Sesamolin Inhibits Lipid Peroxidation in Rat Liver and Kidney

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ABSTRACT Although the sesame lignans, sesaminol and sesamolinol, have been shown to possess antioxidative activity, less is known about the metabolism and antioxidative properties of sesamolin, a major constituent of sesame oil. To determine the ability of sesamolin to act as an antioxidant in vivo, we fed rats a diet containing 1% sesamolin for 2 wk and studied its metabolism and its effects on oxidative stress. About 75% of the ingested sesamolin was excreted unmetabolized in feces, but it was not detected in urine. Sesamolin and its metabolites, sesamol and sesamolinol, were excreted primarily as sulfates and glucuronides. The amount of sesamolin and its metabolites was lower in the plasma than in the liver or kidneys. When we compared rats fed a diet containing 1% sesamolin for 14 d with those fed a control diet, we found that liver weight was significantly greater in the former group. Lipid peroxidation activity, measured as 2-thiobarbituric acid reactive substances, was significantly lower in the kidneys and liver of the sesamolin-fed rats than in the controls. In addition, the amount of 8-hydroxy-2’-deoxyguanosine excreted in the urine was significantly lower in the sesamolin-fed rats. These results suggest that sesamolin and its metabolites may contribute to the antioxidative properties of sesame seeds and oil and support our hypothesis that sesame lignans reduce susceptibility to oxidative stress. J. Nutr. 128: 1018–1022, 1998.

KEY WORDS:  · sesamolin · oxidative stress · antioxidants · lipid peroxidation · rats

Recently, much attention has been focused on the role of the antioxidative defense system in oxidative stress (Sugiyama et al. 1996, Tsuda et al. 1996). Endogenous antioxidants in plants may play an important role in antioxidative defense against oxidative damage (Frei et al. 1988, Naito et al. 1995), possibly preserving the biological functions of cells (Naito et al. 1994, Osawa et al. 1990a). There is increasing interest in the protective biological function of natural antioxidants contained in dietary plants, which are candidates for the prevention of oxidative damage (Frankel et al. 1993, Osawa et al. 1990a, 1990b and 1995).

Sesame seeds (Sesamum indicum, Linn, Pedaliaceae) have long been categorized as a traditional health food in Japan and other East Asian countries. A number of lipid-soluble antioxidants have been isolated from sesame seeds, including sesaminol (Katusuki et al. 1994), sesaminol (Osawa et al. 1985), p1 (Katusuki et al. 1993) and pinoresinol (Katusuki et al. 1992); sesame oil has been found to contain considerable amounts (up to 1.5%) of the sesame lignans, sesamin and sesamolin (Beroza and Kinman 1973). During the industrial bleaching process of unroasted sesame oil, sesamolin is converted to sesaminol (Fig. 1; Fukuda et al. 1986). In addition, although sesamolin is readily converted to sesamol under certain conditions such as hydrolytic scission at the C2 position (Haslam 1970), the metabolism of sesamolin in vivo is not yet known.

The lignans present in sesame oil are thought to be responsible for many of its unique chemical and physiologic properties, including its antioxidant and antimutagenic properties. This is of special interest because sesamin, sesamolin and a phytosterol present in the unsaponifiable fraction of sesame oil have been reported to possess no appreciable in vitro antioxidant activity (Budowski 1950). It is possible, however, that these compounds may act as antioxidants in vivo.

Lipid peroxidation is one of the reactions induced by oxidative stress; it is especially active in those tissues having membranes rich in polyunsaturated fatty acid. There have been few comparative studies, however, on the ability of dietary antioxidants to modify the susceptibility of organs or tissues to oxidative stress and to alter the cellular antioxidant defense system (Tappel 1973). We have recently found that some sesame lignans act effectively in vitro as antioxidants and peroxyl radical scavengers (Kang, M.-H., Naito, M., Tsujihara, N. and Osawa, T., unpublished results). To determine the ability of sesame lignans to act as antioxidants in vivo, we fed rats a diet containing 1% sesamolin and examined the metabolism of this compound as well as its effects on oxidative stress in vitro.

