Effects of Sesamin and α-Tocopherol on the Production of Chemical Mediators and Immunoglobulins in Brown-Norway Rats

Jiong-Yan GU, Michiko NONAKA, Koji YAMADA, Kazunari YOSHIMURA, Mikako TAKASUGI, Yuji ITO, and Michihito SUGANO

Laboratory of Food Science, Department of Food Science and Technology, Kyushu University School of Agriculture, Higashi-ku, Fukuoka 812, Japan

Received May 2, 1994

This study investigated the effects of sesamin and α-tocopherol on eicosanoid production and immune function in the rat. Male Brown-Norway rats, 4 weeks of age, were given either control, 0.5% sesamin, 0.5% α-tocopherol, or 0.5% sesamin plus 0.5% α-tocopherol diets for 3 weeks. When sesamin and tocopherol were given together, the proportion of 20:4n-6 phosphatidylcholine (PC) was significantly lowered in liver and lung, while that of 18:2n-6 and 20:3n-6 was significantly increased in liver. Simultaneous administration of these compounds significantly reduced the production by the lung of leukotriene C4 (LTC4), but there was no effect on the splenic LTC4 production and plasma prostaglandin E2 concentration. Sesamin and tocopherol, either separately or in combination, significantly reduced both the proportion of splenic CD4+ and CD8+ T cells, but the change in the CD4+/CD8+ ratio was not significant. The effects of sesamin and tocopherol feeding on serum levels of IgA, IgE, and IgG were not so marked, but tocopherol significantly decreased the serum IgM level. These results suggest that the feeding of sesamin and tocopherol suppress LTC4 production through the decrease in the arachidonic acid level in lung PC.

Sesamin, a lignan from sesame oil, has been known to exhibit diverse physiological functions in addition to its antioxidant activity. Shimizu et al. reported that sesamin specifically interferes with the metabolism of essential fatty acid at the step catalyzed by Δ5-desaturase in microorganisms. Since such a regulation also occurs in rats, there is the possibility that, in contrast to the possible reduction of the eicosanoid production from arachidonic acid, the production of the 1-series prostaglandin from dihomo-γ-linolenic acid would be stimulated. Prostaglandin E2 has various favorable physiological functions. Alpha-tocopherol also appears to suppress eicosanoid production from arachidonic acid when consumed at a high level.

These observations indicate a possible role of sesamin and tocopherol in the production of chemical mediators. It is therefore interesting to know the interaction of these compounds on the production of eicosanoids involved in the immune function. In this study, we compared the changes in chemical mediator and immunoglobulin levels after administration of sesamin and tocopherol, either separately or in combination, in immunoresponsive Brown-Norway rats.

Materials and Methods

Animals and diets. Four-week-old male Brown-Norway rats (Seiwa Experimental Animals, Fukuoka) weighing an average of 96 g were given free access to one of the four experimental diets; control, sesamin-added, tocopherol-added, and sesamin and tocopherol-added diets. The basal diet according to the recommendation of the American Institute of Nutrition contains following ingredients by weight percent: casein, 20; corn oil, 5; AIN mineral mixture, 3.5; AIN vitamin mixture, 1; choline bitartrate, 0.2; DL-methionine, 0.3; corn starch, 15; cellulose, 5; and sucrose to 100. Sesamin and α-tocopherol were added at the 0.5% level at the expense of sucrose. Sesamin, 99.5% purity as total lignans analyzed by HPLC, was a gift from Suntoy Ltd., Osaka and was an equiweight mixture of sesamin and episesamin. Alpha-tocopherol was the product of Eisai Co., Tokyo. Mineral and vitamin mixtures were purchased from Oriental Yeast Co., Tokyo. Body weight and food intake were recorded every other day. After 3 weeks of feeding, the rats were killed by withdrawing blood from the abdominal aorta in a syringe containing Na2-citrate under light diethyl ether anesthesia. Liver, lung, and spleen were immediately excised, blotted, and weighed.

Analyses of plasma, liver, and lung fatty acids. Plasma, liver, and lung lipids were extracted by the method of Folch et al. and phosphatidylcholine (PC) was separated by thin-layer chromatography (TLC) with chloroform/methanol/water (65:25:4, v/v/v) as a developing solvent. The fatty acid composition of PC was analyzed as methyl esters by gas chromatography on a SALLR 10C column as described previously.

