Effects of Sesamin on Aortic Oxidative Stress and Endothelial Dysfunction in Deoxycorticosterone Acetate-Salt Hypertensive Rats

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In the present study, we evaluated the relationship between the antihypertensive effect of sesamin, a lignan from sesame oil, and its antioxidative activity in deoxycorticosterone acetate (DOCA)-salt hypertensive rats. After a 5-week treatment period, systolic blood pressure was significantly elevated in normal diet-fed DOCA-salt animals compared with cases in sham-operated animals. Sesamin feeding, tempol (a superoxide dismutase mimetic) treatment or antihypertensive drugs combination (triple therapy; reserpine, hydralazine, hydrochlorothiazide) significantly suppressed the development of DOCA-salt-induced hypertension. Compared with sham-operated rats, the normal diet-fed DOCA-salt rats revealed marked increases in aortic superoxide (O$_2^-$) production. These increases in O$_2^-$ production were significantly suppressed by sesamin feeding or tempol treatment, but not by triple therapy. Acetylcholine (Ach)-induced endothelium-dependent relaxation was markedly decreased in normal diet-fed DOCA-salt rats, compared with cases in sham-operated rats. Sesamin feeding and triple therapy significantly improved the DOCA-salt-induced impairment of endothelium-dependent relaxation. However, tempol treatment had no effect on the impaired vasodilator responses induced by DOCA-salt treatment. In DOCA-salt rats with or without sesamin feeding, systolic blood pressure significantly correlated with both aortic O$_2^-$ production and endothelium-dependent vascular relaxation. These findings suggest that sesamin feeding inhibits the enhancement of aortic O$_2^-$ production in DOCA-salt hypertensive rats, and this effect may contribute to the antihypertensive effect of sesamin. Sesamin feeding-induced improvement of endothelial dysfunction seems to result from the above antioxidative and antihypertensive effects.

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

Sesamin is one of the lignans contained abundantly in sesame oil. Previous studies have indicated that sesamin inhibits lipid metabolism, such as desaturation in polyunsaturated fatty acid biosynthesis$^{11}$ and cholesterol absorption.$^{21}$ In recent studies, we have demonstrated the antihypertensive effect of sesamin using several types of experimental hypertensive models.$^{3–5}$ The most efficient antihypertensive activity was observed in the deoxycorticosterone acetate (DOCA)-salt hypertensive rat model. Moreover, a development of cardiovascular hypertrophy in these animals was attenuated by the sesamin-feeding$^{31}$.

There is accumulating evidence indicating that an oxidative stress in vascular tissues is closely related to the development of hypertensive diseases.$^{6}$ Nakazono et al.$^{7,8}$ have shown that blood pressure of spontaneously hypertensive rats (SHR) was decreased markedly by administration of heparin-binding superoxide dismutase (SOD) which bound to vascular endothelial cells. In angiotensin II-induced hypertensive rats, both the development of hypertension and the altered endothelium-dependent vascular relaxation were improved by the treatment with membrane-targeted forms of SOD.$^{8}$ These findings suggest that vascular superoxide (O$_2^-$) production is increased in several animal models of hypertension and contributes to the development and/or maintenance of their high blood pressure and endothelial dysfunction.

Most recently, we noted that the increased vascular O$_2^-$ production in DOCA-salt hypertensive animals was normalized almost completely by the feeding of sesamin-containing diet.$^{9}$ On the other hand, a previous in vitro study has shown that stretching vascular smooth muscle cells results in increased O$_2^-$ production,$^{10}$ 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. Thus in the present study, we further evaluated the relationship between the antihypertensive effect of sesamin and its antioxidative activity in DOCA-salt hypertensive rats.

