Antioxidative Effects of Turmeric, Rosemary and Capsicum Extracts on Membrane Phospholipid Peroxidation and Liver Lipid Metabolism in Mice

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Phospholipid hydroperoxides (PLOOH) in the plasma, red blood cells (RBC) and liver of mice were measured after dietary supplementation for one week (1% w/w of diet) with a turmeric extract (curcuminoid), hexane extract of rosemary, and supercritical CO2-extracted capsicum pigment (supplemented with α-tocopherol to prevent fading). A lower PLOOH level was found in RBC of the spice extract-fed mice (65-74% of the non-supplemented control mice). The liver lipid peroxidizability induced with Fe2+/ascorbic acid was effectively suppressed by dietary supplementation with the turmeric and capsicum extracts to mice. While no difference in the plasma lipids was observed, the liver triacylglycerol concentration of the turmeric extract-fed mice was markedly reduced to one-half of the level in the control mice. These findings suggest that these spice extracts could act antioxidatively in vivo by food supplementation, and that the turmeric extract has the ability to prevent the deposition of triacylglycerol in the liver.

Key words: antioxidant; spice; erythrocyte; triglyceride; curcuminoid

Many spices have been extensively used as natural food additives for flavoring, seasoning, coloring and antiseptic properties. Several spices are known to exhibit antioxidative activities.1,2 In recent years, the antioxidative property of food constituents has been seriously noted by medical and nutritional experts, since the reactive oxygen species-mediated oxidation of biological molecules has been proposed to induce a variety of pathological events such as atherogenesis,3 carcinogenesis4 and even aging.5

Although many in vitro studies on the antioxidative property of food constituents have been reported, little is known about the biological functions of dietary antioxidants in vivo, except for several well-known antioxidants such as tocopherols, β-carotene and ascorbic acid. Since the bioavailability of food constituents is limited by their digestibility and metabolic fate, an oral administration trial of a dietary antioxidant is favored to evaluate its biological function.

In this study, we examined extracts of turmeric (Curcuma longa L.), rosemary (Rosmarinus officinalis L.) and capsicum (red pepper, Capsicum annum L.) as antioxidative food supplements. These spices contain different types of antioxidants from each other. The rhizome of turmeric has been used as a traditional remedy for treating sprains and inflammation in several Asian countries.6 Curcuminoids (curcumin, demethoxycurcumin and bisdemethoxycurcumin), the phenolic yellowish pigments of turmeric, have been suggested to have antioxidative, anticarcinogenic, anti-inflammatory and hypcholesterolemic activities.6 Several phenolic diterpenes isolated from rosemary have been reported to prevent lipid peroxidation in bulk,7 emulsified,7 liposomal9 and microsomal10 systems. The main constituents of capsicum pigment are hydroxylated carotenoids (xanthophylls).9 Both hydroxylated and non-hydroxylated carotenoids are expected to act as potential membrane antioxidants due to their reactivity with singlet molecular oxygen and peroxyl radicals.10

In the present study to investigate the antioxidative properties in vivo, we determined phospholipid hydroperoxides (PLOOH) as key products for oxidative injury in membranous phospholipid layers in the plasma, red blood cells (RBC) and liver of mice. The formation and accumulation of PLOOH have been confirmed in several cellular disorders11-14 and in aging.15-18 We observed by the dietary supplementation that these spice extracts contributed to preventing the oxidative events in some organelle membranes, and that the turmeric extract effectively suppressed liver lipid deposition in mice.

Materials and Methods

Reagents. Three commercial spice extracts, the turmeric extract (>99% curcuminoid), the hexane-extract of rosemary (containing carnosol (~1.5%) and other phenolic diterpenes), and the supercritical CO2-extracted capsicum pigment (containing capsanthin (~3%) and other carotenoids, and supplemented with α-tocopherol (1.5%) to prevent fading) were obtained from Lion Co. (Tokyo, Japan), and were used without further purification. All other reagents and chemicals used were commercially available extra-pure-grade products.

