EFFECT OF DIETARY TURMERIC (CURCUMA LONGA) ON IRON-INDUCED LIPID PEROXIDATION IN THE RAT LIVER

A. CH. PULLA REDDY and B. R. LOKESH
Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore-570 013 India

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Abstract—Male Wistar rats were fed a control diet or the control diet supplemented with 1% (by weight) turmeric for 10 wk. In rats injected with 30 mg Fe²⁺/kg body weight, lipid peroxidation was 29 and 35% lower in liver homogenates and microsomes, respectively, of turmeric-fed rats than in those of rats fed the control diet. The activities of superoxide dismutase, catalase and glutathione peroxidase were higher (by 19, 19 and 20%, respectively) in liver homogenates of rats fed the turmeric-containing diet in comparison with the controls. These studies indicate that dietary turmeric lowers lipid peroxidation by enhancing the activities of antioxidant enzymes.

INTRODUCTION
Curcumin is a component of turmeric, a yellow colouring spice which is used routinely in many of the dishes prepared in Asian countries (Govindarajan, 1980). Curcumin is a good antioxidant and inhibits lipid peroxidation in rat liver microsomes, erythrocyte membranes and brain homogenates (Pulla Reddy and Lokesh, 1992; Salimath et al., 1986; Sharma, 1976). Curcumin is also a good scavenger of reactive oxygen species and lowers the formation of inflammatory compounds such as prostaglandins and leukotrienes (Elizabeth and Rao, 1990; Huang et al., 1991; Unnikrishnan and Rao, 1992). Because of these effects, curcumin has been used to lower carrageenan-induced oedema in rats and to give moderate relief of rheumatoid arthritis in human patients (Deodhar et al., 1980; Srihari Rao et al., 1982). These beneficial effects of curcumin may indicate that turmeric has therapeutic potential. Indeed, feeding turmeric to mice and rats inhibits the formation of tumours in response to benzopyrene, 3-methylcholanthrene and 3'-methyl-4-dimethylaminobenzene (Aruna and Sivaramakrishnan, 1992; Polasa et al., 1991).

Recently, turmeric was also shown to contain a water-soluble peptide termed turmerin (Leela Srinivas et al., 1992). Like curcumin, turmerin has also been reported to be a good antioxidant and protects erythrocyte membranes and phospholipid fatty acids from oxidation in vitro (Leela Srinivas et al., 1992).

Turmerin is present in turmeric at levels up to 0.1%, while curcumin constitutes up to 5% (Govindarajan, 1980). In an earlier, unpublished study we demonstrated that inclusion of lipophilic curcumin in diets containing n-6 and n-3 polyunsaturated fatty acids lowered lipid peroxidation in the serum and liver microsomes of rats (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993). However, the effect of turmerin supplementation in the diet on lipid peroxidation is yet to be ascertained. The presence of curcumin and turmerin makes turmeric an excellent dietary antioxidant. However, no studies have been carried out to test the efficacy of turmeric in lowering lipid peroxidation in vivo. Using a model system developed by Tappel and his colleagues for measuring in vivo lipid peroxidation (Hu et al., 1990), we have demonstrated that dietary turmeric lowers lipid peroxidation in rat liver homogenates and microsomes. Further, we have demonstrated that feeding turmeric to rats also modulates the antioxidant enzymes in a manner that favours the lowering of lipid peroxidation.

MATERIALS AND METHODS
Thiobarbituric acid, vitamin E (DL-α-tocophorol), xanthine oxidase, glutathione, glutathione reductase, cumene hydroperoxide, hydrogen peroxide, 2-cellulose, choline chloride and methionine were obtained from Sigma Chemical Co. (St Louis, MO, USA). NADPH, cytochrome c, xanthine, adenosine diphosphate, trichloroacetic acid and EDTA were purchased from Sisco Research Laboratory (Bombay,
Table I. Diet composition

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Amount (g/100 g diet)</th>
</tr>
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<tbody>
<tr>
<td>Casein*</td>
<td>20.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>5.0</td>
</tr>
<tr>
<td>Sucrose</td>
<td>60.0</td>
</tr>
<tr>
<td>Choline chloride</td>
<td>0.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3</td>
</tr>
<tr>
<td>Vitamin mix†</td>
<td>1.0</td>
</tr>
<tr>
<td>Mineral mix‡</td>
<td>3.5</td>
</tr>
<tr>
<td>Groundnut oil</td>
<td>10.0</td>
</tr>
</tbody>
</table>

*Kaira District Milk Producers Cooperative, Anand, India.
†AIN 76 vitamin and mineral mix.
‡In the experimental diet turmeric (mixed in the groundnut oil) was added at a level of 1% (1 g/100 g diet).

India). Ferrous sulphate (FeSO₄·7H₂O) was obtained from Qualigen Fine Chemicals (Bombay, India). Refined groundnut oil and turmeric powder were purchased from a local supermarket.

Animals. Groups of 12 male Wistar rats weighing 70–75 g were fed the purified diets with or without turmeric for 10 wk. The rats had free access to water. The food intake and growth of the animals were monitored at regular intervals.

Diets. The composition of the basal diet is summarized in Table I. The experimental diet (1% turmeric) was prepared by mixing the basal diet thoroughly with turmeric suspended in groundnut oil. Fresh feed was provided daily.

Liver homogenates, microsomes and lipid peroxidation. Six of the rats in each dietary group were injected IP, 1 hr before they were killed, with 30 mg iron as ferrous sulphate in 1 ml saline/kg body weight (Hu et al., 1990). The remaining rats were injected with the same volume of saline alone. The rats were killed by cardiac puncture and the livers were perfused with saline and homogenized in 10 volumes of 0.15 M KCl. The liver microsomes were prepared as described by Lokesh et al. (1981). The washed microsomes were suspended either in 0.15 M KCl or other appropriate buffers as indicated.