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). Reserpine and hydralazine hydrochloride were purchased from Tokyo Kasei Kogyo Co. (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 five groups: i) normal diet group; ii) 0.1% sesamin-containing diet group; iii) 1% sesamin-containing diet group; iv) tempol (4-hydroxy-2,2,6,6-tetramethyl piperidine-1-oxyl)-treated group; and v) triple therapy 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 sub-
cutaneously (15 mg/kg), and 1% NaCl was added to their tap water for drinking. Tempol is readily soluble in water and was administered in the drinking water (172.2 mg/l). Rats of the triple therapy group were received three antihypertensive drugs combination (triple therapy): reserpine (2 mg/l), hydralazine hydrochloride (40 mg/l), and hydrochlorothiazide (25 mg/l) in their drinking water. These drugs were administered as hypotensive doses, which were determined based on previous studies.12,13) Treatments with sesamin-containing diet and tempol-containing drinking water were started at the beginning of DOCA-salt treatment. Triple therapy was begun at the three weeks after starting the DOCA-salt treatment and given for 2 weeks. Systolic blood pressure (SBP) was monitored weekly with the tail cuff method and a pneumatic pulse transducer (BP-98A, Softron, Tokyo, Japan). Five weeks after the start of DOCA-salt treatments, the thoracic aortas were removed, freed from fat and adherent connective tissue, and then used for measurement of aortic O2 production and for isometric tension studies.

Measurement of Aortic O2 Production  The O2 production was measured using a lucigenin-enhanced chemoluminescence assay.14) The thoracic aorta was isolated and cut into strips with special care to preserve the endothelium. 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 CaCl2, 1.20 mM MgSO4, 1.03 mM KH2PO4, 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 lumino- meter (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 O2 production was expressed as RLU per min per dry tissue weight.

Isometric Tension Studies  About 2-mm aortic segments of the thoracic aorta were suspended in organ chambers containing 10 ml of Krebs–Ringer bicarbonate solution (118.5 mM NaCl, 4.7 mM KCl, 2.5 mM CaCl2, 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM NaHCO3, 10 mM glucose) under a resting tension of 1.5 g at 37 °C and gassed with 95% O2–5% CO2. Contractions and relaxations were expressed as changes in isometric tension by a force transducer (TB-612T, Nihon Kohden, Osaka, Japan) coupled to a polygraph (RM 6000, Nihon Kohden). Approximately a 1.5-h equilibration period was allowed before the start of the experiments. The strips were precontracted with 1-phenylephrine (Phe, 10−6 M). After a plateau was attained, the strips were exposed to acetylcholine (Ach, 10−6 to 10−5 M) to construct dose-response curves. Vasodilator response to Ach was expressed as a percentage of the response to Phe in each tissue.

Results  At the beginning of the experiment, no significant difference in basal level of SBP was observed among all experimental groups. After a 5-week treatment period, SBP was markedly elevated in the DOCA-salt normal diet group (197.9±7.0 mmHg) compared with those in the normal diet-fed sham-operated group (118.3±2.9 mmHg). Sesamin-feeding dose-dependently attenuated the DOCA-salt-induced increase in SBP (0.1% sesamin-containing diet, 170.5±8.0 mmHg; 1% sesamin-containing diet, 153.7±4.5 mmHg). Tempol treatment also significantly suppressed the elevation of blood pressure in response to DOCA-salt treatment (tempol group, 175.5±5.7 mmHg). Triple therapy using three antihypertensive drugs for 2 weeks markedly suppressed the DOCA-salt-induced hypertensive effects, and the level of SBP after 5 weeks was similar to that of the sham-operated group (triple therapy group, 133.2±4.7 mmHg) (Fig. 1). In the sham-operated groups, the blood pressure elevations were not significantly different among all experimental groups (date not shown).

Aortic O2 Production  O2 production in aortic segments was significantly increased in the normal diet-fed DOCA-salt rats compared with the sham-operated groups, whereas this increase in O2 production was dose-dependently suppressed by the feeding of the sesamin-containing diet. Likewise, tempol treatment significantly suppressed the DOCA-salt-induced increase in O2 production. On the other hand, triple therapy failed to suppress the O2 production induced by the
DOCA-salt treatment (Fig. 2).

Vascular Relaxation Ach at concentrations of $10^{-9}$ to $10^{-5}$ M produced a dose-dependent relaxation in aortic rings obtained from all experimental groups (Fig. 3). This effect of Ach was markedly reduced in vessels from DOCA-salt normotensive diet group. Sesamin feeding (1%) and triple therapy significantly improved the reduced responses to Ach, but tempol treatment failed to suppress the DOCA-salt-induced enhancement of vascular $O_2^*$ production despite the fact that the blood pressure lowering effect of triple therapy was the most efficient. Taken together, it is unlikely that suppressive effect of sesamin and tempol on vascular $O_2^*$ production results from decreases in blood pressure. Rather, it is reasonable to consider that the antioxidative activities of sesamin and tempol at least partly contribute to their antihypertensive actions.