Animals and diets. Seventy-two male ddY mice (9

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Abbreviations: RBC, red blood cells; PLOOH, phospholipid hydroperoxides; PCOOH, phosphatidylcholine hydroperoxide; PEOOH, phosphatidylethanolamine hydroperoxide; CL-HPLC, chemiluminescence-high performance liquid chromatography; BHT, butylated hydroxytoluene; TBARS, thiobarbituric acid-reactive substances
Table 1. Composition of the Spice Extract-supplemented Diets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Mouse group</th>
<th>Control</th>
<th>Turmeric</th>
<th>Rosemary</th>
<th>Capsicum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cascin</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>α-Cornstarch</td>
<td>45</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Soybean oil*</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Fiber</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>Mineral mixtureb</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td>3.5</td>
<td></td>
</tr>
<tr>
<td>Vitamin mixturec</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>α-Methionine</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Turmeric extract</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Rosemary extract</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
<td>—</td>
<td>1.0</td>
</tr>
<tr>
<td>Capsicum extractd</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* The tocopherol contents of soybean oil were 4.7, 1.0, 33.5 and 9.8 mg/100 g for α-, β-, γ- and δ-tocopherol, respectively.

b AIN-76 mineral mixture (Oriental Yeast Co., Tokyo, Japan).

c AIN-76A vitamin mixture (Oriental Yeast Co., Tokyo, Japan) containing α-tocopherol acetate (500 mg/100 g).

d The capsicum extract contained α-tocopherol (1.5% w/w) to prevent fading.

weeks old; Clea Japan, Tokyo) were acclimatized with commercial rodent feed (F-2; Funabashi Farm Co., Funabashi, Japan) for one week. The mice were randomly divided into four groups (18 mice each) and were fed on a control diet or the spice extract-supplemented diets (Table 1) for one week. During the experimental feeding period, the mice were housed in stainless steel cages (6 mice/cage) and given free access to the diet and drinking water. The body weights of the mice were measured every other day, and the food intakes were measured every day.

After the experimental feeding period, food was withheld overnight. Heparinized blood was withdrawn by heart puncture under light ether anesthesia and collected into sterile tubes from six mice in each group. The livers were excised and rinsed in ice-cold saline. The plasma and packed RBC were prepared as previously described by Nakagawa et al., and the livers were weighed and homogenized with four volumes of ice-cold saline.

During the experiment, the mice received human care consistent with institutional guidelines.

Phospholipid hydroperoxide assay. Phosphatidylcholine hydroperoxide (PCOOH) and phosphatidylethanolamine hydroperoxide (PEOOH) in the plasma, RBC and liver were simultaneously determined by chemiluminescence-high performance liquid chromatography (CL-HPLC), as previously reported by Miyazawa et al. Total lipids of the plasma and liver were extracted with a mixture of chloroform/methanol (2:1, v/v) containing 0.002% (w/v) butylated hydroxytoluene (BHT). The RBC total lipids were extracted with a mixture of 2-propanol and chloroform containing 0.002% (w/v) BHT. An aliquot of the total lipid sample was submitted to the CL-HPLC assay.

Evaluation of the in vitro peroxidizability of liver lipids. An aliquot of the liver homogenate was incubated with 0.1 mm ferrous sulfate and 0.3 mm ascorbic acid for 30 min at 37°C. PLOOH and thiobarbituric acid-reactive substances (TBARS) were measured before and after the incubation. PLOOH was measured by CL-HPLC as already described. TBARS were measured by the colorimetric method of Okawa et al. with 0.01% BHT in the assay mixture. The content of TBARS is expressed as the malondialdehyde equivalent.

