Antihypertensive Effect of Sesamin. IV. Inhibition of Vascular Superoxide Production by Sesamin

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We previously demonstrated the preventive effect of sesamin, a lignan from sesame oil, on the development of several experimental models of hypertension. In the present study, we explored the mechanisms underlying the antihypertensive effect of sesamin using the deoxycorticosterone acetate (DOCA)-salt hypertensive model. After a 5-week treatment period, aortic superoxide (O_2^-) production was measured in the lucigenin chemiluminescence assay. Chemiluminescence signals significantly decreased in sesamin-containing diet-fed DOCA-salt hypertensive rats compared with those in the normal diet-fed DOCA-salt rats, although the signals in sham-operated control animals were not affected by the sesamin feeding. In addition, there was a positive correlation between systolic blood pressure and aortic O_2^- production. These findings suggest that sesamin feeding inhibits enhanced vascular O_2^- production in DOCA-salt hypertensive rats and that the antioxidative action of sesamin may contribute to its antihypertensive activity.

Key words: sesamin; deoxycorticosterone acetate (DOCA)-salt hypertension; superoxide

We have demonstrated the antihypertensive effect of sesamin, a lignan from sesame oil, in several types of experimental hypertensive models.1–3) The most efficient activity was observed in the deoxycorticosterone acetate (DOCA)-salt hypertensive rat model. When sesamin-containing diets were fed to the animals, the development of hypertension and cardiovascular hypertrophy were markedly attenuated.1) Most recently, we found that dietary sesamin efficiently improved the diminished endothelium-dependent vasorelaxation in DOCA-salt hypertensive animals.5) In salt-loaded stroke-prone spontaneously hypertensive rats (SHR-SP), sesamin feeding also effectively reduced histologic renal damage such that the antioxidative action of sesamin may be due, at least in part, to immunopotentiation and antioxidative activity.5)

Excessive superoxide (O_2^-) production in the vessel wall induces endothelial dysfunction, which causes several pathologic conditions, such as diabetes, hypercholesterolemia, and atherosclerosis. Recent studies have shown that aortic O_2^- production is increased in several animal models of hypertension and that antioxidants, such as vitamin C and vitamin E, attenuate the elevation of blood pressure and the impairment of vascular reactivity in hypertensive animals,9) although antihypertensive therapy did not always prevent the increased oxidative stress.10)

In the present study, we investigated the possible mechanisms of the antihypertensive effect of sesamin in DOCA-salt hypertensive rats. We hypothesized that sesamin feeding inhibits enhanced vascular O_2^- production in DOCA-salt hypertensive rats and that this effect may contribute to the antihypertensive activity of sesamin.

MATERIALS AND METHODS

Materials Sesamin was prepared from refined sesame oil and purified as described previously.11) Sesamin-containing diets (0.1, 1 w/w% in commercial normal diet, NMF) were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents used were obtained from Sigma Chemical Co. (St. Louis, Missouri, U.S.A.) and Nacalai Tesque (Kyoto, Japan).

Animal Experiments Male Sprague-Dawley rats (6 weeks old) (SLC, Inc., Hamamatsu, Japan), were anesthetized with sodium pentobarbital (40 mg/kg, i.p.), and the right kidney was removed via a right flank incision. After a 1-week postsurgical recovery period, rats were separated into a sham-operated group and a DOCA-salt group. Each group was further divided into three groups: i) normal diet group; ii) 0.1% sesamin-containing diet group; and iii) 1% sesamin-containing diet group. The sham-operated groups were given tap water ad libitum. Rats in the DOCA-salt group were treated twice weekly with DOCA suspended in corn oil, which was administered subcutaneously (15 mg/kg), and 1% NaCl was added to their tap water for drinking. Systolic blood pressure (SBP) was monitored weekly with the tail cuff method and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). After 5 weeks, the thoracic aortas were removed, freed from fat and adherent connective tissue, and then used for measurement of aortic O_2^- production.

