Effects of Sesamin and α-Tocopherol, Individually or in Combination, on the Polyunsaturated Fatty Acid Metabolism, Chemical Mediator Production, and Immunoglobulin Levels in Sprague-Dawley Rats

Jiong-Yan Gu, Yoko Wakizono, Akira Tsubita, Beong-Ou Lim, Michiko Nonaka, Koji Yamada, and Michihiro Sugano*

Laboratory of Food Science, Department of Food Science and Technology, School of Agriculture, Kyushu University, Higashi-ku, Fukuoka 812–8, Japan

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Feeding sesamin and α-tocopherol in combination, both at the 0.5% dietary level, to Sprague-Dawley rats for 3 weeks resulted in a trend toward decreasing the proportion of 20:4n-6 and 22:5n-6 and increasing that of 18:2n-6 in phosphatidylycholine from various tissues, suggesting interference with the metabolism of linoleic acid. This dietary manipulation significantly reduced the production of leukotriene C4 in the lung, the splenic production of leukotriene B4, and reduction of the plasma histamine level. Simultaneous administration of sesamin and α-tocopherol significantly increased the production of IgA, IgG, and IgM by mesenteric lymph node lymphocytes, while the IgE level tended to be reduced. These effects were not necessarily apparent by feeding these compounds separately. Thus, sesamin and α-tocopherol in combination would be effective for regulating the eicosanoid production and modifying the immune function.

Sesame has long been extensively used as a traditional health food or as a medicinal plant in oriental countries for various purposes. However, the biochemical background of its effect has not been totally appreciated. It has recently been reported that sesamin, a lignan exclusively occurring in sesame seed, had diverse physiological functions.1) Sesamin has modified the metabolism of linoleic acid to arachidonic acid, and hence, the production of some eicosanoids.2–4) In addition, the antioxidative5) and hypcholesterolemic6) effects of sesamin appear to be influenced synergistically by α-tocopherol.1,5) Alpha-tocopherol is not only an effective antioxidant but also a modulator of eicosanoid production.6,7)

It has been reported that the type of dietary fat is closely involved in regulating the allergic reactivity through a change in the formation of arachidonic acid and eicosanoid production.8,9) Thus, there is a possibility that the combined use of sesamin with tocopherol may be effective in modifying this reactivity. Immunologically hypersensitive Brown-Norway rats have been employed in several laboratories for investigating allergic and autoimmune manifestations.10) We have previously shown that sesamin and α-tocopherol in combination significantly reduced the proportion of arachidonic acid in liver and lung phosphatidylycholine (PC), suppressed leukotriene C4 (LTC4) production in the lung, and reduced the proportion of splenic CD4+ and CD8+ T-cell subsets in Brown-Norway rats.11) However, there is no information as to whether there is such an effect on non-sensitive normal rats. Therefore, in the present study, our aim was to determine whether these effects could be reproduced even in normal rats. The changes in fatty acid profiles and chemical mediator production were monitored in Sprague-Dawley rats given sesamin and α-tocopherol either separately or in combination. The concentration of plasma histamine, as a measure of inflammatory mediation, and the immunoglobulin level in mesenteric lymph node (MLN) lymphocytes, as a measure of the intestinal immune function, were also examined.

Materials and Methods

Animal and diets. Four-week-old male Sprague-Dawley rats (Seiwa Experimental Animals, Fukuoka) weighing an average of 147 g were given free access to one of four experimental diets: control, sesamin-added, α-tocopherol-added, or sesamin and α-tocopherol-added; 6 rats in each dietary group. The basal diet according to the recommendation of the American Institute of Nutrition12) contained the following ingredients by weight percentage: casein, 20; corn oil, 5; AIN mineral mixture, 3.5; AIN vitamin mixture, 1; choline bitartrate, 0.2; N2+ 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.

The sesamin preparation, of 99.5% purity as an equivale,weight mixture of sesamin and episesamin, was presented by Suntory Ltd., Osaka. Alpha-tocopherol was obtained from Eisai Co., Tokyo, and the 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 into a syringe containing 3.8% Na2-citrate13) under light diethyl ether anaesthesia. The liver, spleen, heart, kidneys, and testes were immediately excised, blotted, and weighed.

