Effect of antioxidants (vitamin C, E and turmeric extract) on methimazole induced hypothyroidism in rats

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Received 6 June 2001; revised 22 January 2002

The study was to investigate the protective effect of antioxidants against methimazole (MMI) induced hypothyroidism in rats. Male Wiistar rats were fed MMI, MMI plus vitamin C, MMI plus vitamin E and MMI plus turmeric extract (TE) supplemented diet. At the end of the experiments, thyroid weights, thyroxine (T4), triiodothyronine (T3) and cholesterol levels were determined. It was observed that MMI treated rats showed increase in thyroid weights, very low levels of circulating T4, T3 and increased levels of total cholesterol as compared to controls (P < 0.001). However, rats which received Vit. C, Vit. E or TE along with MMI showed reduced weights (38-55 % less) in thyroid glands (P < 0.01), less suppressed T4 and T3 levels (2-6 % and 7-35% respectively) and less increase in total cholesterol levels (19-52 %) which are statistically significant. The data suggest the positive effect of antioxidants on thyroid gland which could be due to direct involvement of antioxidants on thyroid gland.

The free radicals, such as superoxides, peroxides and hydroxyl radicals are known to play an important role and have been identified as major contributors to cell and tissue damage in many disease conditions. In the thyroid gland, relatively high levels of H2O2 are generated particularly in response to thyrotropin. This H2O2 serves as substrate for the thyroperoxidase enzyme which catalyzes the synthesis of thyroid hormones, namely thyroxine and triiodothyronine. However, in hypothyroidism, hormone synthesis being low, the H2O2 may get accumulated. Such H2O2 might destroy the thyroid cells if protective mechanisms are deficient. Recently it was observed that reactive free radicals appear to play a significant role in the development of goiters in hypothyroidism. The study was undertaken to explore the potential benefits of antioxidants for the treatment of hypothyroid conditions. In the present study, antithyroid drug methimazole (MMI) is used to produce hypothyroidism, and the effects of antioxidants such as vit. C, vit. E and turmeric extract examined on control and hypothyroid rats.

Materials and Methods

Five groups of Wistar male rats (12 per group) of 150-200 g body weight were maintained on normal diet (20% protein) which consisted of 70% wheat, 20% Bengal gram, 5% fish meal powder, 4% dry yeast powder, 0.75% refined til oil and 0.25% shark liver oil and water ad libitum. Group I rats were fed normal laboratory diet and served as control. Group II rats were ingested with 1 g methimazole (1-methyl-2-mercaptoimidazole, Sigma Chemicals, USA) per 1 kg normal diet. Group III rats were fed 1 g MMI + 2.5 g vit. C per kg diet (Ascorbic acid, Loba Chemicals Pvt. Ltd., Mumbai). Group IV rats received 1 g of MMI + 2.5 g vit. E per kg diet (α-tocopherol acetate, Loba Chemicals Pvt. Ltd., Mumbai) and Group V rats were fed with 1 g MMI + 10 g turmeric extract (Saiba Industries Pvt. Ltd., Mumbai) per 1 kg normal diet.

Rats were sacrificed at the end of the 15th and 30th days of the experiment. Blood was collected by cardiac puncture under ether anesthesia. Serum was separated and frozen -20°C till analysis. The liver was perfused with cold normal saline through the hepatic portal vein. They were taken out, dried between filter papers to remove excess blood. Liver was homogenized in a glass-teflon homogenizing tube with cold phosphate buffer pH 8.0 for the determination of lipid peroxidation (malondialdehyde content). The thyroid glands were removed, weighed and fixed in 10% neutral buffered formalin. Sections were prepared by routine methods and stained with hematoxylin and eosin for histopathology of thyroid.
Lipid peroxidation assay

The administration of MMI enhanced hepatic MDA content suggesting that this compound produced hepatotoxicity. Hence the value of lipid peroxidation was estimated by determining malondialdehyde content. Malondialdehyde, one of the end products of the free radicals initiating lipid peroxidation was measured using the thioarbituric acid assay as described by Uchiyama and Mihara. Protein levels were determined by the method of Lowry et al. using bovine serum albumin as standard. The results are expressed as nanomole of MDA per mg of liver protein using MDA bis-(diethylacetate) as the standard.