Measurement of Aortic O_2^- Production The O_2^- production was measured using a lucigenin-enhanced chemiluminescence assay.15) The thoracic aorta was isolated and cut into strips with special care to preserve the endothelium. In some strips, the endothelium was removed by gently rubbing the intimal surface with a cotton ball. Three 5-mm aortic segments were placed in test tubes containing modified Krebs–HEPES buffer (pH 7.4, 99.01 mm NaCl, 4.69 mm KCl, 1.87 mm CaCl_2, 1.20 mm MgSO_4, 1.03 mm K_2HPO_4, 25 mm Na–HEPES, 11.1 mm glucose) and allowed to equilibrate in the dark for 15 min at 37°C before measurement. After 30 s, lucigenin (5 μM) was added to the tube. Luminescence was measured using a luminometer (Sirius-2, Funakoshi, Tokyo, Japan). The relative light unit (RLU) was integrated every 3 s for 15 min and averaged. Background counts were determined from identically treated vessel-free readings and subtracted from the vessel readings. Aortic O_2^- production was expressed as RLU per min per dry tissue weight.

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RESULTS AND DISCUSSION

At the beginning of the experiment, no significant differences in basal levels of SBP and body weights were observed among all experimental groups. After a 5-week treatment period, SBP was markedly elevated in the normal diet-fed DOCA-salt group compared with those in the normal diet-fed sham-operated group. Sesamin-feeding dose dependently attenuated the increase in SBP. The gain in body weight of the normal diet-fed DOCA-salt group was less than that in the normal diet-fed sham group, as described previously.

The DOCA-salt-induced body weight loss was slightly improved by the feeding of the 1% sesamin-containing diet, although the feeding of the 0.1% sesamin-containing diet had no effect. In sham groups, the blood pressure elevations and the body weight gains were not significantly different between the normal-diet and sesamin-diet groups.

Increased O$_2^-$ production in aortic segments obtained from the normal diet-fed DOCA-salt group was significantly suppressed by the feeding of the 1% sesamin-containing diet (Fig. 1). In sham groups, sesamin feeding had no effect on O$_2^-$ production. In addition, there was a positive correlation between SBP and vascular O$_2^-$ production (Fig. 2), thereby indicating a close relationship between the sesamin-induced decrease in vascular O$_2^-$ production and its antihypertensive activity.

In the present study, removal of the endothelium did not affect the aortic O$_2^-$ production (data not shown). This suggests that the increase in vascular O$_2^-$ production in DOCA-salt hypertensive rats was mainly derived from vascular smooth muscle cells and/or adventitia.

We recently demonstrated that dietary sesamin improved the impairment of endothelium-dependent vasorelaxation in DOCA-salt rats, and suggested that, at least in part, an antioxidative activity of sesamin may contribute to the activity. The mechanisms by which sesamin feeding decreases the vascular O$_2^-$ production are unclear. Asami et al. found that dietary sesamin was metabolized in the liver and converted to an antioxidative catechol form. This metabolite might exhibit direct radical scavenging activity in the vascular wall. Alternatively, sesamin may reduce the vascular O$_2^-$ production by inhibiting the activity of an NADPH oxidase, an enzyme which is known to be a main source of O$_2^-$ production in the vasculature, and is increased in the vascular tissues of DOCA-salt hypertensive rats. On the other hand, a previous in vitro study has shown that stretching vascular smooth muscle cells results in increased O$_2^-$ production, thereby suggesting that the high blood pressure by itself may increase the vascular O$_2^-$ production. Therefore the possibility that dietary sesamin-induced decreases in vascular O$_2^-$ production may result from a decrease in blood pressure cannot be ruled out. Further studies are needed to clarify the above problems.

In conclusion, sesamin feeding markedly suppressed the enhancement of aortic O$_2^-$ production in DOCA-salt hypertensive rats, and this effect may contribute to the antihypertensive activity of sesamin.

REFERENCES