The precise mechanisms by which sesamin feeding decreases the vascular $O_2^*$ production cannot be explained from our findings. Several natural products such as quercetin and catechin, which have a catechol moiety in their structures, are known to exhibit a potent radical scavenging activity in vitro. Sesamin itself does not have such chemical structure, and has no antioxidative properties in vitro. However, recent study investigating the metabolic pathway of sesamin administered orally to rats, demonstrated that the methylenedioxyphenyl moiety in the structure of sesamin was changed into a dihydroxyphenyl (catechol) moiety in the liver, and the metabolic products had strong radical scavenging activities in vitro. The authors suggested that sesamin is a prodrug and the metabolites containing the catechol moieties in their structures are responsible for the protective effects of sesamin against oxidative damage in the liver. Thus, further studies are needed to clarify whether the above metabolites and/or related compounds are involved in the decreasing effect of sesamin feeding on vascular $O_2^*$ production in DOCA-salt hypertensive rats.

It has become evident that the NADH/NADPH oxidase represents the important source of $O_2^*$ in vascular tissues of hypertensive animals. Beswick et al. showed that aortic mRNA levels of p22phox, an NADH/NADPH oxidase subunit, in DOCA-salt rats were strikingly increased compared with those in their normotensive controls, and that treatment of apocynin, a selective NADH/NADPH oxidase inhibitor, markedly suppressed the enhancement of aortic $O_2^*$ production and the elevation of blood pressure. These results suggest that the increase in NADPH oxidase expression is responsible for the enhancement of vascular $O_2^*$ production and possibly contributes to the elevation of blood pressure in...
DOCA-salt hypertensive rats. Furthermore, Wu et al.\(^\text{25}\) reported that aortic SOD activity in DOCA-salt rats was markedly reduced. Therefore, in DOCA-salt hypertensive animals, it is suggested that both an enhanced NADH/NADPH oxidase activity and a reduced SOD activity contribute to the higher aortic \(\text{O}_2^\text{2} \) production. It remains to be seen whether the sesamin feeding affects the activity/expression of these enzymes.

It has been well acknowledged that DOCA-salt hypertension is accompanied by cardiovascular hypertrophy.\(^\text{26}\) We recently demonstrated that sesamin feeding can effectively suppress the vascular hypertrophy,\(^\text{27}\) even though the SBP in sesamin-fed animals remained at the hypertensive level. Since hypertension itself is a main causal factor of hypertrophy, it is possible that the blunting of the rise in blood pressure of sesamin-fed rats is associated with the absence of cardiovascular hypertrophy. On the other hand, a previous study\(^\text{28}\) using one-kidney, one-clip hypertensive rats demonstrated that the attenuation of vascular hypertrophy was observed after captopril treatment at a dose which did not lower blood pressure effectively. Moreover, vasorelaxing agents such as hydralazine failed to suppress vascular hypertrophy, even at a hypertensive dose.\(^\text{29}\) These observations suggest that factors other than blood pressure per se are involved in cardiovascular hypertrophy. Recently, it has been shown that \(\text{O}_2^\text{2} \) and its dismutated product \(\text{H}_2\text{O}_2 \) stimulate the proliferation of vascular smooth muscle cells,\(^\text{30,31}\) and that treatment of antioxidant markedly suppress the development of vascular hypertrophy in DOCA-salt rats.\(^\text{32}\) Therefore, it is possible that antioxidative property of sesamin are at least partly involved in the suppression of DOCA-salt-induced vascular hypertrophy.

