb)</td>
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C—Control; I—Iron overloaded

Fig. 1—Erythrocytic lipid peroxides level in rabbits given repeated iron (im) preparation

U/mg of Hb) and thereafter, remained statistically comparable to respective control values (Fig. 2). No significant change in mean catalase activity was noted in group I rabbits during the experimental period. In group II, the catalase activity increased nonsignificantly by day 7 (35.18±2.37 U/mg of Hb) and then declined on day 14 (34.41±2.69 U/mg of Hb) and 28 (30.02±2.75 U/mg of Hb). However, no significant change was observed in catalase activity in group II animals (Fig. 3).

Excess iron is stored in various tissues either as diffused soluble mobile fraction (ferritin) or as insoluble aggregate deposits (hemosiderins)21. The liver and spleen usually have high storage-iron concentration followed by kidney, heart, muscle and brain, and erythrocytes or plasma are not considered as storage depots of excess iron22. Thus, it is suggested that gradual and significant increase in blood iron concentration following its intramuscular administration might be due to increase in iron content in transferring protein (transferrin) or heme. However, there was no significant change in PCV following iron administration, thus suggesting that initial increase in whole blood iron was due to increase in plasma iron than heme iron. Transferrin, cytochromes, peroxidase and catalase present in plasma contain about less than 1% of non-heme iron. Therefore, increased blood iron concentration particularly during the early part of the experiment might be attributed to free iron, but not transferrin that requires further study.

Enhanced erythrocytic lipid peroxides level in iron-administered animals suggested oxidative damage to lipids present in erythrocytic membranes. Iron, being the transition metal, participates in Fenton reaction, converting less reactive superoxide radical and hydrogen peroxide to more reactive hydroxyl radical and iron has been successfully used as a free radical generating system in vitro23,24. Plasma lipid peroxidation has been earlier reported to increase by 28.5% in rats following intraperitoneal administration of iron dextran25. Erythrocytes membrane is rich in polyunsaturated fatty acids and presence of powerful transition metal catalyst and generation of free...
radicals in the present study might have rendered these cells to peroxidative damage. Golberg and coworkers have recorded induction of oxidative stress in liver, kidney, muscle and skin after repeated intramuscular administration of iron dextran (total dose of 825-100 mg/kg) in rats. The initial increase in superoxide dismutase and an increased trend in catalase activity might be attributed to compensatory up-regulation in their synthesis to counteract free radicals. However, non-significant decline in superoxide dismutase and catalase activities after day 7 (activities were 13.90 and 7.34 per cent lower, respectively on day 28 from corresponding day 0 values) might be due to utilization and exhaustion of in-built anti-oxidant defense system with persistence of pro-oxidant in the body and possible generation of reactive oxygen species. This was substantiated by increased erythrocytic lipid peroxidation up to 14 days after the cessation of intramuscular iron administration. It is concluded that repeated intramuscular administration of iron preparations is not free from erythrocytic oxidative damage.

Acknowledgement

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References

24. Sultana S, Saleem M, Sharma S, Khan N, Lupeol, a triterpene, prevents free radical mediated macromolecular