MATERIALS AND METHODS

Animals and diet. All animal protocols were approved by the Laboratory Animal Care Advisory Committee of Nagoya University. Ten Sprague-Dawley male rats (170–200 g; ST, Shizuoka, Japan) were housed individually at 22 ± 1°C in metabolic cages, permitting separate collection of urine and feces, with a 12-h light:dark cycle. All rats were allowed free access to the diet and water for 1 wk for adaptation to the new environment. The rats were divided in two...
groups of five and were fed control or experimental diets containing 1% sesamolin (Table 1), isolated by preparative HPLC from sesame oil (the kind donation of Corunum, Osaka, Japan) (Fukuda et al. 1986). Beginning 1 wk later, urine and feces were collected and stored at −80°C. Urine was collected for 24 h on d 7 after feeding sesamolin and used for measurements of 8-hydroxy-2′-deoxyguanosine (8-OHdG) missed.1

After 2 wk of consuming the diets, the rats were starved for 12 h and killed by anesthetization with diethyl ether. Blood was taken from the abdominal aorta and plasma was separated. The kidneys, liver, stomach, and large and small intestines were removed and washed with cold PBS, pH 7.4, and portions of each were homogenized in cold PBS. Plasma and tissue homogenates were stored at −80°C. Protein concentrations were determined with bicinchonic acid (BCA) protein assay kit (Fierce, Rockford, IL) with bovine serum albumin as the standard.

Measurement of oxidative susceptibility and free radical trapping capacity. A 1–mL aliquot of each homogenate was incubated for 2 h at 37°C with 15 μmol/L ferrous sulfate in 10 mmol/L phosphate buffer (pH 7.4). To measure 2-thiobarbituric acid reactive substances (TBARS) (Hu et al. 1989), 1 mL of each mixture was added to a tube containing 2 mL of 0.46 mol/L trichloroacetic acid (TCA) and mixed. After centrifugation at 700 × g for 10 min, 2 mL of each supernatant was added to 1 mL of 49 μmol/L 2-thiobarbituric acid (TBA; Merck, Darmstadt, Germany). The tubes were boiled for 10 min and allowed to cool, and the absorbance at 532 nm was measured. TBARS were calculated as malondialdehyde (MDA) equivalents, using freshly diluted malondialdehyde bis (dimethyl acetal), i.e., 1,1,3,3-tetraethoxy propane as the standard. MDA was prepared by hydrolysis of 1,1,3,3-tetraethoxypropane (Aldrich Chemical, Milwaukee, WI) with 1 mol/L HCl (Philpot 1963).

Measurement of antioxidative activity in rat liver microsomes. Wistar rats (n = 3, 8-wk old, 180–200 g) were killed, their livers were removed and homogenized, and microsomes were prepared by differential centrifugation (Kornbrust and Mavis 1980). Crude sesame extracts were provided by Takemoto Oil (Aichi, Japan). Sesame lignins were purified by preparative reverse-HPLC to obtain sesaminol, sesamin, sesamolin and pinoselinol from crude sesame extract (Fukuda et al. 1986, Katsuzaki et al. 1994, Osawa et al. 1985). Their purity was confirmed by proton nuclear magnetic resonance, 1H-α-Tocopherol and butylated hydroxytoluene (BHT) were purchased from Wako (Osaka, Japan). Probucol and sesamol were purchased from Sigma Chemical (St. Louis, MO). The compounds were dissolved in methanol (MeOH) by sonication; the final MeOH concentration was <1%. Fresh solutions (1 g/L) of microsomal protein in 50 mmol/L Tris-HCl (pH 7.4) were prepared for each assay. A test compound in MeOH was added to 1 mL of microsomal preparation to a final concentration of 10 μmol/L, and the mixture was incubated at 37°C for 30 min (Osawa et al. 1987). The formation of TBARS was then assayed as described above.

Determination of urinary 8-OHdG by competitive ELISA. Urine samples were centrifuged at 10,000 × g for 15 min, and the supernatant was used for the determination with a competitive ELISA method with the use of an “8-OHdG Check” kit (Japan Institute for the Control of Aging, Fukuroi, Japan). The determination range was 0.64–2,000 μg/mL and the specificity of the monoclonal antibody used in the competitive ELISA kit has been established (Erhola et al. 1997, Osawa et al. 1995, Toyokuni et al. 1997).

