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Alpha-tocopherol supplementation on chromium toxicity: a study on rat liver and kidney cell membrane

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Abstract: Membrane damage is one of the important consequence of chromium, an environmental toxicant, to produce cytotoxicity. α-tocopherol, a membrane protectant can be used to reduce the chromium-induced membrane damage. In the present study, the impact of chromium in presence and absence of α-tocopherol was studied on plasma membrane of liver and kidney in male Wistar rats (80—100 g body weight). Significant increase in membrane cholesterol level as well as significant decrease in membrane phospholipid level in chromium exposed (0.8 mg/100 g body weight/d, i.p., for 4 weeks) animals suggest structural alteration of both liver and kidney plasma memebrane. The alkaline phosphatase, total ATPase and Na^+-K^+-ATPase activities of plasma membrane were significantly decreased in both liver and kidney after chromium treatment. However, α-tocopherol (30 mg/100 g diet) supplementation can restrict the changes in these membrane-bound enzyme activities. Thus, the usefulness of dietary supplementation of α-tocopherol to restrain the chromium-induced membrane damage is suggested.

Keywords: chromium; liver; kidney; membrane; α-tocopherol

Introduction

The chromium plays dual role in nature. The chromium (Ⅲ) is essential for glucose and lipid metabolism (Chorvatovicova, 1993). On the other hand, chromium (Ⅵ) compounds are potent toxic (Dey, 1997 ; 2001) and carcinogenic to the body (DeFlora, 1990). This functional differentiation of chromium (Ⅲ) and chromium (Ⅵ) is largely decided by the ionic permeability of the plasma membrane (DeFlora, 1989). Thus the membrane damage is one of the crucial factors of chromium (Ⅵ) toxicity (Dey, 2001).

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; Standeven, 1991). This reduction process generates reactive oxygen species such as active oxygen radical (Chorvatovicova, 1993). Studies also revealed that vitamin E (α-tocopherol) can protect cells from chromate-induced cytotoxicity (Sugiyama, 1989). Further, dietary pretreatment of rats and guinea pigs with vitamin E showed significant protection of bone marrow cells from chromium (Ⅵ)-induced cytotoxicity (Chorvatovicova, 1991). Wise (Wise, 1994) reported that pretreatment of cells with the vitamin E can block clastogenesis induced by chromate.

Thus, in the present investigation we have tried to reduce the effect of chromium cytotoxicity by the α-tocopherol (Vitamin E) *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 plasma membrane.

1 Materials and methods

1.1 Maintenance and treatment of animals

Male albino rats of Wistar strain (80—100 g) were fed with a laboratory-prepared diet, as described elsewhere (Nayak, 1998) with water *ad libitum*. Laboratory acclimatized rats were divided into three groups of almost equal average body weight. The animals of two groups were injected intraperitoneally (i.p.) with chromium as CrO_3 at a dose of 0.8 mg/100 g body weight per day (20% LD_{50}) for 28 days, as described earlier (Dey, 2001). The animals of one of the chromium-treated groups serving as the supplemented group supplied α-tocopherol in the diet (30 mg/100 g diet) for the same period. The animals of the remaining group received only the vehicle (0.9% NaCl), served as control.

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1.2 Tissue collection

After the experimental period, overnight fasting rats were sacrificed by cervical dislocation. Liver and kidneys were immediately dissected out of the body, wiped off and stored at −20℃.

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 homogenates were then centrifuged at 15000 × g for 15 minutes at 4℃. The supernatants were collected and centrifuged again at 22650 × g for 20 minutes 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 and Ralph (Christopher, 1972) respectively.

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 level of statistical significance employed in all cases was $P < 0.05$.

2 Results

Alterations in membrane cholesterol and phospholipid contents of liver and kidney is depicted in Fig. 1. Significant increase in membrane cholesterol content in chromium treated group and insignificant increase in α-tocopherol supplemented chromium treated group were observed in both liver and kidney (Fig. 1a). Whereas significant decrease (compared to control) in membrane phospholipid content in both organs of chromium treated group was found which is remain unaltered (when compared with chromium treated group) when the animals were supplemented with α-tocopherol (Fig. 1b).

