Antihypertensive Effect of Sesamin. II. Protection against Two-Kidney, One-Clip Renal Hypertension and Cardiovascular Hypertrophy

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We investigated the antihypertensive effect of sesamin, a lignan from sesame oil, using two-kidney, one-clip (2K,1C) renal hypertensive rats. After clipping the left renal artery, animals were assigned to either a normal diet group (control group) or a sesamin-containing (1% (w/w)) diet group (sesamin group). The sham-operated rats (sham group) were fed a normal diet and tap water. The systolic blood pressure of the control group increased progressively in comparison with the sham group. This 2K,1C-induced hypertension was markedly reduced by feeding the sesamin-containing diet. The systolic blood pressure after 4 weeks was 123.6 ± 4.01 mmHg in the sham group, 187.43 ± 5.69 mmHg in the control group and 145.57 ± 6.78 mmHg in the sesamin group, respectively. There were significant increases in left ventricle plus septum weight-to-body weight ratio in the control group compared with the sham group. This rise was also significantly reduced in the sesamin group. When the thoracic aorta was histochromically evaluated, the wall thickness and wall-to-lumen ratio in the control group were significantly increased, compared with the sham group, indicating that vascular hypertrophy had occurred in the control group. The sesamin diet tended to ameliorate this vascular hypertrophy, although its effect was not statistically significant. These findings suggest that sesamin is useful as prophylactic treatment to combat the development of renal hypertension and cardiac hypertrophy.

Key words sesamin; two-kidney, one-clip; hypertension; cardiac hypertrophy; vascular hypertrophy

Sesame seed and oil have been used extensively as traditional health foods. However, little is known about the biologically active components of sesame and their actions. Sesamin is one of the lignans found in high concentration in sesame, but has not attracted much nutritional and biological attention, unlike other lignans such as sesaminol which is known to exhibit antioxidative activity. 1) Recently, several studies have investigated the biological activity of sesamin and found that it affects lipid metabolism, such as desaturation in polyunsaturated fatty acid biosynthesis 2) and cholesterol absorption. 3) Most recently, it has been reported that sesamin exhibits protective effects against liver damage caused by alcohol or carbon tetrachloride 4) and against 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinogenesis. 5) Thus, the biological actions of sesamin may be multifunctional.

We recently demonstrated that dietary sesamin efficiently suppresses the development of hypertension induced by deoxycorticosterone acetate (DOCA) and salt. 6) To further evaluate the antihypertensive activity of sesamin, we decided to examine the effect of this lignan on the development of other types of hypertension. We report here that dietary sesamin can markedly suppress the increased blood pressure and cardiac hypertrophy in two-kidney, one-clip (2K,1C) renal hypertensive rats.

MATERIALS AND METHODS

Materials Sesamin was prepared from refined sesame oil and purified as described previously. 7) Sesamin-containing diet (1% (w/w) in commercial normal diet) was obtained from Oriental Yeast Co., Ltd. The concentration of sesamin was determined based on our previous study. 4) All other reagents used were of analytical grade.

Animal Experiments Male Sprague-Dawley rats (200—240 g) (Charles River Japan Inc.) were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and the left renal artery was partially occluded by a silver clip (0.2 mm i.d.), while the right renal artery was left intact. These rats were assigned to a normal diet group (control group) and a sesamin-containing diet group (sesamin group). The control group was fed normal diet, whereas the sesamin group were given the same diet containing 1% sesamin. The sham-operated rats had the left renal artery dissected free from adherent connective tissue but no clip was placed in position and the animals were fed a normal diet and tap water ad libitum. Systolic blood pressure was monitored weekly with a tail cuff and a pneumatic pulse transducer (BP-98A, Softron). After 4 weeks, animals were killed by exsanguination under anesthesia (40 mg/kg sodium pentobarbital, i.p.), and the heart removed. The weights of the whole heart and left ventricle plus septum were determined. In some rats from the three groups, the thoracic aorta was also removed and free from fat and adherent connective tissue and then used for morphometric analysis.

Morphometric Analysis The thoracic aorta of each rat was placed in a vial of 10%-formaldehyde neutral buffer solution for later analysis. Cross-sections from the thoracic aorta were cut 5 μm thick and stained with Elastica-van Gieson. The vessel wall area and thickness were determined in three to four different cross-sections of each vessel using a computerized digitizing system (IBAS II, Carl Zeiss, Germany).