Determination of sesamolin metabolites. An aliquot (0.5 g) of dried, pulverized feces was extracted twice with five volumes of MeOH by shaking. The MeOH layers were combined and evaporated in vacuo; the resulting precipitates were suspended in 0.1 mol/L citrate phosphate added with 0.1 mol/L vitamin C and 3 mmol/L EDTA, pH 5.0. Each 200-μL aliquot of urine, plasma, fecal extract or tissue homogenate was incubated at 37°C for 45 min with 500 units of β-glucuronidase (Wako) and 40 units of sulfatase (Sigma Chemical) in 0.1 mol/L citrate phosphate buffer (Lee et al. 1995). As controls, tubes containing β-glucuronidase, sulfatase or both enzymes

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Composition of control and experimental diets</th>
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<tr>
<td>Ingredient</td>
<td>Control diet</td>
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<td>g/100 g</td>
<td>g/100 g</td>
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</tr>
<tr>
<td>Casein</td>
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<tr>
<td>Cellulose</td>
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<tr>
<td>Corn oil</td>
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<tr>
<td>Vitamin mixture2</td>
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</tr>
<tr>
<td>Corn starch</td>
<td>61.5</td>
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<tr>
<td>Sesaminol</td>
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1 Composition of mineral mixture (g/100 g): CaHPO4·2H2O, 0.43; KH2PO4, 34.31; NaCl, 25.06; Fe-Citrate, 0.623; MgSO4, 4.8764; ZnCl2, 0.02; MnSO4·5H2O, 0.121; CuSO4·5H2O, 0.156; KI, 0.0005; CaCO3, 29.29; (NH4)6Mo7O24·4H2O, 0.0025; cellulose-powder, 5.1036.

2 Composition of the vitamin mixture (mg/100 g): retinyl acetate, 100.0; cholecalciferol, 0.25; all-rac-α-tocopheryl acetate, 500.0; menadione, 520.0; thiamin-HCl, 120.0; riboflavin, 400.0; pyridoxine-HCl, 80.0; vitamin B-12, 0.05; vitamin C, 3,000.0; biotin, 2.0; folic acid, 20.0; calcium-pantothenate, 500.0; p-aminobenzoic acid, 500.0; nicotinic acid, 600.0; inositol, 600.0; choline chloride, 20,000.0; cellulose powder, 73.057.7.

Table 2: Food intake, weight gain and organ weights of rats fed control or 1% sesamolin diets for 14 d1

<table>
<thead>
<tr>
<th>Group</th>
<th>Food intake</th>
<th>Body weight gain</th>
<th>Liver weight</th>
<th>Kidneys weight</th>
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<tr>
<td>g/d</td>
<td>g/14 d</td>
<td>g</td>
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<tr>
<td>Control</td>
<td>18.6 ± 0.55</td>
<td>5.2 ± 2.77</td>
<td>7.9 ± 0.88*</td>
<td>1.9 ± 0.12</td>
</tr>
<tr>
<td>Experimental</td>
<td>17.3 ± 0.91</td>
<td>6.4 ± 2.79</td>
<td>10.7 ± 0.86</td>
<td>2.0 ± 0.09</td>
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</tbody>
</table>

1 Values are means ± SD, n = 5.

* Significantly different than control, P < 0.05.
were incubated with the buffer. The mixture was vortexed with 1.4 mL ethyl acetate and centrifuged at 750 × g for 15 min. One milliliter of the ethyl acetate layer was transferred to a microfuge tube and evaporated in vacuo. Each dried sample was resuspended in 50 mL ethyl acetate and centrifuged at 750 L.

HPLC was performed by injecting 20-μL samples onto a chromatograph fitted with a 4.6 i.d. × 250 mm ODS-Column (Nomura Chemical, Aichi, Japan). The chromatography was operated at a flow rate of 0.8 mL/min at ambient temperature, using MeOH/H2O (6:4, v/v) as the mobile phase at a wavelength of 290 nm. The major metabolites were isolated by rechromatography on HPLC, and their identities were confirmed by low resolution electron impact mass spectroscopy and by UV spectroscopy in MeOH.

Analysis of statistics. Results are presented as means ± SD. Statistical analysis was performed by Student’s t test. Differences of P < 0.05 were considered significant.

RESULTS

Growth and health. Food intake, body weight gain and kidneys weight did not differ in the two groups of rats (Table 2). In contrast, liver weight was significantly higher in the rats fed sesamolin (Table 2). We observed no specific histological changes in the livers of these rats (data not shown).