α-Tocopherol determination. α-Tocopherol in the plasma, RBC and liver was determined by HPLC with fluorometric detection. α-Tocopherol was extracted with 5.0 ml of n-hexane from the plasma (0.2 ml), packed RBC (0.3 ml), and liver homogenate (1.0 ml) after adding 2.0 ml of ethanol containing 2,2,5,7,8-pentamethoxy-6-hydroxychroman as an internal standard. In the case of RBC, esterified α-tocopherol was saponified with potassium hydroxide before extraction.

Lipid analyses of the plasma and liver. Phospholipids, triacylglycerols and total cholesterol of the plasma and liver were colorimetrically determined by Phospholipid-test, Triglyceride-E-test and Cholesterol-E-test kits (Wako Pure Chemical Co., Osaka, Japan), respectively. For the liver lipid analysis, the liver total lipids were dissolved in Triton X-100 before colorimetric assays of the triacylglycerols and cholesterol according to the method of Carr et al.

Statistical analyses. Each data value is expressed as the mean value and standard deviation (SD). Student’s t-test was used for a statistical analysis between the control diet group and each experimental diet group.

Results

No differences in the food intake and body weight gain were observed among the four dietary groups of mice after one week of experimental feeding. The food intake (mean ± SD) for the control, turmeric, rosemary, and capsicum extract-fed mice was 4.7 ± 0.6, 4.5 ± 0.2, 4.2 ± 0.1 and 4.2 ± 0.3 g/day/mouse, and the body weight gain (mean ± SD) was 3.4 ± 0.5, 1.6 ± 1.1, 2.0 ± 0.7 and 2.8 ± 2.2 g/week/mouse, respectively. No difference in the liver weight was apparent among the four dietary groups (data not shown).

The CL-HPLC assay showed PCOOH to be the predominant phospholipid hydroperoxide present in mouse plasma (Table 2). On the other hand, both PCOOH and PEOOH were detected as major types of PLOOH in RBC and liver. In RBC, a lower level of PLOOH (65–74% of the control) was shown in the spice extract-fed mice. Changes in the PLOOH levels of the plasma and liver of the spice extract-supplemented mice were not significantly different from those of the control mice.

The liver susceptibility to in vitro lipid peroxidation was strongly influenced by the dietary supplements given to the mice. Accumulations of PLOOH and
Table 2. Phospholipid Hydroperoxide Contents in the Plasma, Red Blood Cells (RBC) and Liver of Mice Fed with the Spice Extract-supplemented Diets for One Week

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Plasma</th>
<th>RBC</th>
<th>Liver</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOOH</td>
<td>PEOOH</td>
<td>PCOOH</td>
</tr>
<tr>
<td>Control</td>
<td>78±7</td>
<td>n.d.</td>
<td>70±13</td>
</tr>
<tr>
<td>Turmeric</td>
<td>76±10</td>
<td>n.d.</td>
<td>49±2</td>
</tr>
<tr>
<td>Rosemary</td>
<td>77±10</td>
<td>n.d.</td>
<td>52±5</td>
</tr>
<tr>
<td>Capsicum</td>
<td>89±4</td>
<td>n.d.</td>
<td>51±7</td>
</tr>
</tbody>
</table>

Each value is the mean±SD (n=3 for six pooled mice).
* Significantly different from the control group (p<0.05).

n.d., not detected (<1 pmol/ml).
PCOOH, phosphatidylcholine hydroperoxide; PEOOH, phosphatidylethanolamine hydroperoxide.

Fig. 1. Peroxidizability of Liver Lipids in the Spice Extract-fed Mice.
Phospholipid hydroperoxides (PLOOH: A) and thiobarbituric acid-reactive substances (TBARS: B) in the liver homogenates were measured before (0 min) and 30 min after Fe²⁺/ascorbic acid-induced lipid peroxidation as described in the Materials and Methods section. Each value is the mean±SD (n=3 for six pooled mice).
*Significantly different from the control group after the 30-min reaction (p<0.05). PLOOH, combined value for the hydroperoxides of phosphatidylcholine and phosphatidylethanolamine.