Statistical Analysis All values were expressed as the mean ± S.E.M. For statistical analysis, we performed analysis of variance (ANOVA) followed by Duncan's
multiple range test. In all comparisons, differences were considered to have statistical significance at $p<0.05$.

RESULTS

Effect of Dietary Sesamin on Cardiac Hypertrophy in 2K,1C Renal Hypertensive Rats The comparative data on cardiac hypertrophy in the three groups of animals after an experimental period of 4 weeks are summarized in Table 1. The gain in body weight by the 2K,1C rats (control and sesamin) was smaller than in the sham group. The heart weight-to-body weight ratio and left ventricle + septum (L.V. + S) weight-to-body weight ratio were markedly increased in control group, but sesamin feeding (1% (w/w) in normal diet) significantly reduced these effects, although not completely abolishing them.

Effects of Dietary Sesamin on Blood Pressure in 2K,1C Renal Hypertensive Rats At the beginning of the experiment, the systolic blood pressure of the sham, control and sesamin groups was 110.52 ± 2.35, 107.54 ± 2.83 and 108.63 ± 2.86 mmHg, respectively. At 1 week after surgery, there were significant increases in systolic blood pressure in the control group compared with the sham group and, thereafter, this hypertensive effect gradually accelerated (Fig. 1). The increase in blood pressure in rats fed the sesamin-containing diet was much smaller than in the control group, and significant hypotensive effects of sesamin were observed at 1, 3 and 4 weeks. The systolic blood pressure after 4 weeks was 123.60 ± 4.01 mmHg in the sham group, 187.43 ± 5.69 mmHg in the control group and 145.57 ± 6.78 mmHg in the sesamin group, respectively.

Effects of Dietary Sesamin on Vascular Hypertrophy in 2K,1C Renal Hypertensive Rats The results of morphometric analysis are summarized in Table 2. In the control group, there were significant increases in wall thickness and wall-to-lumen ratio compared with the sham group, indicating the occurrence of vascular hypertrophy in the control group. Sesamin feeding tended to reduce the above increases, but this effect of sesamin was not statistically significant.

DISCUSSION

We found that dietary sesamin efficiently reduced the development of hypertension in 2K,1C rats. Since sesamin feeding (1% (w/w) in normal diet) was started at the prehypertensive stage, our results suggest that sesamin treatment may be a prophylactic regimen combating the development of 2K,1C renal hypertension.

The renin-angiotensin system plays an important role in the development and maintenance of hypertension and

Table 1. Comparative Data on Body and Heart Weights in Sham-Operated, Control and Sesamin-Fed Rats after 4 Weeks of Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>Sham (n = 5)</th>
<th>Control (n = 10)</th>
<th>Sesamin (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW, g)</td>
<td>398.20 ± 11.83</td>
<td>302.30 ± 16.44***</td>
<td>307.27 ± 11.34</td>
</tr>
<tr>
<td>Heart weight (HW, mg)</td>
<td>1074.80 ± 40.29</td>
<td>1112.90 ± 55.94</td>
<td>999.82 ± 51.54</td>
</tr>
<tr>
<td>L.V. + S. weight (mg)</td>
<td>728.40 ± 29.73</td>
<td>816.10 ± 39.66</td>
<td>732.55 ± 39.06</td>
</tr>
<tr>
<td>HW/BW (mg/g)</td>
<td>2.70 ± 0.04</td>
<td>3.71 ± 0.15**</td>
<td>2.94 ± 0.08*</td>
</tr>
<tr>
<td>L.V. + S. weight/BW (mg/g)</td>
<td>1.83 ± 0.02</td>
<td>2.72 ± 0.09**</td>
<td>2.37 ± 0.07***</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M. ** $p<0.01$, compared with the sham group.  * $p<0.05$,  ** $p<0.01$, compared with the control group. L.V., left ventricle; S., Septum.

Table 2. Morphological Analysis of the Aorta in Sham-Operated, Control and Sesamin-Fed Rats after 4 Weeks of Treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>(n)</th>
<th>Wall thickness (μm)</th>
<th>Wall area (mm²)</th>
<th>Wall to lumen ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5</td>
<td>84 ± 2</td>
<td>0.48 ± 0.02</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>2K, 1C (Control)</td>
<td>6</td>
<td>107 ± 4**</td>
<td>0.55 ± 0.03</td>
<td>0.32 ± 0.02**</td>
</tr>
<tr>
<td>2K, 1C (Sesamin)</td>
<td>6</td>
<td>93 ± 7</td>
<td>0.50 ± 0.05</td>
<td>0.29 ± 0.01</td>
</tr>
</tbody>
</table>

Values are the mean ± S.E.M.  ** $p<0.01$, compared with the sham group.