There is increasing evidence that \(\text{O}_2^\text{2} \) rapidly reacts with nitric oxide (NO) to diminish endothelium-dependent vasorelaxation.\(^\text{33}\) Laursen et al.\(^\text{34}\) showed that Ach-induced vasodilation was impaired in angiotensin II-induced hypertensive rats compared with that in normotensive control rats, and that both angiotensin II-induced hypertension and the impaired vasodilatory responses to Ach were restored by \textit{in vivo} treatment with liposome-encapsulated SOD. The authors suggested that hypertension caused by chronically elevated angiotensin II is mediated in part by \(\text{O}_2^\text{2} \), likely via degradation of endothelium-derived NO. However, in our separate experiments, pretreatment with exogenous SOD, tempol or tiron (an \(\text{O}_2^\text{2} \) scavenger) had no effect on impaired vasodilatory responses in aortic rings from DOCA-salt rats (unpublished data). Furthermore, in the present study, vasodilatory responses in the DOCA-salt triple therapy group were markedly improved compared with the responses in the DOCA-salt normal diet group despite the fact that increases in vascular \(\text{O}_2^\text{2} \) production in the DOCA-salt normal diet group and in the DOCA-salt triple therapy group were nearly equal. The reason for this difference remains unclear. However, several researchers reported that lowering of blood pressure improved endothelial dysfunction in DOCA-salt hypertensive model\(^\text{27}\) and Dahl salt-sensitive model.\(^\text{28}\) Thus, at least in salt-sensitive hypertensive model, the impaired Ach-induced endothelium-dependent vasodilatory responses might be secondary to the elevation of blood pressure.

In the present study, endothelium-dependent vasodilatory responses in the DOCA-salt tempol-treated group were close to those in the DOCA-salt normal diet group despite the fact that the blood pressure in the DOCA-salt tempol-treated group was lower than that in the DOCA-salt normal diet group. This result might be due to the SOD mimetic effect of tempol. Most recently, Chen et al.\(^\text{29}\) reported that renal medullary interstitial infusion of tempol increased the formation of \(\text{H}_2\text{O}_2 \) in the renal medulla in anesthetized rats, and that this increase in \(\text{H}_2\text{O}_2 \) formation counteracted the renal vasodilatory effect of tempol. Therefore, in the present study, long-term treatment with tempol might increase the vascular \(\text{H}_2\text{O}_2 \), which is capable of impairing the endothelium-dependent vasodilatory response in the DOCA-salt tempol treated group.

In the present study, sesamin feeding and tempol treatment suppressed the DOCA-salt-induced vascular \(\text{O}_2^\text{2} \) production at almost the same potencies. However, sesamin feeding showed a greater inhibition of blood pressure elevation, compared with the case of tempol treatment. Thus, it is possible that an unknown property of sesamin, which is independent
of antioxidative effect, is partly involved in its antihypertensive effect. Several humoral factors such as the renin-angiotensin system are known to play an important role in the development of hypertension. However, it is unlikely that sesamin exerts antihypertensive action by interfering with the renin-angiotensin system, since this lignan is more effective in renin-independent DOCA-salt hypertension than on the renin-dependent two-kidney, one-clip renal hypertensive model. On the other hand, in our separate experiments using the rat aortic ring, sesamin produced Ca\(^{2+}\)-antagonistic vasorelaxing activity (unpublished data). This pharmacological action, at least in part, may contribute to its antihypertensive activity.

In summary, both sesamin feeding and tempol treatment, which inhibited the DOCA-salt-induced enhancement of vascular \(O_2\) production, suppressed the blood pressure elevation in DOCA-salt hypertensive model. In addition, triple therapy, which showed the potent blood pressure lowering effect, failed to suppress the DOCA-salt-induced enhancement of vascular \(O_2\) production. These results suggest that antioxidative activities of sesamin and tempol, at least partly, contribute to their antihypertensive actions. On the other hand, triple therapy, which mainly lowers the blood pressure through adrenergic nerve inhibitory effect, direct vasodilatory effect and diuretic effect, improved the impairment of DOCA-salt-induced endothelium-dependent vasodilatory responses, suggesting that the impaired endothelium-dependentvasodilatory responses in DOCA-salt rats may be secondary to the elevation of blood pressure. In conclusion, sesamin feeding inhibits the enhancement of aortic \(O_2\) production in DOCA-salt hypertensive rats, and this antioxidative property is closely related to the antihypertensive effect of sesamin. The improvement of endothelial dysfunction in sesamin-fed DOCA-salt animals seems to result from the blood pressure lowering effect.

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