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Chromium-induced membrane damage: protective role of ascorbic acid

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Abstract: Importance of chromium as environmental toxicant is largely due to impact on the body to produce cellular toxicity. The impact of chromium and their supplementation with ascorbic acid was studied on plasma membrane of liver and kidney in male Wistar rats (80 – 100g body weight). It has been observed that the intoxication with chromium (i.p.) at the dose of 0.8 mg/100g body weight per day for a period of 28 days causes significant increase in the level of cholesterol and decrease in the level of phospholipid of both liver and kidney. The alkaline phosphatase, total ATPase and Na^+-K^+-ATPase activities were significantly decreased in both liver and kidney after chromium treatment, except total ATPase activity of kidney. It is suggested that chromium exposure at the present dose and duration induce for the alterations of structure and function of both liver and kidney plasma membrane. Ascorbic acid (i.p. at the dose of 0.5 mg/100g body weight per day for period of 28 days) supplementation can reduce these structural changes in the plasma membrane of liver and kidney. But the functional changes can not be completely replenished by the ascorbic acid supplementation in response to chromium exposure. So it is also suggested that ascorbic acid (nutritional antioxidant) is useful free radical scavenger to restrain the chromium-induced membrane damage.

Key words: chromium; liver; kidney; membrane; ascorbic acid

Introduction

The chromium plays dual role in nature. The chromium (Ⅲ) is essential for glucose and lipid metabolism (Chorvatovicova, 1993). On the other hand, excessive intake of chromium (Ⅵ) compounds are potent toxic and carcinogenic to the body (DeFlora, 1990). Hepatic and renal toxicity is the most common toxicity faced in chromium (Ⅵ)-exposed workers or animals (Laborda, 1986; Goyer, 1990; Hojo, 1991). This different functions of the chromium (Ⅲ) and chromium (Ⅵ) is largely depend on their ionic permeability of the plasma membrane (DeFlora, 1989).

Inside the cells, chromium (Ⅵ) is reduced through reactive intermediates such as chromium (Ⅴ) and (Ⅳ) to the more stable chromium (Ⅲ) by cellular reductants (DeFlora, 1989; Sugiyama, 1991; Standeven, 1991). This reduction process generates reactive oxygen species such as active oxygen radical (Chorvatovicova, 1993). Different studies have shown that ascorbic acid is responsible for ~80% of chromium (Ⅵ) reductase activity in rat liver and kidney (Susa, 1997). In addition to this, Susa (1997) showed that the ascorbic acid level was reduced after chromium (Ⅵ) treatment. Recently we have shown that ascorbic acid is an important nutritional antioxidant to prevent the chromium-induced tissue toxicity (Dey, 1997). Hence, supplementation with additional ascorbic acid may reduce the effect of chromium (Ⅵ).

Recent studies indicate that both chromium (Ⅵ) and chromium (Ⅲ) are biologically active oxidation states (Stohs, 1995). Earlier suggestion of oxidative impact of chromium (Ⅵ) on membrane phospholipids indicates the probable structural alteration of the membrane (Ginter, 1989). On the other hand, activation of the membrane bound enzyme (Bagchi, 1997) indicate the functional alteration of the membrane. Thus, in the present investigation we have tried to reduced the effect of chromium cytotoxicity by the ascorbic acid in vivo in terms of certain structural and functional components like cholesterol and phospholipid level as well as alkaline phosphatase (ALP), total ATPase and Na^+-K^+-ATPase activities of the liver and kidney membrane.

1 Materials and methods

1.1 Maintenance and treatment of animals

Male albino rats of Wistar strain weighing 80 – 100g were used for the present investigation. The rats were fed with a diet containing protein (Casein) 18%, carbohydrate (Amylum) 72%, fat (Groundnut) 7%, salt mixture 4% and vitamins mixture as reported elsewhere (Chatterjee, 1976; 1984). Water was fed ad libitum. All rats were acclimated to this diet and laboratory environment for 4 – 5 days. Then rats were divided into three groups of equal average body weight. They were housed individually in cages and light-controlled room (12 h/d). The animals of one of the groups were injected intraperitoneally (i.p.) with chromium as CrO_3 at a dose of 0.8 mg/100g body weight per day (20% LD_{50}) for 28 days. The animals of the another group serving as the supplemented group treated with both chromium and ascorbic acid i.p. at a dose of 0.8 mg/100g body weight per day and 0.5 mg/100g body weight per day respectively at an interval of six hours for a period of 28 days. The animals of the remaining group received only the vehicle (0.9% NaCl), served as control. The animals of the control group were pair-fed with those of the chromium-treated and supplemented group.