Serum thyroxine (T₄), triiodothyronine (T₃) and total cholesterol

Serum T₄ and T₃ concentrations were measured by radioimmunoassay (using RIA kits from BRIT, Board of Radiation and Isotope Technology, Vashi, Mumbai) with minor modification. Rat serum was used instead of human thyroid hormone free serum to construct the standard curve. Total cholesterol concentrations were determined by standard enzyme colorimetric procedures using commercially available kits from Boehringer Mannheim Diagnostic (Mannheim, Germany).

Data were presented as mean±SD and were statistically analysed by the unpaired Student’s t-test. P-value of <0.05 was considered significant.

Results and Discussion

Free radical production, a natural event in cells, becomes highly dangerous if overproduction occurs. However, a number of antioxidants defence systems are present within the cell, which helps to protect it from the deleterious effects of oxidative stress. The most important chain breaking antioxidant inhibitor of lipid peroxidation is α-tocopherol, a physiological antioxidant and membrane stabilizer. As a major water soluble antioxidant, vit. C is capable of maintaining sulphydryl compounds in a reduced state, particularly in several redox reactions.

The blood levels of T₄, T₃, total cholesterol as well as the weights of thyroid gland and MDA/mg of liver protein in control rats, MMI-induced hypothyroid rats and rats treated with vit. C, vit. E or TE, along with MMI for 15 days and 30 days are shown in Tables 1 and 2 respectively.

### Table 1—Effect of vit. C, vit. E and TE on MMI induced hypothyroid rats for 15 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thyroid wt (mg)</th>
<th>Serum T₄ (µg/dl)</th>
<th>Serum T₃ (ng/dl)</th>
<th>Serum T₃ cholesterol (mg/dl)</th>
<th>Liver MDA (nM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11 ± 1.2</td>
<td>3.7 ± 0.6</td>
<td>51 ± 5.5</td>
<td>64 ± 4.5</td>
<td>1.02 ± 0.32</td>
</tr>
<tr>
<td>MMI</td>
<td>53 ± 6.8</td>
<td>0.76 ± 0.3</td>
<td>12.5 ± 2.9</td>
<td>112 ± 8.2</td>
<td>1.3 ± 0.12</td>
</tr>
<tr>
<td>MMI + vit. C</td>
<td>36 ± 2.2</td>
<td>0.96 ± 0.18</td>
<td>15.7 ± 1.7</td>
<td>105 ± 8.0</td>
<td>0.74 ± 0.21</td>
</tr>
<tr>
<td>MMI + vit. E</td>
<td>37 ± 2.9</td>
<td>0.93 ± 0.2</td>
<td>14 ± 5.4</td>
<td>87 ± 6.1</td>
<td>0.92 ± 0.15</td>
</tr>
<tr>
<td>MMI + TE</td>
<td>35 ± 2.8</td>
<td>0.98 ± 0.36</td>
<td>26 ± 9.9</td>
<td>91 ± 8.4</td>
<td>1.1 ± 0.12</td>
</tr>
</tbody>
</table>

P values: *< 0.001; < 0.01 as compared with control; c < 0.001; d < 0.01 as compared with MMI.

### Table 2—Effect of vit. C, vit. E and TE on MMI-treated hypothyroid rats for one month

<table>
<thead>
<tr>
<th>Groups</th>
<th>Thyroid weights (mg)</th>
<th>Serum T₄ (µg/dl)</th>
<th>Serum T₃ (ng/dl)</th>
<th>Serum T₃ cholesterol (mg/dl)</th>
<th>Liver MDA (nM/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>14 ± 1.8</td>
<td>4.3 ± 0.5</td>
<td>48 ± 10</td>
<td>60 ± 6.8</td>
<td>0.96 ± 0.32</td>
</tr>
<tr>
<td>MMI</td>
<td>63 ± 12</td>
<td>0.7 ± 0.15</td>
<td>6.8 ± 2</td>
<td>131 ± 21</td>
<td>1.27 ± 0.11</td>
</tr>
<tr>
<td>MMI + vit. C</td>
<td>45 ± 4.7</td>
<td>0.78 ± 0.16</td>
<td>9.7 ± 1.7</td>
<td>108 ± 11</td>
<td>0.96 ± 0.24</td>
</tr>
<tr>
<td>MMI + vit. E</td>
<td>37 ± 3.4</td>
<td>0.73 ± 0.2</td>
<td>10.8 ± 4.4</td>
<td>105 ± 14</td>
<td>1.13 ± 0.15</td>
</tr>
<tr>
<td>MMI + TE</td>
<td>36 ± 3.5</td>
<td>0.8 ± 0.36</td>
<td>15 ± 9.9</td>
<td>95 ± 24</td>
<td>0.8 ± 0.07</td>
</tr>
</tbody>
</table>

