THE INFLUENCE OF ASCORBIC ACID ON NICKEL-INDUCED HEPATIC LIPID PEROXIDATION IN RATS

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ABSTRACT

We studied the effect of oral ascorbic acid treatment on nickel sulfate-induced lipid peroxidation in the liver of Wister strain male albino rats. Lipid peroxide and glutathione levels and the activities of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) were estimated in liver. Nickel sulfate administration significantly increased the level of lipid peroxides and decreased glutathione, SOD, CAT, and GSH-Px activities in liver. The simultaneous administration of ascorbic acid with nickel sulfate resulted in a remarkable improvement of lipid peroxide, glutathione, SOD, CAT, and GSH-Px status in liver in comparison with rats treated with nickel alone. Nickel sulfate has an adverse effect on hepatic lipid peroxidation in animals, but simultaneous treatment with ascorbic acid offers a relative protection against nickel-induced hepatotoxicity.

KEYWORDS

nickel sulfate, protein restriction, lipid peroxide, glutathione, catalase, superoxide dismutase, glutathione peroxidase

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INTRODUCTION

The rapid development of science, industry, medicine, and agriculture has exposed man and his environment to number of exotic heavy metals. Nickel is considered one of the most toxic heavy metals having an adverse affect on biological activity /1/. Mankind is exposed to nickel produced from such sources as mining, extraction and refining, electroplating, grinding and polishing, nickel powder metallurgy, nickel alloys, nickel cadmium batteries, chemical industry, food processing; and nickel waste disposal via food, water, and air /2, 3/. Bones, kidney, and liver are the organs in which nickel accumulates. Nickel reportedly causes severe liver and kidney damage by altering several marker enzymes and ascorbate cholesterol metabolism /4/. Recent studies have shown that metals like iron, copper, cadmium, lead, and nickel exhibit the ability to produce reactive oxygen species (ROS) or free radicals, resulting in lipid peroxidation, DNA damage, depletion of sulfhydryls, and altered calcium homeostasis /5/. Liver, the major site for detoxification, is the primary target for environmental and occupational toxicity. Protection of the hepatic cell membrane from lipid peroxidation becomes a necessity to prevent, to cure, or to delay the aforesaid pathologies. As free radical-induced lipid peroxidation has been reported to be associated with various deleterious effects, including tissue damage and necrosis, we have chosen to study the effect of ascorbic acid, a potent antioxidant and a good scavenger of free radicals, on lipid peroxidation and on the activities of antioxidant enzymes during nickel sulfate-induced hepatotoxicity in rats.

EXPERIMENTAL

Adult male albino rats of the Wister strain weighing 160±5g each were fed with laboratory stock diet and water ad libitum for 1 wk. The acclimatized animals were divided into four groups of six animals each. Group I served as an untreated control. Group II rats were administered nickel sulfate in double distilled water, at a dose of 2.0

mg/100 g b.wt. intraperitoneally (i.p) /6/ on alternate days until the tenth dose. Group III rats were treated orally with ascorbic acid at a dose of 50 mg/100 g. b.wt /7/, and Group IV rats were given both nickel sulfate (2.0 mg/100 g b.wt. i.p.) and ascorbic acid simultaneously (50 mg/100 g b.wt. orally) on alternate days until the tenth dose. The rats were sacrificed by cervical decapitation at the end of the last dose after overnight fasting. The liver was dissected out, washed in ice-cold saline, and weighed. Approximately 100 to 150 mg tissue was homogenized in 0.05 M Tris-Cl/1mM EDTA (pH 7.0) buffer by Polytron (setting 7, 20 sec). The homogenate was centrifuged for 20 min at 3,000 × g at 40°C, and the supernatant was used for assaying antioxidant enzymes as follows:

Superoxide dismutase: SOD activity was determined according to the method of Misra and Fridovich /8/. Briefly, 100 µL of tissue extract (20 to 30 µg protein) was added to 900 µL of 50 mM carbonate buffer (pH 10.2) containing 0.7 mM epinephrine; the absorbance was measured for 4 min at 480 nm. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of epinephrine by 50%.

Catalase: CAT activity was determined by the method of Aebi /9/. In this assay, 10 µL of tissue extract (20 to 30µg protein) was placed for 30 min into an ice bath. Triton X-100 (10µL) was then added to the mixture. The sample (100 µL) was then mixed with 500 µL of 66 mM of hydrogen peroxide and 400 µL of 50 mM phosphate buffer (pH 7.0) containing 1 mM EDTA; the absorbance was monitored for 30 sec at 240 nm. The molar extinction coefficient of 43.6 mM cm^{-1} was used to determine CAT activity. One unit of CAT activity was defined as millimoles of degraded hydrogen peroxide/min/mg protein.

