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Role of Testosterone in Ameliorating the Cadmium Induced Inhibition of Thyroid Function in Adult Male Mouse

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Cadmium (Cd) has been recognized as one of the toxic metals, widely used in the different industries (Fleischer et al. 1974; Hiatt and Juff 1975). This metal is known to be thyroid inhibitory in rats and mice (Yoshida et al. 1987; Yoshizuka et al. 1991; Gupta et al. 1995). It has also been suggested that Cd induced decrease in serum thyroxine (T_4) is the result of direct inhibition of hormone synthesis in the gland by the metal and the decrease in serum triiodothyronine (T_3) is because of the inhibition of hepatic T_4 to T_3 conversion, the major pathway of T_3 generation (Gupta et al. 1995). It is often claimed that Cd induced cellular damage is the result of alteration in membrane structure caused by peroxidation of composite lipids following the metal administration (Stacey et al. 1980).

Testosterone (17-ß-hydroxy-4-androsten-3-one), the principal male androgen, is produced and secreted largely by testes. This has been shown to have antiperoxidative property against xenobiotics (Jaya et al. 1995). The present experiment was therefore designed to investigate the ameliorating effects of testosterone, if any, against Cd induced peroxidative tissue damage that ultimately leads to thyroid dysfunction (Gupta et al. 1996).

MATERIALS AND METHODS

Twenty eight adult male Swiss mice weighing 25-30 g were housed in temperature (27±1°C) and light controlled (14 hr light :10 hr dark) room with the provision of food (Gold Mohur Mice feed, Hindustan Lever Ltd., Bombay, India) and water *ad libitum.* They were randomly divided into four groups of seven each. Groups 2, 3 and 4 were administered subcutaneously (s.c.) with cadmium chloride ($CdCl_2$, 1.0 mg/ kg body weight, dissolved in distilled water); testosterone propionate (TP, 2.5 mg/ kg body weight, dissolved in corn oil) and equivalent doses of $CdCl_2$ + TP, one after another, respectively every day for 15 days. Group 1, receiving equivalent

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amount of vehicle (corn oil and distilled water) only, served as control. Dose of TP was taken from Kumar and Rana (1988) and Jaya et al. (1995); and that of $CdC1_2$ from Yoshida et al. (1987).

On the last day of experimentation, animals were sacrificed, blood from each was collected, centrifuged and serum samples were stored at -20°C for the estimations of T_4 and T_3 by radioimmunoassay (RIA). The liver was removed quickly, washed thoroughly with phosphate buffered saline (PBS, pH 7.4) and processed for biochemical assays.

The assay of lipid peroxidation (LPO) was done by the method of Ohkawa et al. (1979). Liver was homogenized in ice cold 0.1 *M* phosphate buffer (pH 7.4, 10% wt/volume) and the homogenate was centrifuged at 2000 g at 4°C for 30 min. LPO was expressed as the amount of malondialdehyde (MDA) formed (nmole) per hr per mg protein.

Hepatic superoxide dismutase (SOD) activity was determined by the measurement of the ability of the enzyme to inhibit the autoxidation of pyrogallol according to the method of Marklund and Marklund (1974). One unit of SOD was defined as the enzyme activity that inhibits the autoxidation of pyrogallol by 50%. Catalase (CAT) activity was assayed by the decomposition of hydrogen peroxide (H_2O_2) following the method of Aebi (1983). CAT activity was expressed as µmole of H_2O_2 decomposed per min per mg protein.

For the assay of type-I iodothyronine 5'-monodeiodinase (5'-D), the liver was homogenized in 3 volume (wt/volume) ice cold phosphate buffer (O. 15 *M*, pH 7.4) containing 0.25 *M* sucrose and 5 m*M* ethylenediamine tetra acetic acid (EDTA) using a Potter-Elvehjem glass-teflon homogenizer. The homogenate was centrifuged at 2000 g at 4°C for 30 min. The supernatant was used for the assay of 5'-D according to the method of Kahl et al. (1987) as followed earlier (Maiti et al. 1995). The enzyme activity was expressed as ng of T_3 generated per hr per mg protein.