1.2 Tissue collection

After the experimental period, the rats were fasted overnight and sacrificed by cervical dislocation. Liver and kidney were immediately dissected out of the body wiped off the blood and weight. The tissues were then stored at −20℃ until

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analysis.

1.3 Isolation of crude membrane fraction

Membrane fractions of the liver and kidney were isolated according to the method described by Ghosh Chowdhuri (Ghosh Chowdhuri, 1995). Tissues were homogenized with a glass homogenizer in 0.25 mol/L cold sucrose solution. The homogenate were then centrifuged at 15000g for 15 min at 4℃. The supernatants were collected and centrifuged again at 22650g for 20 min at 4℃. The supernatants, thus obtained, were discarded and the pellets were suspended in 1 ml chilled Tris buffer (pH 7.0) after three washing with the same buffer.

1.4 Assay of membrane protein

Membrane protein was estimated using Folin-Ciocalteau reagent and following the method described by Lowry (Lowry, 1951) using bovine serum albumin as the standard.

1.5 Estimation of membrane cholesterol and phospholipid

Cholesterol and phospholipid contents of the isolated membrane fractions were estimated by the methods of Zlatkis (Zlatkis, 1953) and Christopher (Christopher, 1972).

1.6 Measurement of alkaline phosphatase activity

Alkaline phosphatase activity of the isolated membrane was assayed using *P*-nitrophenyl phosphate (PNPP) as substrate according to the method of Linhardt and Walter (Linhardt, 1963).

1.7 Determination of total ATPase and Na^+-K^+-ATPase activities

Total ATPase and Na^+-K^+-ATPase activities were measured by the method of Sen (Sen, 1981).

1.8 Statistical analysis

Data for each group were subjected to analysis of variance (ANOVA). The data are expressed as the mean ± standard error of six animals. The level of statistical significance employed in all cases was $P < 0.05$.

2 Results

Table 1 shows the alterations in membrane cholesterol and phospholipid contents of liver kidney. Cholesterol content increased and phospholipid content decreased significantly in both liver and kidney studied in chromium treated group only when compared with the control group of animals (Table 1). On the other hand the chromium plus ascorbic acid treated group showed significant decreases the membrane cholesterol content and significant increases the membrane phospholipid content in liver and kidney when compared with chromium treated group (Table 1).

Table 1 Membrane cholesterol and phospholipid level of liver and kidney following exposure to chromium and the impact of ascorbic acid supplementation on their effects

Parameters	Tissues	Groups of animals		
		Control	Chromium treated	Chromium and ascorbic acid treated
Cholesterol,	Liver	11.79 ± 0.52	24.31 ± 0.73*	13.73 ± 0.66#
mg/100 mg protein	Kidney	19.23 ± 0.95	35.48 ± 0.53*	22.74 ± 0.84#
Phospholipid,	Liver	26.06 ± 3.22	10.62 ± 0.58*	24.97 ± 1.35#
mg/100mg protein	Kidney	21.81 ± 1.35	8.61 ± 0.45*	28.83 ± 5.63#

The values are means of six observations ± *SEM*; *. Indicates significant changes when compared with control group ($P < 0.05$); #. Indicates significant changes when compared with chromium treated group ($P < 0.05$)

The data presented in Table 2 revealed that the ALP, total ATPase and Na^+-K^+-ATPase activities in plasma membrane of both liver and kidney were significantly decreased in the chromium-treated group only when it compared with control group, except total ATPase activity of kidney. On the other the chromium plus ascorbic acid treated group only showed significant increases the ALP activity of kidney and Na^+-K^+-ATPase activity of liver and kidney when compared with chromium treated group (Table 2).