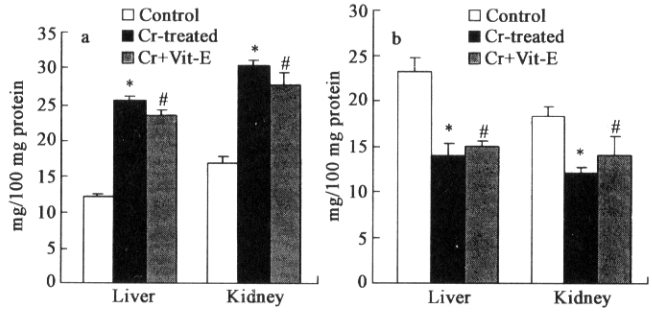

Fig. 1 Membrane cholesterol (a) and phospholipid (b) level of liver and kidney following exposure to chromium and the impact of α-tocopjerol supplementation on their effects

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 Fig. 2 revealed that the ALP (Fig. 2a), total ATPase (Fig. 2b) and Na^+-K^+-ATPase (Fig. 2c) activities in plasma membrane of both liver and kidney were significantly decreased in the chromium-treated group when it compared with control group. On the other hand, after supplementation with α-tocopherol the ALP, total ATPase and Na^+-K^+-ATPase activities of both liver and kidney membranes showed significant increase when compared with chromium treated group (Fig. 2).

3 Discussion

Recent studies indicated that both chromium (Ⅵ) and chromium (Ⅲ) are biologically active oxidation states (Stohs,

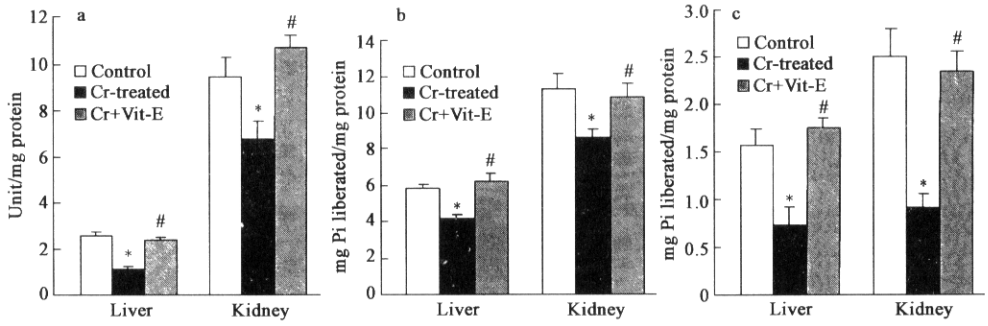

Fig.2 Membrane ALP(a), ATPase(b) and Na^+-K^+-ATPase(c) activities of liver and kidney following exposure to chromium and the impact of α-tocopherol supplementation on their effects

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($P < 0.05$)

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.

In the present investigation, the chromium-induced membrane damage is clearly indicated by significant increases of the membrane cholesterol content in both liver and kidney(Fig. 1). This increase may be due to imbalance in cholesterol incorporation into the membrane. Thus chromium may impair the function of lecithin cholesterol acetyl transferase. 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, 1995). 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 (Fig. 1). But the impact of chromium on membrane cholesterol and phospholipid contents were not found to be disappearing when chromium was accompanied by α-tocopherol(Fig. 1a and 1b). From this result it has been observed that these structural changes of the liver and kidney plasma membrane can not be attenuated by the α-tocopherol supplementation (Fig.1).

The reports of impact of chromium on the alkaline phosphatase activity of tissue membrane are contradictory (Chorvatovicova, 1993; Kumar, 1984; Susa, 1997). After chromium treatment, the activity of alkaline phosphatase in plasma membrane of both liver and kidney was found to be decreased(Fig.2a) as observed in our earlier studies (Dey, 1997; 2001). On the other hand, the chromium plus α-tocopherol treated group showed significant increase in the alkaline phosphatase activity of both liver and kidney membrane when compared with chromium treated group(Fig.2a). This inhibition of alkaline phosphatase reflects selective damage of the plasma membrane(Kumar, 1984) which is also supported by alteration of cholesterol and phospholipid contents(Fig.1). Thus, the present investigation indicates that the supplementation with α-tocopherol an attenuation of chromium-induced inhibition of the alkaline phosphatase activity. This observation is in agreement with the earlier observation by Tezuke (Tezuke,1991) who demonstrated the protective effects of vitamin E against chromium (Ⅵ).

The total ATPase activity of membrane were reduced significantly in the chromium-treated group in both liver and kidney. However, α-tocopherol supplementation has an effect on the chromium-induced changes of total ATPase activity (Fig. 2b). 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 both the organs(Fig. 2c). When the chromium-treated group was supplemented with α-tocopherol, the Na^+-K^+-ATPase was found to be increased significantly in both liver and kidney in comparison to the chromium-treated group (Fig. 2c). The ATP hydrolyzing activity of Na^+-K^+-ATPase is inversely proportional with the cholesterol/phospholipid

ratio of the membrane(Yeagle, 1985). Thus, in the present investigation, the decreased Na^+-K^+-ATPase activity may be related to altered cholesterol and phospholipid contents and may not be the direct effect of chromium itself.

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 not be attenuated but the functional changes can be fully attenuated by the α-tocopherol supplementation. However, more detailed studies are needed to know the exact mechanism of chromium-induced membrane damage. The study with varied dose and duration and 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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