JOURNAL OF APPLIED TOXICOLOGY
J. Appl. Toxicol. **18**, 317–320 (1998)

Role of Ascorbic Acid in Cadmium-induced Thyroid Dysfunction and Lipid Peroxidation

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Key words: cadmium; ascorbic acid; triiodothyronine; thyroxine; type I iodothyronine 5′-monodeiodinase; lipid peroxidation; superoxide dismutase; catalase; mice.

A study on the effects of ascorbic acid (AA) on heavy metal (cadmium)-induced thyroid dysfunction and lipid peroxidation (LPO) was carried out in Swiss male mice. The animals were administered with either cadmium (1.0 mg kg^{-1} body wt.) alone or in combination with AA (1 mM) every day for 15 days. While cadmium treatment led to a decrease in the serum concentrations of thyroid hormones and hepatic type I iodothyronine 5′-monodeiodinase (5′D-I) activity, an increase in the level of lipid peroxidation was observed. The metal-induced decrease in hepatic 5′D-I activity and serum triiodothyronine (T_3) concentration was restored by treatment with AA. However, AA could not restore the serum thyroxine (T_4) concentration. The increased level of LPO was also ameliorated by AA. It appears that the protective effect of AA against cadmium-induced thyroid dysfunction is mediated through its antioxidative action.

INTRODUCTION

Cadmium (Cd) is a divalent metal toxicant that preferentially becomes localized in hepatocytes and causes liver injury.[1] Involvement of free radical production is associated with Cd toxicity because the peroxidation of membrane lipids is enhanced and intoxication is potentiated by the inhibition of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).[2] On one hand, Cd has been found to inhibit thyroxine (T_4) synthesis and/or its release and to depress type I iodothyronine 5′-monodeiodinase (5′D-I) activity, resulting in a decreased serum triiodothyronine (T_3) level.[3–6] On the other hand, some evidence suggests that membrane damage due to increased lipid peroxidation (LPO) is responsible for the depressed hepatic 5′D-I enzyme activity in the presence of xenobiotics.[4–9]

One of the effective ways of preventing thyroid dysfunction is through the regulation of the peroxidative process in the cells. In fact, we have recently reported that Cd-induced thyroid dysfunction can be ameliorated to some extent by the use of antioxidants such as testosterone propionate and α-tocopherol.[5,6] Ascorbic acid (AA) is known as a potential scavenger of reactive oxygen species (ROS)[10–12] and it may protect the lipids from detectable peroxidative damage induced by aqueous peroxyl radicals.[11] These observations led us to investigate the role of AA, if any, in the regulation of thyroid abnormality in Cd-intoxicated mice.

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CCC 0260–437X/98/050317–04$17.50

Received 4 November 1997
Revised 30 April 1998
Accepted 4 May 1998

EXPERIMENTAL

Reagents and chemicals

Radioimmunoassay (RIA) kits for the estimation of serum concentrations of T_4 and T_3 were supplied by Bhabha Atomic Research Centre, Bombay. L-Thyroxine, L-triiodothyronine and diethylenetriamine pentaacetic acid (DTPA) were purchased from Sigma Chemical Co., USA; TRIS, thiobarbituric acid (TBA) and dithiothreitol (DTT) were from E. Merck India Ltd., Bombay; pyrogallol, ethylenediamine tetraacetic acid (EDTA) and all other chemicals were of reagent grade and were obtained from Loba Chemie, Bombay.

Animals and dosing

Two-month-old colony-bred male Swiss mice (30–35 g) of the departmental animal house were used for this experiment. They were maintained under a controlled temperature (27 ± 1°C) and light schedule (14 h light/10 h dark) and were provided with food (Gold Mohur Mice Feed, Hindustan Lever Ltd., Bombay, India) and water *ad libitum*. The mice were divided randomly into four groups of seven each. The animals of group 2 were administered subcutaneously (s.c.) 0.1 ml of cadmium chloride at the dose of 1.0 mg kg^{-1} body wt. dissolved in distilled water, daily for 15 days, and those of group 3 received 0.1 ml of AA (dissolved in distilled water) only at the dose of 1 mM (nearly equivalent to the human therapeutic dose)[13] on alternate days. Group 4 mice were administered simultaneously with equivalent doses of cadmium chloride and AA. Group 1, receiving an equivalent amount of vehicle alone (distilled water), served as a control. All the treatments were given between 09.00 and 10.00 h to avoid circadian variation.