P values: *< 0.001 as compared with control; b < 0.01; < 0.05 compared with MMI.
A marked increase in thyroid weight was observed in MMI-induced hypothyroid rats when compared to an average weight of 11 mg/rat for controls (Table 1). vit. C, vit. E or TE along with MMI treatment also showed an increase in thyroid weights (MMI-382% vit. C -227%, vit. E -236% and TE-218% increase of controls); \( P<0.001 \). However, these increased weights of thyroid glands were 38-43% less in vit. C, vit. E or TE treated group as compared to the MMI treated group (MMI-100%, vit. C -60%, vit. E-62% or TE-57%) \( P<0.001 \). Similar increase was observed at 30 days of MMI treatment (Table 2). Here the weights of thyroid gland were 47-55% less increased in vit. C, vit. E or TE treatment along with MMI as compared to the MMI treated group (MMI-100%, vit. C -63%, vit. E-47% or TE-45%) \( P<0.01 \).

Thyroid histology was normal in control rats. In contrast, MMI induced obvious thyroid hypertrophy. This hypertrophy was characterized by decrease in follicular size. Follicular lumen disappeared completely with significant increase in cell size. However, slight thyroid hypertrophy was seen in the thyroids of

Fig. 1 — Photomicrographs of thyroid gland in control (a), MMI treated hypothyroid rats (b), MMI + vit. C (c), MMI + vit. E (d) and MMI + TE (e) after 15 days of the treatment.
MMI-treated rats along with either vitamin C, vitamin E or turmeric extract as compared to controls (Fig. 1).

A drastic decrease in both T4 and T3 values in serum was seen in rats given 0.1% MMI for 15 days and 30 days. Serum TSH concentrations could not be decreased in MMI treated rats (74-79% of controls in 15 days and 81-84% of controls in 30 days). These values were also decreased in vit. C, vit. E or TE along with MMI treated rats and were not statistically significant as compared to MMI alone.

MMI treatment resulted in significant reduction of serum T3 concentrations in all groups as compared to controls, \( P<0.001 \) (49 – 75% in 15 days and 69-86% in 30 days). However, there was less suppression of T3 values in vit. C, vit. E and TE groups as compared to MMI group. (MMI- 75%, vit. C-70%, vit. E-72%, TE-49% in 15 days, MMI-86%, vit. C-80%, vit. E-78%, TE-69% in 30 days). Although vit. C and vit. E supplement diet did not improve serum T3 values for 15 days, significant differences in T3 levels was observed in TE treated rats for 15 days. Supplementation of vit. C, vit. E and TE for 30 days showed more significant effect in the T3 values in serum.

Hypothyroidism causes pronounced hypercholesterolaemia\(^9\). In our study rats treated with vit. C, vit. E and TE showed hypocholesterolaemic responses with respect to only MMI treatment. Total cholesterol levels were increased significantly in MMI induced hypothyroidism in rats as compared to controls \( (P<0.01) \). However, vit. C, vit. E and TE treatment resulted in significant reduction of total cholesterol concentration, Tables 1 and 2 (19-52% less in 15 days and 32-51% less in 30 days), as compared to those of treated with MMI only (MMI-100%, vit. C-81%, vit. E-64%, TE-56% in 15 days and MMI -100%, vit. C-68%, vit. E-64% TE 49% in 30 days).

The increased levels of hepatic MDA observed in rats with hypothyroidism as compared to controls, were not statistically significant (Tables 1 and 2).

However, in vit. C, vit. E and TE treatment showed significantly decreased MDA levels in 15 days as compared to the MMI treated group (Table 1). Similar results were not observed after 30 days of treatment. Only TE along with MMI treatment group showed significantly decreased levels of MDA as compared to the MMI treated group (Table 2).

In view of the above results it may be concluded that treatment of hypothyroidism with vit. C, vit. E or TE increased the antioxidant defences of the cells and attenuate injurious effects of reactive oxygen metabolites.

Acknowledgement

The authors thank Dr. (Mrs) R.Chinoy Head Pathology Department, TMH for kindly providing facilities for histopathology of thyroid.

References