Analysis of lipids. Tissue lipids were extracted and purified by the method of Folch et al.14) and phospholipid, cholesterol and triglyceride were measured as described previously.15) Phosphatidylycholine (PC) was separated by thin-layer chromatography with chloroform–ethanol–water–triethylamine (30:35:6:3.5, v/v/v/v) as the developing solvent.16) The fatty acid composition of PC was analyzed by gas-liquid chromatography (GLC) in a SILAR 10C column.17)

Measurements of eicosanoids. A sample was cut from the spleen or lung and immediately homogenized in 10ml of phosphate-buffered saline (PBS, pH 7.4), before being incubated at 37°C for 20 min. LTBA and LTC4 were extracted by the method of Moskbel et al.18) and measured by a radioimmunoassay, using a commercial kit (NEK-030 and NEK-037, Du Pont NEN Research Products, Boston, MA for LTBA and LTC4, respectively). Eicosanoids were measured under a linear relationship with respect to the tissue weight and incubation time.11)

* To whom correspondence should be addressed.
**Measurement of plasma histamine.** Plasma (1.5 ml) was placed in a centrifuge tube containing 1 ml of 1 N HClO₄ and then mixed. After centrifuging at 3000 rpm for 30 min, histamine was extracted by the method described elsewhere.³⁹

**Measurements of the mesenteric lymph node (MLN) immunoglobulins.** The mesenteric lymph node was excised, and the lymphocytes 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 resulting cell suspension was layered on 4 ml of Lympholyte-Rat (Cedarlane, Hornby, Canada) and centrifuged at 1500 g for 30 min. The lymphocyte band at the interface was recovered, and the cells were rinsed three times with the RPMI 1640 medium. The lymphocytes were cultured in a 10% fetal bovine serum (FBS, Intergen Co., U.S.A.) RPMI 1640 medium. The MLN lymphocytes were adjusted to 2 × 10⁶ cells/ml, and after incubating at 37°C for 6 h, the concentrations of IgE, IgA, IgM, and IgG were measured by an enzyme-linked immunosorbent assay (ELISA).³⁹

**Statistical analyses.** Data were analyzed by one-way ANOVA, before inspecting all differences by Duncan’s new multiple-range test.²¹ Differences are considered statistically significant at p < 0.05.

**Results**

**Growth parameters and relative tissue weights**

Table I shows the body weight, food intake, and relative weight of several tissues. The food intake and, hence, the body weight gain tended to be low in the group receiving sesamin and α-tocopherol simultaneously. However, these parameters were comparable among the other three groups. Sesamin significantly increased the relative liver weight irrespective of the presence or absence of α-tocopherol, while α-tocopherol did not influence the liver weight. The relative weights of the spleen, heart, and kidney, but not of the tests, were also greater in the combined sesamin and α-tocopherol-added group.

**Fatty acid composition of tissue phosphatidylcholine**

Table II shows the fatty acid composition of liver and spleen phosphatidylcholine (PC). Liver PC had an increasing trend in the proportion of 18:2n-6 and a decreasing trend of 20:4n-6 in those rats given α-tocopherol when compared to the control and sesamin groups. When sesamin and α-tocopherol were given together, these differences become significant. Consequently, the ratio of (20:3n-6 + 20:4n-6)/18:2n-6, an index of linoleic acid desaturation, was lowest in the sesamin plus α-tocopherol group. The proportion of 22:5n-6 was decreased by dietary manipulation and was most marked in the combination group. A similar but lesser degree of response was observed in spleen PC. However, the effect of dietary manipulation

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

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Relative tissue weight (g/100 g body wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Final</td>
<td>Liver</td>
</tr>
<tr>
<td>Control</td>
<td>146 ± 3a</td>
<td>312 ± 9abc</td>
<td>20.3 ± 0.7a</td>
</tr>
<tr>
<td>Sesamin</td>
<td>147 ± 3a</td>
<td>328 ± 7a</td>
<td>21.1 ± 0.55</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>147 ± 3a</td>
<td>316 ± 6abc</td>
<td>20.1 ± 0.6a</td>
</tr>
<tr>
<td>Sesamin + α-Tocopherol</td>
<td>146 ± 3a</td>
<td>298 ± 11b</td>
<td>18.2 ± 1.0b</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats.