Table 2 Membrane ALP, ATPase and Na^+-K^+-ATPase activities of liver and kidney following exposure to chromium and the impact of ascorbic acid supplementation on their effects

Parameters	Tissues	Groups of animals		
		Control	Chromium treated	Chromium and ascorbic acid treated
Alkaline phosphatase,	Liver	2.95 ± 0.25	1.14 ± 0.08*	2.02 ± 0.59
unit/mg protein	Kidney	11.81 ± 0.54	9.32 ± 0.14*	11.65 ± 0.38#
Total ATPase,	Liver	6.25 ± 0.42	4.58 ± 0.48*	5.06 ± 0.38
mg Pi liberated/mg protein	Kidney	11.43 ± 0.93	9.52 ± 0.29	10.58 ± 0.33
Na^+-K^+-ATPase,	Liver	1.49 ± 0.18	0.74 ± 0.24*	1.67 ± 0.15#
mg Pi liberated/mg protein	Kidney	2.68 ± 0.69	1.01 ± 0.12*	2.13 ± 0.30#

The values are means of six observations ± *SEM*; *. Indicates significant changes when compared with control group ($P < 0.05$); #. indicates significant changes when compared with chromium treated group ($P < 0.05$)

3 Discussion

From the present experimental results it was demonstrated that in response to chromium, a significant increase in cholesterol level and a significant decrease in phospholipid level in both liver and kidney plasma membrane (Table 1). The

increased membrane cholesterol level may be due to its utilization or increased synthesis. On the other hand, the decreased membrane phospholipid level indicates the damage of the membrane structure of the cell. The probable impact of chromium on the lipid catabolising enzymes can not be ruled out as evidenced by increased excretion of urinary lipid metabolites (Bagchi, 1995a). This increased catabolism of lipids may result in accumulation of acetyl-CoA which in turn may lead to increased synthesis of cholesterol in the tissues particularly in non-steroid producing tissues. Thus, chromium by altering the relative proportion of cholesterol and phospholipid may cause damage to the cell membrane structure. Interestingly these alterations were found to be disappearing when chromium was accompanied by ascorbic acid (Table 1). From this result it has been observed that these structural changes of the liver and kidney plasma membrane can be attenuated by the ascorbic acid supplementation.

After chromium treatment the deceased activity of alkaline phosphatase in plasma membrane of both liver and kidney (Table 2). On the other hand the chromium plus ascorbic acid treated group only showed significant increase the alkaline phosphatase activity of kidney when compared with chromium treated group (Table 2). These observation are in appear to be in conformity with the earlier reports (Dey, 1997). It is well known that the inhibition of alkaline phosphatase reflects selective damage of the plasma membrane (Kumar, 1984). With respect to chromium (Ⅵ)-induced nephrotoxicity in animals, an inhibition of alkaline phosphatase localized in the cell membrane of liver cells was reported (Kumar, 1984). On the other hand, the elevation of alkaline phosphatase localized in the cell membrane of liver cells was reported in animals treated with chromate (Chorvatovicova, 1993). But, Susa(1997) observed inhibitory effect of chromium (Ⅵ) on alkaline phosphatase activity of the hepatocyte plasma membrane. In the present investigation, results indicate that the supplementation with ascorbic acid an partially attenuated chromium-induced inhibition of the alkaline phosphatase activity.

The total ATPase activity of membrane was reduced significantly in the chromium-treated group in case of liver only. However, ascorbic acid supplementation has no effect on the chromium-induced changes (Table 2). The inhibition of the energy production by the cytotoxic concentration of chromium (Stohs, 1995) may have some role in the chromium-induced changes of the ATPase activity. The Na^+-K^+-ATPase activity was found to be reduced significantly in chromium treated group in liver and kidney. When the chromium-treated group was supplemented with ascorbic acid, the Na^+-K^+-ATPase was found to be increased significantly in both liver and kidney in comparison to the chromium-treated group. The observed decrease of the Na^+-K^+-ATPase activity induced by chromium intoxication may be supported by the reports on chromium-induced hamper of the membrane transport (Standeven, 1991). Thus, chromium by altering the total ATPase and Na^+-K^+-ATPase activities may cause damage the cell membrane in the present dose and duration. But these changes cannot be completely attenuated by the ascorbic acid supplementation.

Thus the present studies indicate that chromium treatment at the present dose and duration induces structural and functional alterations in the plasma membrane of both liver and kidney. These structural changes can be attenuated by the ascorbic acid supplementation but the functional changes cannot be replenished completely by the ascorbic acid supplementation. However, more detailed studies are needed to know the exact mechanism of chromium-induced membrane damage. The study with other free radical scavangers and antioxidative supporters are also valuable to get the means by which toxic impacts of chromium can be restrained.

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