Fig. 1. Changes in Systolic Blood Pressure of Sham-Operated, Control and Sesamin-Fed Rats

Columns and bars are the mean ± S.E.M.  ** $p<0.01$, compared with the sham group.  * $p<0.05$,  ** $p<0.01$, compared with the control group.
cardiac hypertrophy in the 2K,1C rat model. In this model, the increase in blood pressure is thought to be initially induced by increased formation of angiotensin II and renal sympathetic nerve activity is also involved in the development of hypertension. The mechanism by which sesamin prevents the development of 2K,1C renal hypertension is unclear, but this lignan may affect the above humoral and/or neural factors. For example, we have noted that sesamin has an inhibitory action on angiotensin converting enzyme (ACE) (unpublished data). However, since the inhibitory action of sesamin on this enzyme is much less potent than that of captopril (IC_{50} value: sesamin, 100 μM vs. captopril, 20 nm), it is reasonable to believe that unknown mechanisms other than ACE inhibition are mainly responsible for the antihypertensive activity of sesamin.

It is generally acknowledged that development of hypertension is accompanied by cardiovascular hypertrophy. Since hypertension itself is the main causal factor of hypertrophy, the blunting of the elevation in the blood pressure of sesamin-fed rats may help prevent cardiovascular hypertrophy. In this study, sesamin feeding markedly reduced cardiac hypertrophy, whereas the suppression of vascular hypertrophy was considerably less potent. A previous study demonstrated that vasorelaxing agents such as hydralazine failed to suppress vascular hypertrophy, even at hypertensive doses. On the other hand, using the 2K,1C renal hypertensive model, attenuation of vascular hypertrophy was observed after captopril treatment at a dose which did not lower blood pressure effectively. These observations suggest that factors other than blood pressure per se, such as local AII production in blood vessels, may be involved in vascular hypertrophy. A recent study demonstrated that in vivo gene transfer of ACE to blood vessels (rat carotid artery) induced vascular hypertrophy without affecting systemic blood pressure and that this vascular hypertrophy was abolished by the in vivo administration of losartan, an AII receptor antagonist.

We recently demonstrated that dietary sesamin can markedly suppress the increased blood pressure, cardiac hypertrophy and vascular hypertrophy in DOCA-salt hypertensive rats. However, in 2K,1C rats, dietary sesamin did not effectively suppress vascular hypertrophy. This discrepancy in the above hypertensive models might be explained by differences in the mechanisms that contribute to the development and/or maintenance of hypertension, or in the effects of sesamin on cardiac muscle and/or vascular wall. In general, 2K,1C hypertension is a renin-angiotensin-dependent renal hypertensive model, whereas DOCA-salt hypertension is independent of the renin-angiotensin system. In DOCA-salt hypertensive rats, the increase in blood pressure is thought to be induced by sodium retention and increased sympathetic nerve activity. In addition, recent studies have noted the impairment of synthesis or release of endothelium-derived relaxing factor, and increased endothelin-1 production in DOCA-salt hypertensive rats. Thus, neural and/or humoral factors appear to be implicated in the difference in effectiveness of sesamin between the two models, although the antihypertensive mechanism of sesamin is still unknown.

Hirose et al. noted that sesamin lowered both serum and liver cholesterol levels by inhibiting the absorption and synthesis of cholesterol, thereby suggesting that sesamin may be an efficient hypocholesterolemic agent. Most recently, it has been reported that sesamin exhibits protective effects against liver damage caused by alcohol or carbon tetrachloride and against 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinogenesis. Thus, sesamin also exhibits biologically beneficial effects.

In the present study, we demonstrated that sesamin efficiently prevents the development of hypertension and cardiac hypertrophy in 2K,1C rats. Further study to examine whether sesamin may be useful as a therapeutic tool in the established hypertension, is in progress in our laboratory. In addition, the possibility that sesamin is a useful protective agent against clinical hypertension warrants further attention.

REFERENCES