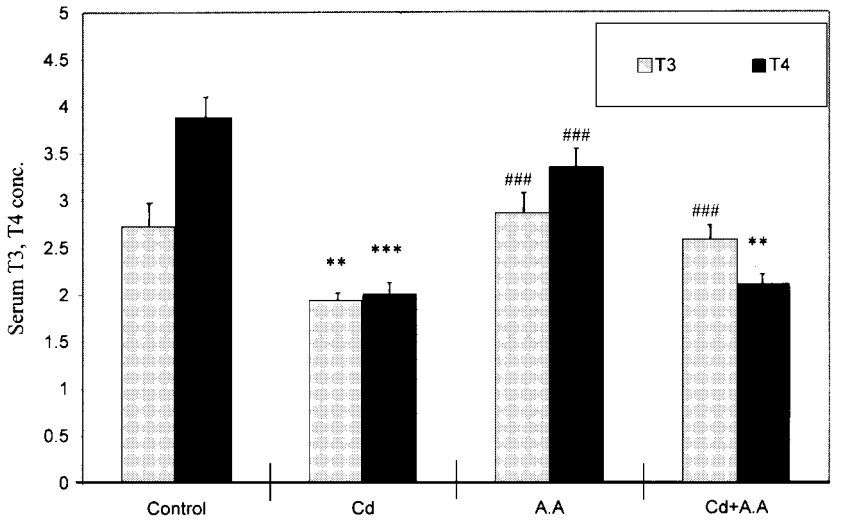

Figure 1. Effects of ascorbic acid (AA) on serum concentrations of T_4 (ng $ml^{-1} \times 10$) and T_3 (ng ml^{-1}) in cadmium-intoxicated mice. Vertical lines indicate standard errors of means.** $P < 0.01$ and *** $P < 0.001$ compared to control group. ### $P < 0.001$ compared to cadmium-treated group.

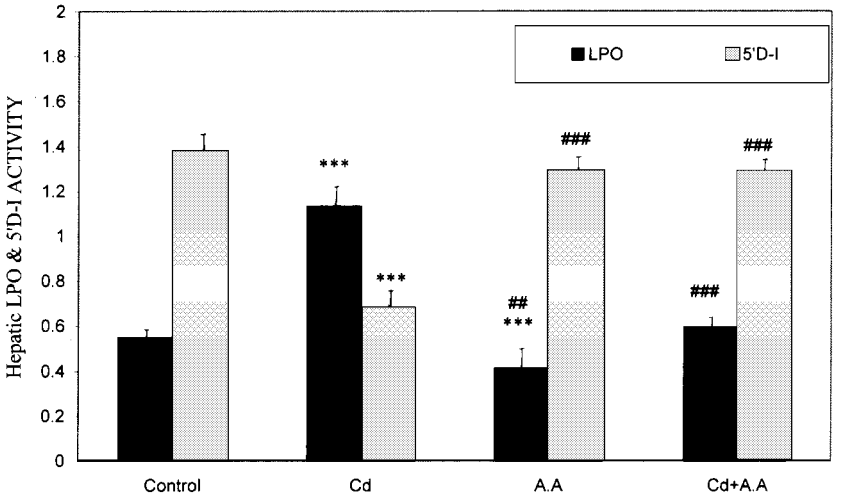

Figure 2. Effects of ascorbic acid (AA) on hepatic 5′D-I activity (ng T_3 generated $h^{-1}\,mg^{-1}$ protein) and lipid peroxidation (nM MDA formed $h^{-1}\,mg^{-1}$ protein) in cadmium-intoxicated mice. Vertical lines indicate standard errors of means. *** $P < 0.001$ compared to control group. ## $P < 0.01$ and ### $P < 0.001$ compared to cadmium-treated group.