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**Table II. Effect of Sesamin and α-Tocopherol on the Fatty Acid Composition of Liver and Spleen Phosphatidylcholine**

<table>
<thead>
<tr>
<th>Fatty acids (weight %)</th>
<th>Control</th>
<th>Sesamin</th>
<th>α-Tocopherol</th>
<th>Sesamin + α-Tocopherol</th>
<th>Control</th>
<th>Sesamin</th>
<th>α-Tocopherol</th>
<th>Sesamin + α-Tocopherol</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.4 ± 0.0a</td>
<td>0.1 ± 0.0b</td>
<td>0.4 ± 0.1a</td>
<td>0.0 ± 0.0b</td>
<td>0.4 ± 0.0ab</td>
<td>0.4 ± 0.0b</td>
<td>0.5 ± 0.0b</td>
<td>0.5 ± 0.1ab</td>
</tr>
<tr>
<td>16:0</td>
<td>22.0 ± 1.0b</td>
<td>26.0 ± 1.3b</td>
<td>24.3 ± 1.1ab</td>
<td>26.2 ± 0.7a</td>
<td>39.2 ± 0.1a</td>
<td>42.3 ± 0.3b</td>
<td>40.6 ± 0.9b</td>
<td>41.2 ± 1.1ab</td>
</tr>
<tr>
<td>18:0</td>
<td>16.5 ± 0.7abc</td>
<td>19.6 ± 0.4b</td>
<td>16.2 ± 1.3a</td>
<td>19.1 ± 0.9bc</td>
<td>9.4 ± 0.2a</td>
<td>8.8 ± 0.1b</td>
<td>9.0 ± 0.2b</td>
<td>8.6 ± 0.1b</td>
</tr>
<tr>
<td>18:1</td>
<td>2.5 ± 0.2a</td>
<td>1.1 ± 0.1b</td>
<td>3.0 ± 0.3a</td>
<td>0.9 ± 0.1b</td>
<td>2.3 ± 0.0a</td>
<td>2.0 ± 0.1b</td>
<td>2.5 ± 0.1b</td>
<td>2.3 ± 0.0b</td>
</tr>
<tr>
<td>18:2:6:2</td>
<td>9.5 ± 0.4b</td>
<td>7.6 ± 0.3a</td>
<td>9.1 ± 0.4a</td>
<td>8.3 ± 0.8b</td>
<td>13.2 ± 0.2b</td>
<td>12.5 ± 0.3a</td>
<td>13.1 ± 0.3b</td>
<td>12.6 ± 0.5b</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>7.7 ± 0.4b</td>
<td>4.6 ± 0.1b</td>
<td>9.4 ± 0.5b</td>
<td>11.4 ± 2.1a</td>
<td>8.5 ± 0.3b</td>
<td>8.3 ± 0.2b</td>
<td>10.3 ± 0.2b</td>
<td>9.9 ± 0.5b</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>1.3 ± 0.2b</td>
<td>1.3 ± 0.1b</td>
<td>1.4 ± 0.1a</td>
<td>1.7 ± 0.2b</td>
<td>1.1 ± 0.0b</td>
<td>1.0 ± 0.1b</td>
<td>1.1 ± 0.0b</td>
<td>1.2 ± 0.1b</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>31.9 ± 1.1bc</td>
<td>33.9 ± 0.7a</td>
<td>29.3 ± 1.2bc</td>
<td>27.5 ± 2.3b</td>
<td>19.7 ± 0.3b</td>
<td>19.7 ± 0.3b</td>
<td>18.0 ± 0.4b</td>
<td>18.7 ± 0.6b</td>
</tr>
<tr>
<td>22:6n-6</td>
<td>0.3 ± 0.0ab</td>
<td>0.2 ± 0.0ab</td>
<td>0.3 ± 0.1b</td>
<td>0.2 ± 0.0b</td>
<td>1.0 ± 0.1a</td>
<td>0.9 ± 0.0b</td>
<td>0.9 ± 0.0b</td>
<td>1.0 ± 0.0b</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>26.0 ± 0.2b</td>
<td>13.2 ± 0.2b</td>
<td>19.2 ± 0.2b</td>
<td>0.5 ± 0.2b</td>
<td>0.6 ± 0.0b</td>
<td>0.3 ± 0.0b</td>
<td>0.5 ± 0.1b</td>
<td>0.2 ± 0.0b</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>0.3 ± 0.1b</td>
<td>0.1 ± 0.0b</td>
<td>0.3 ± 0.1b</td>
<td>0.2 ± 0.1ab</td>
<td>0.9 ± 0.0b</td>
<td>0.9 ± 0.0b</td>
<td>0.9 ± 0.0b</td>
<td>0.8 ± 0.1b</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.0 ± 0.0b</td>
<td>0.1 ± 0.0b</td>
<td>0.1 ± 0.0b</td>
<td>0.0 ± 0.0b</td>
<td>0.8 ± 0.1b</td>
<td>0.7 ± 0.1b</td>
<td>0.5 ± 0.1b</td>
<td>0.5 ± 0.0b</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.2 ± 0.0b</td>
<td>0.2 ± 0.0b</td>
<td>0.3 ± 0.1b</td>
<td>0.3 ± 0.0b</td>
<td>0.1 ± 0.0b</td>
<td>0.1 ± 0.0b</td>
<td>0.2 ± 0.0b</td>
<td>0.2 ± 0.0b</td>
</tr>
<tr>
<td>(20:3 + 20:4)/18:2</td>
<td>4.4 ± 0.3a</td>
<td>3.3 ± 0.2b</td>
<td>4.2 ± 0.3b</td>
<td>3.2 ± 0.3b</td>
<td>0.7 ± 0.0b</td>
<td>0.5 ± 0.0b</td>
<td>0.7 ± 0.0b</td>
<td>0.5 ± 0.0b</td>
</tr>
<tr>
<td>(20:4/20:3)</td>
<td>43.0 ± 3.0b</td>
<td>7.7 ± 0.2b</td>
<td>3.1 ± 0.2b</td>
<td>2.6 ± 1.3b</td>
<td>2.4 ± 0.1b</td>
<td>2.5 ± 0.1b</td>
<td>1.9 ± 0.0b</td>
<td>2.0 ± 0.2b</td>
</tr>
</tbody>
</table>