Identification of sesamolin metabolites in feces. We detected four peaks in the feces of rats fed sesamolin that were not present in rats fed the control diets (Fig. 2). Three peaks were identified as sesamol, sesamolinol and sesamolin by liquid chromatography mass spectrometry (Fig. 2). Each of these sesamol metabolites was apparently excreted as conjugates of β-glucuronic acid and sulfates (data not shown). We were unable to purify a sufficient amount of the metabolite in the second peak to identify this compound.

Concentration of sesamolin metabolites in plasma and tissues. We detected ~80% of the sesamolin in the large intestine (Table 3). Only trace amounts of sesamolin metabolites were detected in plasma and in all of the other organs (Table 3). In each organ and in plasma, most of the sesamolin and its metabolites was present as conjugated glucuronides and sulfates; little was present in free form (Fig. 3).

Effects of sesame lignans on lipid peroxidation in vitro. When we assayed the effect of individual sesame lignans on lipid peroxidation activity of rat liver microsomes induced by ADP-Fe2+/NADPH, we found that sesamolin and sesamin did not inhibit lipid peroxidation (Fig. 4). In contrast, sesamol and sesamolinol significantly decreased lipid peroxidation (Fig. 4).

Susceptibility of tissues to lipid peroxidation. When we assayed rat liver and kidney homogenates for their ability to inhibit FeSO4-induced lipid peroxidation, we found that TBARS formation was significantly influenced by diet (Fig. 5). TBARS formation was significantly lower in both the liver and kidneys of rats fed the 1% sesamolin diet than in controls (Fig. 5). In contrast, we observed no difference in antioxidative activity between plasma samples from the two groups of rats (Fig. 5).

Urinary excretion of 8-OHdG. There was a significant difference in urinary excretion of 8-OHdG between the control group and 1% sesamolin-fed group (7.84 ± 3.72 vs. 3.26 ± 1.92 μmol/d, P < 0.05).

DISCUSSION

In oriental countries, sesame seed is a traditional health food and has long been thought to possess the ability to prevent various diseases including atherosclerosis and hypertension and to retard aging (Budowski and Markley 1951, Namiki 1995). We focused our attention on the lignan type of antioxidants present in sesame seeds and oil because of their unique structure and function. We have previously shown that sesaminol is the principal antioxidative component in sesame seed oil (Osawa et al. 1990a) and that sesaminol is formed from sesamolin during the bleaching of unroasted sesame oil. This reaction has been studied with the use of an in vitro system (Fukuda et al. 1986). In this experiment, we have demonstrated that sesamolin can be metabolized to sesamol and sesamolinol in vivo, and that all of these compounds exist primarily as

### TABLE 3

| Table 3: Concentration of sesamolin and its metabolites in plasma and organs of rats fed 1% sesamolin diet
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<tr>
<td></td>
<td>Free</td>
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<tr>
<td>Plasma</td>
<td>0.01 ± 0.01</td>
<td>0.90 ± 0.43</td>
<td>0.04 ± 0.03</td>
<td>0.40 ± 0.02</td>
<td>0.01 ± 0.02</td>
<td>0.37 ± 0.03</td>
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<tr>
<td>Stomach</td>
<td>3.28 ± 2.97</td>
<td>9.40 ± 8.14</td>
<td>2.42 ± 3.38</td>
<td>4.68 ± 5.23</td>
<td>0.56 ± 0.90</td>
<td>2.54 ± 0.01</td>
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<tr>
<td>Liver</td>
<td>0.25 ± 0.26</td>
<td>3.68 ± 1.65</td>
<td>0.11 ± 0.10</td>
<td>1.50 ± 0.31</td>
<td>0.76 ± 0.76</td>
<td>4.04 ± 0.68</td>
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<tr>
<td>Kidneys</td>
<td>1.29 ± 1.32</td>
<td>3.33 ± 0.95</td>
<td>0.47 ± 0.09</td>
<td>1.32 ± 0.07</td>
<td>0.11 ± 0.17</td>
<td>1.73 ± 0.34</td>
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<tr>
<td>Small intestine</td>
<td>64.19 ± 30.4</td>
<td>29.7 ± 10.98</td>
<td>7.26 ± 7.68</td>
<td>3.05 ± 0.67</td>
<td>3.13 ± 21.86</td>
<td>18.36 ± 5.99</td>
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<tr>
<td>Large intestine</td>
<td>28.07 ± 45.95</td>
<td>76.98 ± 1.26</td>
<td>0.00 ± 6.50</td>
<td>4.40 ± 0.04</td>
<td>0.00 ± 62.57</td>
<td>67.06 ± 63.92</td>
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1 Values are means ± so, n = 5.
SESAMOLIN INHIBITS LIPID PEROXIDATION