Glutathione peroxidase: GSH-Px activity was determined using a modification of the method of Flohe and Gunzler /10/. In this assay, 100 µL of the tissue extract (20 to 30 µg protein) was added to 300 µL of reaction mixture containing 100 µL phosphate buffer, 100 µL 100 mM reduced glutathione, 100 µL 1.5 mM NADPH, 0.34 units of glutathione reductase, 500 µL of 50 mM phosphate buffer, pH 7.0, 1 mM (EDTA) and incubated for 10 min at 37°C. This was followed by the addition of 100 µL of 12 mM t-butyl hydroperoxide and

determining the absorbance of the reaction mixture for 3 min at 340 nm. The millimolar extinction coefficient of 6.22 mM cm^{-1} was used to determine the activity of GSH-Px. One unit of activity was equal to the millimole of oxidized NADPH/min /mg of protein. The glutathione level was measured by the method of Moron et al. /11/, and liver lipid peroxide levels were estimated in terms of 2-thio-barbituric acid reactants, using 1,1/,3,3/, tetramethoxypropane as standard /12/. Protein was determined by the method of Lowry /13/.

The mean±SEM values were calculated for each group. For determining the significance of intergroup differences, each parameter was analyzed separately. A one way analysis of variance (ANOVA) was carried out at 1% of Fischer's distribution to determine which of the groups differed among themselves. Duncan's multiple range test was applied with the level of significance fixed at $P<0.05$.

TABLE 1

Effect of nickel on hepatic lipid peroxidation and antioxidant enzyme activities in rats

Treatment group	Lipid peroxide (µm TBA reactants/g protein)	Glutathione (µm/g tissue)	Enzyme activities (un ts/mg)[1]		
			SOD	CAT	GSH-Px
I	3.81±0.21[a]	4.56±0.24[a]	61.25±3.10[a]	7.53±0.34[a]	0.087±0.011[a]
II	6.34±0.28[b]	2.80±0.02[b]	48.32±0.28[b]	5.82±0.28[b]	0.059±0.009[b]
III	3.78±0.18[a]	4.81±0.28[a]	62.12±2.82[a]	7.62±0.38[a]	0.084±0.010[a]
IV	4.07±0.12[a]	3.92±0.44[c]	56.24±3.28[c]	6.82±0.28[c]	0.071±0.010[c]

[1]SOD, superoxide dismutase ; CAT, catalase ; GSH-Px, glutathione peroxidase; TAB, 2-thio-barbituric acid

Each value is the mean ±SEM of six observations in each group. In each column, values with different superscripts (a, b) were significantly different from each other ($p<0.05$). Treatment groups: I-untreated control; II-nickel sulfate; III-ascorbic acid; IV-nickel sulfate+ascorbic acid

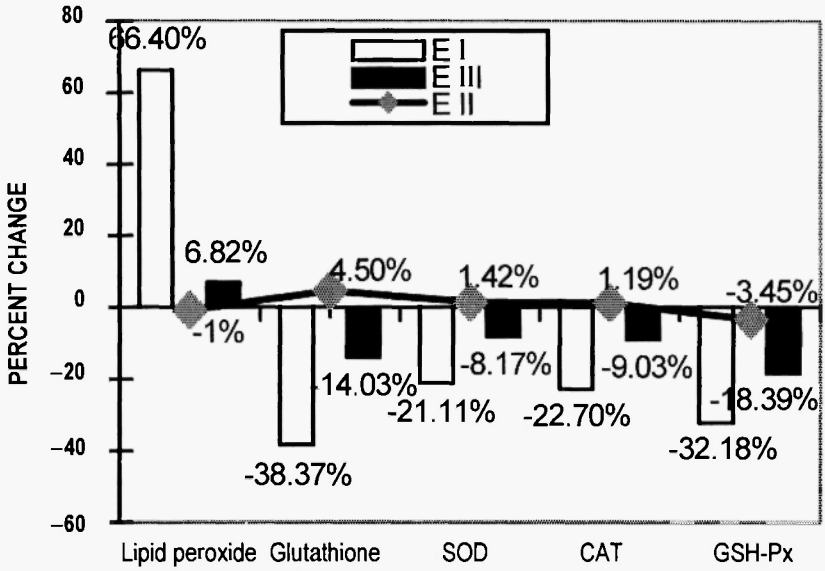

Fig. 1: Percent change chart of lipid peroxide, glutathione, and anti-oxidant enzyme activities in liver of nickel sulfate treated rats.
SOD, superoxide dismutase; CAT, catalase; GSHPx, glutathione peroxidase. E I, Group I (control) vs. Group II (+$NiSO_4$); E II, Group I vs. Group III (+ascorbic acid); E III, Group I vs. Group IV ($NiSO_4$+ ascorbic acid)