Values are means ± SE of 6 rats.

Values without the same superscript letter are significantly different at p < 0.05.
was not apparent on the fatty acid profiles of heart, kidney, testis, and plasma PC when compared to that on liver and spleen PC (data not shown). There was a decreasing trend of 22:6n-3 in all tissue PC examined, except for plasma PC, in the rats given sesamin either alone or together with α-tocopherol. The response in the other n-3 polyunsaturated fatty acids was inconsistent depending on the tissues examined.

Concentration of liver lipids
Sesamin significantly increased the concentration of liver phospholipid (Fig. 1A), the effect being more marked when α-tocopherol was simultaneously given. In contrast, sesamin alone or together with α-tocopherol lowered liver cholesterol significantly (Fig. 1B). The concentration of liver triglyceride was also reduced by sesamin, although the difference was not significant (Fig. 1C).

Eicosanoid production and histamine concentration
Figure 2 shows that the splenic production of LTB₄ was reduced by α-tocopherol but not by sesamin (Fig. 2A). This effect was not influenced even when α-tocopherol was given with sesamin. There was no effect on the splenic production of LTC₄ (data not shown). Simultaneous administration of sesamin and α-tocopherol significantly reduced the production of LTC₄ by the lung (Fig. 2B), while this effect was not found when either additive was given individually. No effect was observed in the production of LTB₄ by the lung (data not shown). The plasma histamine concentration was significantly lowered by the combination of sesamin and α-tocopherol (Fig. 2C).

Immunoglobulin production in MLN lymphocytes
Table III shows that the IgA, IgG, and IgM levels in MLN lymphocytes were significantly increased when sesamin was given together with α-tocopherol, while the IgE
level was not changed significantly by this manipulation. The significant IgA elevating effect was also found when sesamin alone was given to rats. In contrast, α-tocopherol alone increased the concentration of IgG and IgM. Both sesamin and α-tocopherol tended to reduce the concentration of IgE when each was given individually.

Discussion

The combined immunomodulative effect of sesamin and α-tocopherol was apparent in the present study when using Sprague-Dawley rats. The production of splenic LTB₄ and lung LTC₄, both potent chemical mediators, was significantly reduced by this combination, while the concentration of plasma histamine, a typical inflammatory mediator, was also significantly lowered. In this context, Sugano et al. have described that α-tocopherol synergistically enhanced the hypcholesterolemic activity of sesamin in rats. Our results also confirm this observation. The response in leukotriene production could not simply be attributed to a change in the polyunsaturated fatty acid profile, since the proportion of arachidonic acid in lung and spleen PC remained apparently unchanged. It is well known that the availability of the substrate fatty acid determines eicosanoid production.⁹

The reducing effect of α-tocopherol on the proportion of 20:4n-6 in liver PC was enhanced when administered together with sesamin (Table II), although it was not always consistent in other tissues. This effect of sesamin can at least in part be attributed to a change in the Δ5 and Δ6 desaturase activities, as estimated from the desaturation indices. In contrast, modification to the profile of the n-3 polyunsaturated fatty acids was only moderate. The tissue-dependent difference in the response of the fatty acid composition is difficult to interpret at present.