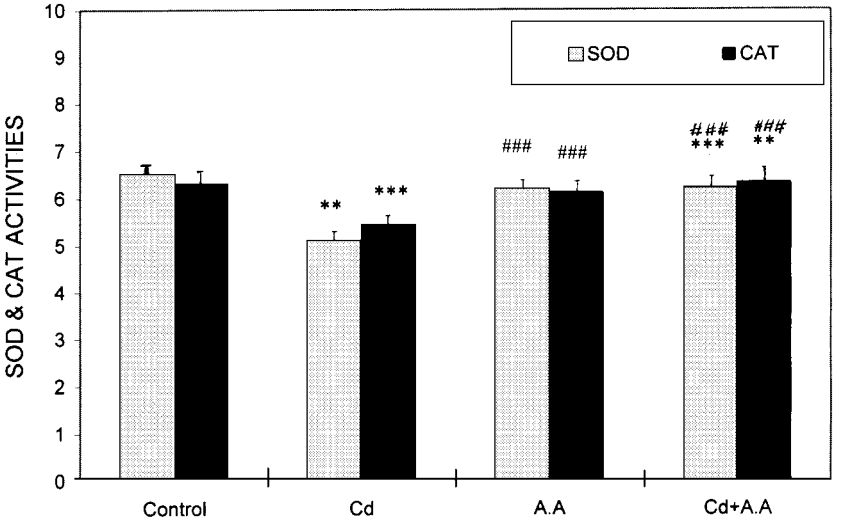

Figure 3. Effects of ascorbic acid (AA) on hepatic activities of superoxide dismutase (SOD, U mg^{-1} protein) and catalase (CAT, μM H_2O_2 decomposed $h^{-1}\,mg^{-1}$ protein $\times$ 10) in cadmium-intoxicated mice. Vertical lines indicate standard errors of means. ** $P < 0.01$ and *** $P < 0.001$ compared to control group. ### $P < 0.001$ compared to cadmium-treated group.

Sample preparation

Twenty-four hours after the last dose, the animals were sacrificed by cervical dislocation. Blood was collected from each mouse and serum samples were stored at –20°C until assayed for T_4 and T_3. The liver was removed immediately, washed thoroughly with phosphate-buffered saline (PBS, pH 7.4) and processed for biochemical assays.

Biochemical assays

The liver was homogenized in three volumes (w/v) of ice-cold PBS (0.15 M, pH 7.4) containing 0.25 M sucrose and 5 mM EDTA using a Potter–Elvehjem glass–teflon homogenizer. The homogenate was centrifuged at 2000 *g* and 4°C for 30 min and the supernatant was used for the assay of 5′D-I following the method described earlier.[14] In brief, the supernatant was incubated with exogenous T_4 (4 μM) for 1 h at 37°C in the presence of DTT (2 mM final concentration). The incubation was terminated with 1 ml of 100% ethanol and kept at –20°C overnight. After 18 h, the samples were centrifuged at 3000 *g* and 4°C for 40 min and the resulting supernatants were kept at –20°C until the *in vitro* generation of T_3, due to the incubation of liver homogenate with exogenous T_4, was measured. Each incubation assay included a blank, in which T_4 was replaced with assay buffer. The 5′D-I activity is expressed as ng T_3 generated h^{-1} mg^{-1} protein.

For the assay of LPO, SOD and CAT the liver was homogenized (10% w/v) in ice-cold PBS (0.1 M, pH 7.4) and the homogenate was centrifuged at 15 000 *g* for 30 min. The LPO was determined in the supernatant by reaction of TBA with malondialdehyde (MDA), a product formed due to the peroxidation of lipids, following the method of Ohkawa *et al*.[15] The amount of MDA was measured by taking the absorbance at 532 nm (extinction coefficient $\epsilon = 1.56 \times 10^5$) using a Shimadzu UV-160A sectrophotometer (Japan).

The activity of hepatic SOD was estimated by measuring the percentage inhibition of pyrogallol autoxidation by the enzyme according to the method of Marklund and Marklund.[16] One unit of SOD is defined as the enzyme activity that inhibits the autoxidation of pyrogallol by 50%.

Catalase activity was estimated following the method of Aebi[17] and was expressed as μM H_2O_2 decomposed min^{-1} mg^{-1} protein.