Measurements of eicosanoids. A sample of the spleen and lung was cut off and immediately homogenized in 2ml of phosphate-buffered saline (PBS, pH 7.2). The homogenates were incubated in the same buffer at 37°C for 30 min. LTC4 was extracted by the method of Moqbel et al. and measured by radioimmunoassay using a commercial kit (NEK-030, New England Nuclear, Boston, MA). Plasma prostaglandin E2 (PEG2) was also measured by radioimmunoassay (NEK-020, New England Nuclear, Boston, MA). Eicosanoids were measured under the linear relationship with respect to the tissue weight and incubation time.

Measurements of plasma immunoglobulins. Immunoglobulins (Igs) were measured by sandwich ELISA. Goat anti-rat IgA, rabbit anti-rat IgG (Fab') 2, goat anti-rat IgM (these from BioSoft, Paris), and mouse anti-rat IgA (Zymed Lab, San Francisco, CA) were used to fix respective Igs. These antibodies were diluted 1000 times with 30 mm carbonate buffer (pH 9.6), and 96-well plates were treated with 100 μl of each solution for 1 h at 37°C. After blocking with 300 μl of blocking solution (Block Ace, Dainihon Pharmaceutical Co., Osaka) for 1 h at 37°C, each well was treated with 100 μl of the culture supernatant for 1 h at 37°C. Then bound IgE was detected by reacting with biotin-conjugated mouse anti-rat IgE (2000 times diluted, Bethyl, Montgomery, TX) followed by POD-conjugated avidin (5000 times diluted, Dakopatts) for 1 h at 37°C. Bound IgA was detected by reacting stepwise with 100 μl of POD–mouse anti-rat IgA (1000 times diluted, Zymed Lab.), IgG with 100 μl of POD–conjugated rabbit anti-rat IgG (Fab') 2 (2000 times diluted, BioSoft) for 1 h at 37°C and

To whom correspondence should be addressed.
bound IgM with 100 µl of POD-conjugated goat anti-rat IgM (1000 times diluted, Zymed Lab). The wells were rinsed three times with 0.05% Tween 20 in PBS between each step. After incubating at 37°C for 15 min with 100 µl of the substrate solution, the reaction was stopped by adding 100 µl of 1.5% oxalic acid, and A_{415} was measured with a MPR-A_{415} ELISA reader (Tohso, Tokyo).

Analyses of CD4+ and CD8+ T cells. The spleen was excised from the rats and the spleen cells were squeezed out into RPMI 1640 medium (Nissui Pharmaceutical Co., Tokyo). After incubating the cells at 37°C for 30 min to remove fibroblasts, 5 ml of the cell suspension was layered on 4 ml of Lympholyte-Rat (Cedarlane, Hornby) and centrifuged at 1500 x g for 30 min. The lymphocyte band at the interface was recovered and the cells were rinsed three times with the RPMI1640 medium. Then the cells were resuspended in PBS containing 10% fetal bovine serum (FBS, Gibco, Grand Island, N.Y.). The cell concentration was adjusted to 1 x 10^6 cells/100 µl, and 5 µl of either CD4-FTC or CD8-PE monoclonal antibodies (Serotec Ltd., Kidlington, Oxford) was added. After incubation for 30 min at 4°C, lymphocytes were rinsed three times with PBS containing 10% FBS and centrifuged at 1200 rpm for 5 min. The stained lymphocytes were fixed by 2% paraformaldehyde and were counted by flow cytometry (Epics Plofile II, Coulter Electronics Ltd., Luto, Beds).

Statistical analysis. Data were analyzed by ANOVA followed by Duncan’s new multiple-range test to evaluate significant differences of the means. 18

Results

Growth parameters and tissue weights

As shown in Table I, food intake and body weight gain were similar in the four groups of rats. However, sesamin significantly increased liver weight, and this effect was not totally ameliorated by tocopherol, which did not increase the liver weight. There were no differences in spleen and lung weights in the different groups.