RESULTS

Table 1 shows that in Group II, nickel induced a significant increase in hepatic lipid peroxide level in comparison with that in the control Group I, whereas in Group IV (nickel sulfate+ascorbic acid), no significant change was noticed when compared with the control Group I. Figure 1 shows that the percentage change of lipid peroxide was almost at a normal level when compared with the respective control Group I (E I vs. E III). The results depicted in Table 1 show that after nickel treatment, the liver SOD, CAT, and GSH-Px activities were significantly suppressed in the experimental group Group II in comparison with the control Group I. In Group IV (nickel sulfate+

ascorbic acid), a significant fall of all antioxidant enzyme activities were noticed, but the percent change of reduction was less than that of Group I (E I vs. E III). After nickel treatment, the liver glutathione level also was significantly decreased in Group II in comparison with the control Group I. Group IV (nickel sulfate+ascorbic acid) showed a percentage change in reduction of glutathione level in comparison with the control Group I, but when compared with Group-II, the level of reduction was found to be remarkably less (E I vs. E III). Rats receiving ascorbic acid alone (Group III) did not show any significant variation in the parameters studied above when compared with their respective dietary controls.

DISCUSSION

Lipid peroxidation is a free radical-mediated process that has been implicated in a variety of disease states. Lipid peroxidation involves the formation and propagation of lipid radicals, the uptake of oxygen, and a rearrangement of the double and unsaturated lipids, resulting in a variety of degraded products that eventually causes destruction of membrane lipids. Increased peroxidation can result in changes in the cellular metabolism of hepatic and extra-hepatic tissues. The increased lipid peroxidation seen here in the liver of nickel-treated rats (Group II) suggests an increase of phospholipase activities during peroxidic decomposition of different suborganelle and plasma membrane lipids. The peroxide generated must stimulate phospholipase A2 (PLA2) activities on the cell membrane. Activation of PLA2 causes the production of a variety of eicosanoids, which are responsible for different metabolic disorders and cell injury /14/. The rats treated concurrently with ascorbic acid and nickel sulfate (Group IV) showed a significant reduction of the lipid peroxide level in comparison with that in the group receiving nickel alone (Group II). Therefore, we may postulate from this observation that ascorbic acid can protect –SH groups from nickel-induced oxidative damage by inhibiting the peroxidation rate of membrane lipids /15/.

Good evidence supports the view that superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) are enzymes that scavenge free radicals during lipid peroxidation. Clearly, the cytotoxicity of molecular oxygen is checked by the delicate balance between the rate of generation of the partially reduced oxygen species and the rate of their removal by different defense mechanisms. A shift in this delicate balance can lead to cellular damage /16/.

In the nickel-treated experimental Group II studied here, the decreased activities of hepatic SOD, CAT, and GSH-Px, the primary antioxidant enzymes, suggests an interaction between the accumulated free radicals and the active amino acids of these enzymes /17/. During hepatotoxicity, these enzymes are structurally and functionally impaired by free radicals, resulting in liver damage. In Group IV (nickel sulfate+ ascorbic acid), a significant improvement in the activity of these antioxidant enzymes was noticed when compared with that of Group II. Such improvement could be due to the relative ability of ascorbic acid to scavenge reactive oxygen species within the lipid region of the membrane. Glutathione comprises up to 90% of the non-protein thiol content of mammalian cells and performs a pivotal role in maintaining their metabolic and transport functions. Glutathione acts as a nucleophilitic 'scavenger' of many compounds and their metabolites via enzymatic and chemical mechanisms, converting electrophilic centers to ether bonds. Glutathione depletion to about 20% to 30% of total glutathione levels can impair cell defenses against toxic actions, which may lead to cell injury and death /18/. Furthermore, glutathione is considered a crucial factor in maintaining the structural integrity of cell membranes, largely through reactions that protect the membrane against free-radical formation /19/. The nickel-induced decrease in glutathione levels seen here may be due to its increased use in protecting –SH-containing proteins from lipid peroxides. In Group IV (nickel sulfate+ascorbic acid), the significant improvement of the glutathione level when compared with that of Group II was due to an ascorbic acid-induced relative protection from nickel abuse of the cellular thiol status in the liver.

In conclusion, although nickel sulfate has an adverse effect on rat liver antioxidant enzyme systems, simultaneous treatment with ascorbic acid offers a relative protection against nickel-induced lipid peroxidation.

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