Hepatic protein content was determined in duplicate using bovine serum albumin (BSA) as the standard following the method of Lowry *et al*.[18]

Assay of hormones

The circulating levels of T_4 and T_3 were estimated by RIA in serum samples using 8-anilino-1-naphthalene sulphonic acid (ANS) following the method of Brown *et al*.[19] with little modification.[20] However, mice hormone-free serum was used in the preparation of hormone standards. The T_4 and T_3 antibodies and labelled hormones were supplied by Bhabha Atomic Research Center, Bombay, India. Lower limit sensitivity was determined to be 0.14 ng ml^{-1} for T_4 and 0.072 ng ml^{-1} for T_3. Interassay variation was less than 5% for both hormones. Specific RIA for the estimation of T_3 in ethanol extract (for the assay of 5′D-I) was according to the method of Visser *et al*.[21]

Statistical analysis

The data were analysed using Student's *t*-test and statistical significance was ascribed at $P < 0.05$.

RESULTS

Administration of Cd decreased serum T_4 and T_3 concentrations and hepatic 5′D-I enzyme activity (Figs 1 and 2). When Cd and AA were administered together thyroid function was almost normalized, as indicated by an increase in serum T_3 concentration and hepatic 5′D-I activity, which were not significantly different from the control values. However, serum T_4 concentration was still significantly less compared to the control value. When AA was administered alone, all the aforesaid parameters remained unaltered and were comparable to the control values but were significantly more compared to those of the Cd-treated group.

Following, administration of Cd, LPO was elevated and SOD and CAT activities were decreased significantly. However, when AA was administered to the Cd-treated animals, LPO decreased and SOD and CAT activities increased significantly compared to the animals treated with Cd alone. When AA was administered alone, it decreased hepatic LPO significantly compared to the values of both control and Cd-treated groups. Both SOD and CAT activities were also significantly more than the values of Cd-treated animals.

DISCUSSION

In the present study, Cd was found to inhibit the thyroid function with respect to serum T_4 and T_3 concentrations and hepatic 5′D-I enzyme activity. These observations are consistent with our previous findings.[4,7,22] Others too have reported the thyroid inhibitory nature of Cd.[23,24] Also, in the Cd-treated group hepatic LPO increased and SOD and CAT activities were decreased, as observed in earlier studies.[25,26]

When AA was administered along with Cd, no change in serum T_3 concentration and hepatic 5′D-I activity was observed compared to the control group, indicating that AA is capable of stimulating the 5′D-I enzyme activity and thus normalizing the serum T_3 concentration. In our earlier studies it was suggested that the decrease in 5′D-I activity is LPO-mediated.[4,7] Interestingly, in the present study AA supplementation not only increased 5′D-I activity but also decreased LPO, suggesting its antiperoxidative property. In fact, some reports have already indicated the antioxidative nature of AA.[10–12] However, as simultaneous administration of Cd and AA did not cause any change in serum T_4 concentration in comparison to mice treated with Cd alone, it appears that AA has no pronounced effect on the thyroid gland, the only organ responsible for T_4 synthesis. This postulation may further be con-

solidated by the fact that administration of AA alone also did not alter the serum T_4 concentration. Yet another possibility is that AA might have stimulated T_4 synthesis/secretion to some extent, which in turn has been utilized as a substrate for the production of T_3, leading to lesser availability in the circulation.

While LPO was decreased by AA alone, no changes in SOD and CAT were observed, suggesting that vitamin C may neutralize free radical species effectively, as has been argued by Yadav *et al.*[27] in relation to alloxan-induced free radical generation.

As postulated by Halliwell and Gutteridge,[28] the mechanism involved in the AA-induced decrease in LPO in Cd-treated mice may be due to the fact that AA reacts rapidly with O_2^- and $HO^\bullet$ to give semidehydroascorbate, which can be further oxidized to dehydroascorbate, the latter product is unstable and breaks down rapidly in a very complex way, eventually producing oxalic and L-threonic acids.

Whatever the mode of action of AA, from our findings it appears that AA may ameliorate the deleterious effects of Cd in relation to circulating serum T_3 concentration and 5′D-I activity. Further investigations on the mode of action(s) of AA on thyroid physiology and biochemistry will certainly be fruitful.

Acknowledgement

Financial support from the University Grants Commission, New Delhi, India is gratefully acknowledged.

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