LTB₄ is one of the most potent leukotactic substances, and its presence in inflamed tissues could represent a local control mechanism for the accumulation of inflammatory leukocytes. LTC₄ accounts for the biological activity of the slow-reacting substance of anaphylaxis (SRS-A), which has long been proposed as an important mediator of allergic bronchoconstriction in humans. In the present study, the contrasting observation was that LTB₄ was significantly decreased in the spleen, while LTC₄ was significantly decreased in the lung by feeding sesamin and α-tocopherol simultaneously. Thus, the effect was specific to those tissues in which different hypersensitive reactions would proceed. Although we do not have direct evidence for the allergic manifestations, our results in this study suggest that the combined use of sesamin with α-tocopherol could improve some allergic responses.

Histamine is also intimately involved in the allergic response. We found a significant reduction in plasma histamine by the combined administration of sesamin and α-tocopherol. Since histamine not only has a potent bronchoconstricting effect, but also increases vascular permeability, and hence, promotes oedema and pain that are characteristic of inflammation response, the combined use of these two components may be effective in reducing this response.

Regarding MLN lymphocytes immunoglobulin levels, IgE is the antibody causing anaphylaxis, while IgA and IgG suppress allergic reactions through interference with allergen absorption by IgA and competition of IgG with IgE. We show here that sesamin and α-tocopherol significantly increased the MLN IgA, IgG, and IgM levels. Contrastingly, the level of IgE was in no way increased by the administration of sesamin and α-tocopherol, either alone or in combination. Thus, the results suggest that the combined use of sesamin and α-tocopherol could modify the allergic reaction in a favorable manner.

A number of animal experiments have shown that polyunsaturated fatty acid is the immunoregulator. Hashimoto et al. have shown that increasing the n-3/n-6 ratio of dietary fatty acids was effective for suppressing the production of chemical mediators. In our previous study with Brown-Norway rats, the simultaneous administration of sesamin and α-tocopherol significantly lowered the proportion of 20:4n-6 in the liver and lung phosphatidylcholine and significantly reduced the production of LTC₄ by the lung. These effects were confirmed in the present experiment with Sprague-Dawley rats. However, compared to the effect in Brown-Norway rats, a reduction in the proportion of 20:4n-6 was found more commonly in Sprague-Dawley rats, and the production of LTC₄ and LTB₄ by the lung and spleen was specifically lowered, respectively. In addition, the concentration of plasma histamine was significantly lowered, and the production in MLN lymphocytes of IgA, IgM, and IgG was significantly increased in those rats fed sesamin together with α-tocopherol. It is therefore suggested that the combined effect of sesamin and α-tocopherol would be a common phenomenon in rats.

The mechanism for the synergism between sesamin and α-tocopherol is still not well understood. Sesamin has shown antioxidative potential in vivo and influenced the activity of peripheral monocytes. We have recently observed that α-tocopherol maintained the level of sesamin at a high concentration in the liver. The interference with the metabolism of polyunsaturated fatty acids through inhibition of Δ5 desaturase by sesamin, and the reducing effect of α-tocopherol on eicosanoid production may modify the wide range of metabolic consequence.

The simultaneous administration of sesamin and α-tocopherol moderately but significantly reduced the food intake and body weight gain. However, it is unlikely that these small changes would greatly modify the results obtained in the present study. In addition, sesamin increased the relative liver weight, and the concentration of liver phospholipid. However, Hirose et al. observed no microscopically histological abnormalities in the liver of
rats fed on a purified diet containing 0.5% sesamin. Accompanying this change was an improvement in the liver functions. Therefore, there is a possibility that this modified liver function may directly and/or indirectly be responsible for the changes in the various parameters analyzed here.

The dose level of sesamin and α-tocopherol employed in this study seemed to be high. However, this combination produced clear effects on various lipid parameters, especially on the concentration of serum cholesterol. A dose-dependent study is needed for further evaluation of the combined effect.

In conclusion, the combined effects of administering sesamin and α-tocopherol on the production of chemical mediators were observed even in Sprague-Dawley rats. Sesamin and α-tocopherol when given together could be effective in regulating the eicosanoid production and modifying the immune indices. Sesamin and α-tocopherol appear to have diverse functions, and more comprehensive studies of the regulative role of these compounds on the immune function are warranted.

References