The basal and incubated lipid peroxidation levels (Mathias and Dupont, 1979) in liver homogenates and microsomes was measured as thiobarbituric acid reactive substances (TBARS) following the method described by Buege and Aust (1978).

Antioxidant enzymes. Superoxide dismutase was measured by the inhibition of cytochrome c reduction mediated by superoxide anions generated by xanthine–xanthine oxidase and monitored at 550 nm (Flohe and Otting, 1984). One unit of superoxide dismutase was defined as the amount required to inhibit the reduction of cytochrome c by 50%. Catalase activity was assayed according to the method of Aebi (1984) by following the decomposition of H₂O₂ at 240 nm. Glutathione peroxidase activity was determined by NADPH oxidation in a coupled reaction system containing cumene hydroperoxide and oxidized glutathione (Tappel, 1978). Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as reference standard.

Statistical analysis. Significant differences in the experimental values were determined using Student’s t-test. Correlation coefficients were calculated to determine the relationship between TBARS and the activities of protective enzymes (Dowdy and Wearden, 1983).

Fig. 1. Lipid peroxidation in (a) liver homogenate and (b) liver microsomes. Rats were injected with 1 ml saline (bars I and II) or Fe²⁺ (30 mg kg body weight in 1 ml saline; bars III and IV). Lipid peroxidation was measured at zero (bars I and III) or at 60 min (bars II and IV) of incubation as described in Materials and Methods. Rats were fed the control diet (□) or turmeric-supplemented diet (■). Results are means ± SD for six rats per group and those marked with an asterisk differ significantly (Student’s t-test) from the corresponding value for rats fed the control diet (*P < 0.01).
**Lipid peroxidation**

Injection of Fe^{2+} into the rat peritoneum significantly enhanced lipid peroxidation in liver homogenates and microsomes (Fig. 1). However, the levels of thiobarbituric acid reactive substance (TBARS) were lower in rats fed the turmeric-supplemented diet: these levels were 29% lower in the homogenates and 35% lower in the microsomes in comparison with the controls. Rats injected with saline did not show any significant enhancement in TBARS in liver homogenates and microsomes, indicating the importance of Fe^{2+} in the induction of lipid peroxidation in hepatic tissues. This is in agreement with the results published by other workers (Galleano and Puntarulo, 1992; Hu et al., 1990).

**Antioxidant enzymes**

The effect of dietary turmeric on the activities of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase was measured. The activities of superoxide dismutase, catalase and glutathione peroxidase were 19, 19 and 20% higher, respectively, in rats fed the turmeric-containing diet than in those fed the control diet (Table 2). The correlation coefficient between lipid peroxidation and the activities of the antioxidant enzymes is shown in Table 3. These studies indicate that turmeric may lower lipid peroxidation by maintaining higher levels of activities of antioxidant enzymes.

**RESULTS**

There were no significant differences in feed consumption (15 g/day/rat), body weight gain (180 ± 12.5 g) and liver weights (3.6 ± 0.37/100 g body weight) between rats on the control diet and those fed the turmeric-supplemented diet. (These values are combined means ± SD, for six rats/group.)

**Lipid peroxidation**

**Antioxidant enzymes**

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Control diet</th>
<th>Turmeric-supplemented diet</th>
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<tbody>
<tr>
<td></td>
<td>Uninduced rats</td>
<td>Induced rats</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>-0.815</td>
<td>-0.080</td>
</tr>
<tr>
<td>Catalase</td>
<td>-0.456</td>
<td>-0.639</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>-0.788</td>
<td>-0.879</td>
</tr>
</tbody>
</table>

Table 3. Linear regression correlation between lipid peroxidation and activities of antioxidant enzymes in liver homogenates from rats fed control or turmeric-containing diets and injected with saline (uninduced rats) or Fe^{2+} (induced rats).
that whole turmeric can also have a similar inhibitory effect on lipid peroxidation in vitro. However we could not quantitate the individual contributions made by the curcumin and turmerin present in turmeric in lowering lipid peroxidation.

On average, curcumin is present to an extent of 2% in turmeric (Govindarajan, 1980). Based on this value, the rats in the study reported here consumed 3 mg curcumin/day. Our unpublished results (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993) indicated that rats consuming 3 mg curcumin per day showed 23% less inflammation in foot pads in response to carrageenan injection. The macrophages isolated from rats fed 3 mg curcumin/day also showed a decrease of 22% in superoxide anion formation (B. Joe and B. R. Lokesh, unpublished data, 1993).

Turmerin is reported to be present at a level of up to 0.1% in turmeric (Leela Srinivas et al., 1992). From this it was calculated that the rats in our study consumed 0.067 mg turmerin/day. However, we have no reported data on turmerin to compare its efficacy with that of curcumin as an antioxidant when given orally or as a dietary supplement.

Turmeric may lower lipid peroxidation by maintaining the activities of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) at higher levels (Table 2). These enzymes play an important role in the regulation of lipid peroxidation (Harris, 1992). We found similar effects on these antioxidant enzymes in rats fed 1% curcumin in the diet (A. Ch. Pulla Reddy and B. R. Lokesh, unpublished data, 1993). The mechanism by which turmeric or curcumin provides an environment for the enhanced activities of these enzymes is yet to be ascertained. In addition, we have also observed that turmerin can scavenge/quench oxygen free radicals such as superoxide anions and hydroxyl radicals which play an important role in the initiation of lipid peroxidation. Curcumin also prevented the oxidation of Fe

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REFERENCES