TBARS during Fe²⁺/ascorbic acid-induced lipid peroxidation were effectively suppressed by dietary supplementation of the turmeric and capsicum extracts (Fig. 1). In contrast, no difference was apparent between the rosemary extract-fed mice and the control mice in their in vitro liver lipid peroxidizability.

The α-tocopherol concentrations in the plasma, RBC and liver of the rosemary extract-fed mice were significantly lower than those of the control mice (Table 3). The RBC α-tocopherol level of the turmeric extract-fed mice was also lower than that of the control mice (Table 3). However, the plasma α-tocopherol level of the capsicum extract-fed mice was significantly higher than that of the control mice.

While no difference was apparent in the plasma lipid contents among the four dietary groups, the liver lipid contents were greatly influenced by turmeric extract supplementation to the mice (Table 4). The triacylglycerol and cholesterol contents in the liver of the turmeric extract-fed mice were significantly lower (45% and 82% of the control value for the triacylglycerol and cholesterol contents, respectively), the triacylglycerol reduction being especially notable.

Discussion
Several minor compounds in legumes and vegetables have been shown to prevent the peroxidative degeneration of plasma lipoproteins and tissue organelles additively or synergistically with endogenous antioxidants. The major candidates for these dietary antioxidants are phenolic compounds and carotenoids (14,22,30-33).

In this study, dietary supplementation with spice extracts was used to demonstrate that the intact PLOOH level (the combined values of PCOOH and PEOOH) of RBC was kept as low as 65–74% equivalence compared to that of the control mice (Table 2). Such an antioxidative effect of dietary spice extracts was confirmed only on the RBC membrane, and not on the plasma or liver. In our previous study, the specific antioxidative effect on
Table 4. Phospholipid (PL), Triacylglycerol (TG) and Total Cholesterol (Cho) Contents in the Plasma and Liver of Mice Fed with the Spice Extract-supplemented Diets for One Week

<table>
<thead>
<tr>
<th>Mouse group</th>
<th>Plasma</th>
<th></th>
<th></th>
<th>Liver</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PL</td>
<td>TG</td>
<td>Cho</td>
<td>PL</td>
<td>TG</td>
<td>Cho</td>
</tr>
<tr>
<td>Control</td>
<td>301±33</td>
<td>176±7</td>
<td>181±6</td>
<td>17.0±1.3</td>
<td>39.9±2.5</td>
<td>3.8±0.2</td>
</tr>
<tr>
<td>Turmeric</td>
<td>323±57</td>
<td>165±20</td>
<td>224±62</td>
<td>20.9±1.0*</td>
<td>18.1±4.0*</td>
<td>3.1±0.3*</td>
</tr>
<tr>
<td>Rosemary</td>
<td>327±28</td>
<td>198±32</td>
<td>180±25</td>
<td>17.9±1.0</td>
<td>28.7±11.5</td>
<td>3.5±0.2</td>
</tr>
<tr>
<td>Capsicum</td>
<td>364±24</td>
<td>153±27</td>
<td>208±29</td>
<td>17.7±0.8</td>
<td>28.4±7.1</td>
<td>3.7±0.4</td>
</tr>
</tbody>
</table>

Each value is the mean±SD (n=3 for six pooled mice).
* Significantly different from the control group (p < 0.05).

the RBC membrane was also demonstrated in mice by dietary supplementation with β-carotene. 22) Although the inhibitory effect of spice extracts on PLOOH formation in the RBC membrane was not as potent as that observed with β-carotene, 22) it is interesting that the antioxidative effect observed in this present study was brought about by short-term feeding (one-week supplementation) with the spice extracts. RBC is rich in polyunsaturated fatty acids in the phospholipid bilayer, and involves higher concentrations of molecular oxygen and ferrous ion as constituents of oxyhemoglobin. The oxidation of hemoglobin accompanies the formation of superoxide that is the source of more reactive oxygen species. 24) Therefore, RBC membrane phospholipids would be more susceptible to peroxidation than other organelle membranes, although being protected by several potent endogenous antioxidative systems such as superoxide dismutase, catalase, glutathione peroxidase and α-tocopherol. 25) The age-related accumulation of PLOOH in human RBC has been confirmed. 26)