The hepatic protein content was determined in duplicate using bovine serum albumin (BSA) as the standard by the method of Lowry et al. (1951).

Serum concentrations of total T_4 and T_3 were estimated by RIA following the method of Brown et al. (1970) with little modification (Kar and Chandola-Saklani 1985). RIA for the specific measurement of *in-vitro* generation of T_3 (for the assay of 5'-D) was done according to the method described earlier (Visser et al. 1975).

The data were analyzed subjecting them to analysis of variance (ANOVA) followed

by student's t-test.

RESULTS AND DISCUSSION

All the animals were healthy and active throughout the experiment. Serum T_4, T_3 concentrations and hepatic 5'-D activity were decreased significantly following Cd administration (Fig 1). These observations are consistent with our earlier findings (Gupta et al. 1995; 1996). Other scientists have also reported thyroid inhibitory nature of Cd (Shrivastava and Sathyanesan 1988; Yoshizuka et al. 1991) in mammals. However, no attempt was made earlier to investigate the ameliorating effect of TP, if any.

When TP was treated alone, no significant change was observed in any of the parameters compared to that of control values. But a significant increase in serum T_4, T_3 concentrations and 5'-D activity was observed as compared to Cd treated group, when TP was administered along with Cd. Thus, TP was found to ameliorate the Cd induced thyroid dysfunction in mice. Of course, other than the serum T_4 concentration that was marginally elevated, serum T_3 concentration and 5'-D enzyme activity were still less compared to their respective control values.

While T_4 is synthesized only by the thyroid gland, the most potent thyroid hormone, 3,3',5 triiodothyronine (T_3) is mainly produced peripherally by enzymatic monodeiodination of T_4 (Chopra et al. 1978). Thus, it has been suggested that 5'-D activity is an important control point for regulating the activity of thyroid hormones (Visser 1978) and the alteration in its activity is reflected on the amount of T_4 and T_3 hormones present in the circulation. Interestingly, in the present study testosterone was found to raise the Cd induced decrease in hepatic 5'-D enzyme activity indicating that TP regulates Cd induced changes in T_3 concentration by altering the peripheral conversion of T_4 to T_3.

The hepatic LPO increased significantly in Cd treated mice. On the other hand, when TP was administered alone or with Cd, almost normal LPO value was maintained (Fig 2). In contrast to LPO, the activities of SOD and CAT in liver tissue were decreased significantly following Cd administration. Interestingly, nearly normal activities of these two enzymes were maintained following TP administration. These observations too indicate ameliorating role of TP on Cd toxicity.

LPO is an oxidative deterioration of polyunsaturated lipids that tends to reduce membrane fluidity which is known to be essential for proper functioning of the biological membranes (Halliwell and Gutteridge 1989). Initiation of LPO is a process solely carried out by free radicals causing cell injury (Ashwood-Smith 1975). SOD and CAT enzymes are important scavengers of these free radicals. The