Table I. Effect of Sesamin and a-Tocopherol on Food Intake, Growth, and Tissue Weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Tissue weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>95 ± 4</td>
<td>172 ± 6</td>
<td>13.8 ± 0.3</td>
</tr>
<tr>
<td>Sesamin</td>
<td>96 ± 2</td>
<td>171 ± 2</td>
<td>13.6 ± 0.4</td>
</tr>
<tr>
<td>a-Tocopherol</td>
<td>96 ± 2</td>
<td>163 ± 5</td>
<td>13.3 ± 0.3</td>
</tr>
<tr>
<td>Sesamin + a-tocopherol</td>
<td>96 ± 3</td>
<td>166 ± 8</td>
<td>12.8 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6-8 rats.

a,b Values without the same superscript letter are significantly different at P < 0.05.

Fatty acid compositions of plasma, liver, and lung phosphatidylcholine

As shown in Table II, sesamin alone modified the fatty acid spectrum of liver phosphatidylcholine (PC), and there was a significant reduction of 18:2n-6 and a significant increase in 20:4n-6. Consequently, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, an index of linoleic acid desaturation, increased significantly. Tocopherol increased the proportion of 18:2n-6, but not 20:4n-6. When these two compounds were given together, the proportion of 20:4n-6 was significantly lowered and that of 18:2n-6, 20:3n-6, and 22:6n-3 increased significantly compared to the corresponding values in control rats. Consequently, the desaturation index for 18:2n-6 was reduced significantly. This reduction was also observed in plasma PC (data not shown). In lung PC, the proportion of 20:4n-6 was significantly lowered in rats given sesamin and tocopherol, but the increases in 18:2n-6 and 20:3n-6 were not significant.

Eicosanoid production

As shown in Fig. 1A, the simultaneous administration of sesamin and tocopherol significantly reduced the production by the lung of LTC_4, but there was no effect on the splenic production of LTC_4 (Fig. 1B). The plasma PGE_2 concentration was not influenced by dietary manipulations (Fig. 1C).

Populations of spleen CD4+ and CD8+ T cells

Sesamin and tocopherol, either individually or in combination, significantly reduced the proportion of both CD4+ and CD8+ T cells in the spleen (Table III). The CD4+/CD8+ ratio tended to increase after dietary manip-

Table II. Effect of Sesamin and a-Tocopherol on Fatty Acid Composition of Liver and Lung Phosphatidylcholine

<table>
<thead>
<tr>
<th>Tissue and group</th>
<th>Fatty acid (weight %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>14:0</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.4</td>
</tr>
<tr>
<td>Sesamin</td>
<td>0.2</td>
</tr>
<tr>
<td>a-Tocopherol</td>
<td>0.4</td>
</tr>
<tr>
<td>Sesamin + a-Tocopherol</td>
<td>0.3</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.7b</td>
</tr>
<tr>
<td>Sesamin</td>
<td>2.8b</td>
</tr>
<tr>
<td>a-Tocopherol</td>
<td>4.0b</td>
</tr>
<tr>
<td>Sesamin + a-Tocopherol</td>
<td>5.5b</td>
</tr>
</tbody>
</table>

Values are means of 6 rats.

a,b Values without the same superscript letter are significantly different at P < 0.05.
Sesamin, Tocopherol, and Immunoresponse in Rats

1857

Fig. 1. Effects of Sesamin and α-Tocopherol on LTC₄ Production in Rat Lung (A) and Spleen (B) and the Concentration of Plasma PGE₂ (C). Means ± SE of 6 rats. Values without a common letter are significantly different at p < 0.05. Cont; control; Ses; sesamin; Toc, α-Tocopherol.

Fig. 2. Effects of Sesamin and α-Tocopherol on Immunoglobulin Concentrations in Rat Plasma. Means ± SE of 6 rats. Values without a common letter are significantly different at p < 0.05. Cont; control; Ses; sesamin; Toc, α-Tocopherol.