**FIGURE 3** Concentration of sesamolin and its metabolites in plasma, kidneys and liver of rats fed 1% sesamolin diet. Free and conjugated sesamolin were determined as described in Materials and Methods. Results are the mean ± SD of five rats.

glucuronides and sulfates in the plasma and tissues. We found that <25% of the ingested sesamolin is absorbed or metabolized, but is excreted directly. Our results also suggest that any absorbed sesamolin is probably excreted through the bile after metabolism in the liver.

Although sesamolin had no effect on body weight, it was associated with liver weight gain in these rats. Although we do not know if sesamolin feeding directly caused this increased liver weight, we regard it as likely, inasmuch as Sugano et al. (1990) and Hirose et al. (1991) have reported liver enlargement in sesamin-fed rats. Furthermore, as with sesamin-fed rats, the livers of sesamin-fed rats showed no specific histological changes, lipid accumulation or alteration in drug-metabolizing enzymes (Hirose et al. 1991, Sugano et al. 1990). However, the importance of this observation remains to be determined.

The addition of iron-chelate complex has been reported to be required for promotion of peroxidation (Aust and Svingen 1982), especially in NADPH-dependent microsomal lipid peroxidation (Hochstein and Ernster 1963). Although sesamin

**FIGURE 4** Inhibitory effects of sesame lignans on the lipid peroxidation activity of rat liver microsomes. Rat liver microsomes (1.0 g protein/L) were incubated at 37°C for 30 min with 10 μmol/L of each compound, and the formation of 2-thiobarbituric acid reactive substances (TBARS) was determined at 532 nm. A positive control, without any added antioxidant, was defined as 100% lipid peroxidation. Each bar represents the mean ± SD (n = 3). * Significantly different than the control group, P < 0.05. BHT, butylated hydroxytoluene

**FIGURE 5** Antioxidative activity in liver (A), kidneys (B) and plasma (C) of rats fed control and 1% sesamolin diet. Each value is the mean ± SD (n = 5). * Significantly different than the control group, P < 0.05. MDA, malondialdehyde.
and sesamolin showed only weak antioxidative activity in the ADP-Fe\textsuperscript{3+}/NADPH system, metabolites of sesamolin, such as sesamol and sesamolinol, strongly inhibited lipid peroxidation. The antioxidative activity of sesamol and sesamolinol can be explained by the presence of the hydroxyl group.

8-OHdG, a DNA base-modified product (Halliwell 1994, Kasai and Nishimura 1984) generated by reactive oxygen species, is mutation prone (Shibutani et al. 1991) and has been shown to be a good marker for oxidative damage (Halliwell and Aruoma 1993). It has been hypothesized that oxidative damage can occur in DNA during the peroxidative breakdown of membrane polyunsaturated fatty acids (Park and Floyd 1992). Because lipid peroxidation can mediate 8-OHdG formation in vitro (Hattori et al. 1997), this process may play a role in carcinogenesis by inducing 8-OHdG generation in vitro. In this study, the amount of 8-OHdG in the urine of rats fed 1% sesamolin was significantly lower than that in rats fed the control diet. We previously observed that sesame lignan feeding in rats effectively reduced the excretion of 8-OHdG in urine after administration of carbon tetrachloride (CCL\textsubscript{4}) (Osawa 1992). These data suggest a possible role for sesame lignans in the prevention of oxidative DNA damage caused in vivo systems.

In conclusion, this study suggests that sesamolin is metabolized to sesamol and sesamolinol in vivo and that both of those compounds strongly inhibit lipid peroxidation. Thus the metabolites of sesamolin may contribute to the antioxidative properties of sesame lignans and reduce susceptibility to some forms of oxidative stress.

**LITERATURE CITED**


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