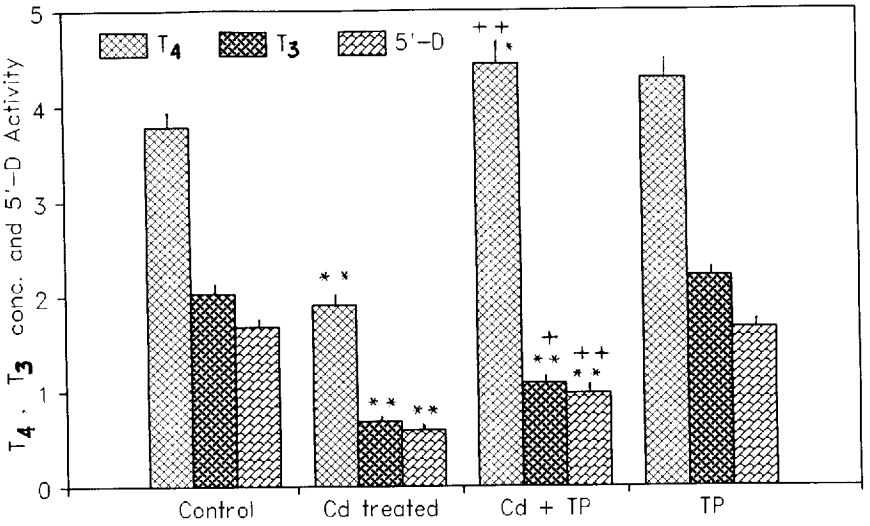

Figure 1. Effect of Cd, Cd + TP and only TP on serum T_4 (µg/ 100 mL), T_3 (ng/mL) concentrations and on hepatic 5'-D activity (ng T_3/ hr /mg protein) in adult male mouse. Vertical lines indicate standard errors of the means. * P<0.01, ** P<0.001 compared to the respective control values; + P<0.0 1, ++ P<0.001 compared to the Cd treated group.

decreased activity of 5'-D enzyme in mice has earlier been suggested to be peroxidation mediated (Huang et al. 1987; Maiti et al. 1995). Thus, in the present study it appears that Cd might have induced free radical generation (Gupta et al. 1996) that in turn decreased 5'-D activity in the tissues hence contributing to the reduced generation of T_3. Earlier free radicals have also been shown to alter the activity of some membrane bound tissue enzymes (Chan et al. 1982). Thus, on one hand, Cd induced inhibition in thyroid function is considered to be peroxidation mediated (Gupta et al. 1996) and on the other hand TP is found to inhibit the LPO. Similar observation has been made by Jaya et al. (1995).

As mentioned earlier, administration of TP to Cd treated mice returned SOD and CAT activities to control values with concomitant decrease in MDA levels. It therefore appears that decreased peroxidative damage to membrane lipids following TP exposure might have contributed to the stimulated 5'-D activity, thus bringing back serum T_3 concentration to near normal level. This concept is further supported by the findings of a previous study in fish that reported increased plasma T_3 concentration following androgen treatment (Hunt and Eales 1979).

Thus, the present study clearly indicates that TP can offer protection from the deleterious effects of Cd toxicity in relation to circulating T_4, T_3 concentrations and 5'-D activity.

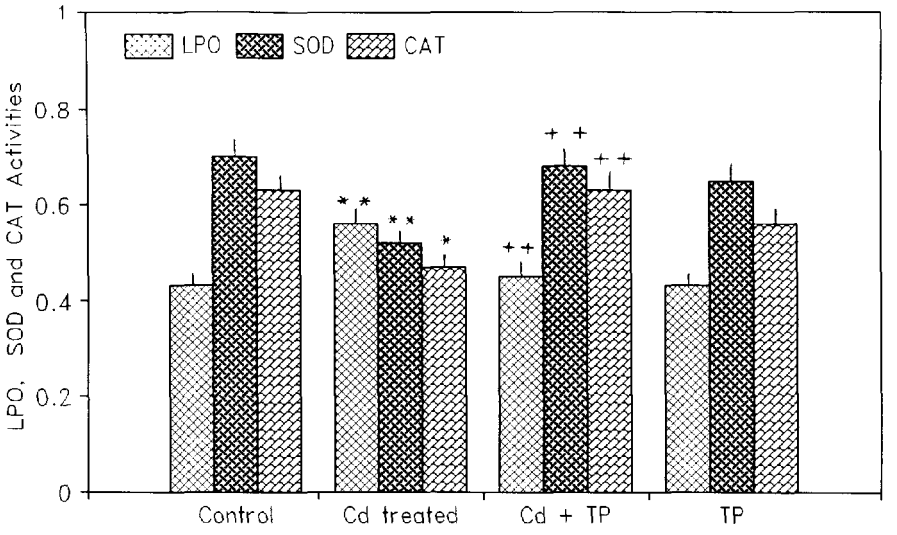

Figure 2. Effect of Cd, Cd + TP and only TP on hepatic LPO (nmole of MDA formed/ hr/ mg protein), SOD (units/mg protein x 10), CAT (µmole of H_2O_2 decomposed/ min/ mg protein x 10^2) activities in adult male mouse. Vertical lines indicate standard errors of the means. * $P<0.01$, ** $P<0.001$ compared to the respective control values; + $P<0.01$, ++ $P<0.001$ compared to the Cd treated group.

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