Table III. Effects of Sesamin and α-Tocopherol on Splenic T Cell Subsets

<table>
<thead>
<tr>
<th>T cells</th>
<th>Groups (positive cells %)</th>
<th>Control</th>
<th>Sesamin</th>
<th>α-Tocopherol</th>
<th>Sesamin + α-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4⁺</td>
<td></td>
<td>41.1 ± 3.3a</td>
<td>33.4 ± 1.7b</td>
<td>32.6 ± 1.6b</td>
<td>37.9 ± 2.0b</td>
</tr>
<tr>
<td>CD8⁺</td>
<td></td>
<td>9.6 ± 0.6a</td>
<td>7.4 ± 0.5b</td>
<td>7.1 ± 0.5b</td>
<td>7.3 ± 0.2b</td>
</tr>
<tr>
<td>CD4⁺/CD8⁺</td>
<td></td>
<td>4.3 ± 0.1a</td>
<td>4.6 ± 0.2b</td>
<td>4.6 ± 0.4b</td>
<td>5.2 ± 0.4b</td>
</tr>
</tbody>
</table>

Values are means ± SE of 8 rats. 
ab Values without the same superscript letter are significantly different at p < 0.05.

Discussion

This study demonstrated that sesamin and tocopherol specifically influenced eicosanoid production. Sesamin, a major lignan constituent contained exclusively in sesame oil, has been shown to have diverse physiological functions including modulation of polyunsaturated fatty acid metabolism and eicosanoid production,²¹ in addition to its various applications. Tocopherol also modifies eicosanoid production and the immune functions.²⁰ Although Shimizu et al. and Hirose et al. observed that sesamin interferes with the desaturation of linolenic acid in Sprague-Dawley rats, in particular at the steps catalyzed by Δ5-desaturase, the change in the polyunsaturated fatty acid spectrum in tissue PC was not necessarily clear in Brown-Norway rats. In contrast, the combination of sesamin with tocopherol showed an evident interaction with various parameters including the fatty acid profile.

Tocopherol appears to potentiate the immune function, possibly by inhibiting the oxidation of arachidonic acid.²²,²³ Although information regarding the effect of sesamin on the immune function is scarce, Hirose et al. showed that sesamin stimulates the activity of the monocytes in peripheral blood of rats as judged by the MTT assay. We demonstrated that tocopherol, when simultaneously administered with sesamin, significantly reduced the proportion of arachidonic acid in liver and lung PC. The feeding of tocopherol and sesamin significantly reduced LTC₄ production in lung, but not in spleen.
Both LTC₄ and PGE₂ are the typical chemical mediators, and are involved in the allergic response. The reduced production of LTC₄ in the lung seems to be in favor of mitigating the allergic response, since the LTC₄ not only has a potent bronchoconstricting effect but also increases vascular permeability and hence promotes mucus secretion in the lung, these are all characteristic features of bronchial asthma. Thus, the combination of sesamin and tocopherol is at least one possible approach to improve these disorders. However, the effect on splenic LTC₄ and plasma PGE₂ concentration was not altered by this dietary manipulation.

T-lymphocytes and IgE are important in the allergic response. We showed that the proportion of CD4⁺ helper T cells was lower in rats given sesamin and/or tocopherol than control rats, and that of CD8⁺ suppressor T cell was also decreased by dietary manipulations. However, the CD4⁺/CD8⁺ ratio remained unchanged. IgE is the antibody causing anaphylaxis, where as IgA and IgG suppress allergic reactions, through inhibition of allergen absorption by IgA and competition with IgG by IgG. We showed here that sesamin increased the plasma IgG and IgE levels but the effect was canceled by tocopherol. On the other hand, tocopherol significantly decreased serum IgM level irrespective of the presence of sesamin. These results suggest that sesamin and tocopherol affect serum Ig levels, but their effect on allergy reaction seemed to be neutral in respect to Ig production.

In conclusion, sesamin and tocopherol in combination exerted various effects which were not necessarily attained individually. The possible beneficial effect of the combined use for the prevention or amelioration of immune disorders was suggested. Further study on the effective dose level and the mechanism causing these effects deserves consideration.

Acknowledgments. This study was supported in part by a Grant-in-Aid for Scientific Research C (No. 05660143) from the Ministry of Education, Science, and Culture of Japan.

References
9) D. Diaz-Sanchez and D. M. Kemeny, Immunology, 72, 297–303 (1991).