As shown here, liver lipid peroxidizability was effectively suppressed in mice by dietary supplementation with the turmeric and capiscum extracts (Fig. 1). The findings suggest that these dietary spice constituents and/or their metabolites can act as antioxidants in the liver. Although the basal levels of PLOOH and TBARS in the liver were not changed by spice supplementation (Table 2 and the data given at 0 min in Fig. 1), dietary supplementation is recognized to contribute to increasing the antioxidative potential of organelle membranes of the liver.

As shown in Table 3, the α-tocopherol levels in the plasma, RBC and liver of the rosemary extract-fed mice were unexpectedly lower than those of the control mice, although the reason is not clear. However, in the rosemary extract-fed mice, the intact PLOOH levels in the plasma, RBC and liver were no higher than the levels in the control mice (Table 2), and the in vitro lipid peroxidizability of the liver was almost equivalent to that of the control mice (Fig. 1). These findings suggest that some components in the rosemary extract and/or their metabolites acted as antioxidants in the liver and partially made up for the loss of α-tocopherol. A similar phenomenon may be expected for the antioxidative effect on RBC observed in the turmeric extract-fed mice (Tables 2 and 3).

On the other hand, the plasma α-tocopherol level of the capsicum extract-fed mice was higher than that of the control mice (Table 3), which is ascribed to a diet high in tocopherols (twofold higher than in the other three diets: see the footnote in Table 2). Therefore, the antioxidative effect observed in the capsicum extract-fed mice would have involved the additive or synergistic action between α-tocopherol and carotenoids contained in the capsicum pigment. To evaluate clearly the antioxidative effect of carotenoids in the capsicum pigment, an α-tocopherol content-matched diet group will be required.

Dietary supplementation with the turmeric extract significantly reduced the triacylglycerol and cholesterol contents in the liver (Table 4). Since a triacylglycerol accumulation in the liver is accompanied by biochemical modification of the mitochondrial and endoplasmic functions, 30) the present findings may partially support the usefulness of turmeric in preventing liver disorders. 6) Since, in the present study, curcuminoids (> 99%) were the predominant constituent of the turmeric extract (see the Materials and Methods section), such action as preventing the deposition of triacylglycerols in the liver should be provided with the curcuminoids. Until now, the reduction of liver triacylglycerols with curcuminoids has been reported only in streptozotocin-induced diabetic rats, 37) but its mechanism has remained unexplained. A similar reducing effect on the liver cholesterol level with curcuminoids has been reported for cholesterol-fed rats 38) and diabetic rats. 37) Babu and Srinivasan 37) have suggested that such effect with curcuminoids could be mediated by stimulation of hepatic cholesterol-7α-hydroxylase activity. On the other hand, Yasni et al. 39) have observed that the liver lipid composition of exogenous hypercholesterolemic rats was not altered by the curcuminoid intake. These controversial results are probably due to disparities in the animals examined and to the diet compositions.

In conclusion, the present study has demonstrated that a turmeric extract (curcuminoid), a hexane extract of rosemary, and the α-tocopherol-supplemented capsicum pigment exhibit their antioxidative effects in vivo by dietary supplementation, and that the turmeric extract had the ability to reduce liver triacylglycerol deposition as well as cholesterol in mice. Further research into the effective dose, chronic effect and bioavailability are required to confirm the nutritional and therapeutic importance of the spices and spice extracts.

Acknowledgment
The spice extracts presented by Lion Co. (Tokyo,
Japan) are gratefully acknowledged. This work was supported by Grant-in-Aid from Yamazaki Spice Foundation.

References


