Symposium

Effects of Vitamin E and Sesamin on Hypertension and Cerebral Thrombogenesis in Stroke-Prone Spontaneously Hypertensive Rats

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The preventive effects of sesamin, a lignan from sesame oil, and vitamin E on hypertension and thrombosis were examined using stroke-prone spontaneously hypertensive rats (SHRSP). At 5 weeks of age the animals were separated into four groups: (i) a control group; (ii) a vitamin E group, which was given a 1,000 mg α -tocopherol/kg diet; (iii) a sesamin group, given a 1,000 mg sesamin/kg diet; and (iv) a vitamin E plus sesamin group, given a 1,000 mg α -tocopherol plus 1,000 mg sesamin/kg diet for 5 weeks from 5 to 10 weeks of age. Resting blood pressure was measured by the tail-cuff method once weekly. A closed cranial window was created and platelet-rich thrombi were induced *in vivo* using a helium-neon laser technique. The number of laser pulses required for formation of an occlusive thrombus was used as an index of thrombotic tendency. In control rats, systolic blood pressure and the amount of urinary 8-hydroxy-2'-deoxyguanosine (8-OHdG) became significantly elevated with age. However, the elevation in blood pressure and 8-OHdG were significantly suppressed in rats administered vitamin E, sesamin, or vitamin E plus sesamin. At 10 weeks, the number of laser pulses required to induce an occlusive thrombus in arterioles of the control group was significantly lower than in the other groups ($p < 0.05$). These results indicate that chronic ingestion of vitamin E and sesamin attenuated each of elevation in blood pressure, oxidative stress and thrombotic tendency, suggesting that these treatments might be beneficial in the prevention of hypertension and stroke. (*Hypertens Res* 2001; 24: 735–742)

Key Words: vitamin E, sesamin, blood pressure, cerebral thrombus, SHRSP

Introduction

Stroke-prone spontaneously hypertensive rats (SHRSP), which are characterized by severe spontaneous hypertension and the development of cerebrovascular disease, were originally isolated from a colony of spontaneously hypertensive rats (SHR) and have been used widely to study hypertension

and stroke (1–3). McIntyre *et al.* showed that free radicals are associated with the maintenance of hypertension in SHRSP (4). We demonstrated that free radicals heavily damaged neurons from SHRSP under conditions of hypoxia and oxygen reperfusion (5). In our previous study, mean red blood cell velocities (MRBCV) in cerebral pial arteries were significantly lower in SHRSP than in Wistar Kyoto Rats (WKY) at the age of 16 weeks and were markedly lower at

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32 weeks (6). Alkayed *et al.* suggested that changes in cerebral blood flow were one of the important factors in neuronal cell damage after cerebral ischemia and reperfusion in SHRSP (7). Oxygen free radicals or oxidants have been proposed to be involved in acute central nervous system injury produced by ischemia and reperfusion in the cerebral circulation (8). Many findings have indicated that oxidative stress is associated with aging and severe age-related degenerative diseases, including cancer and cardiovascular disease (9–12). The possible involvement of oxidative stress in hypertension, the most serious risk for cardiovascular disease, was recently reported in both a hypertensive rat model and in man (13, 14). Some epidemiological studies reported that vitamin E (α -tocopherol) attenuates this elevation in blood pressure (15, 16). The same studies suggested that this anti-hypertensive effect was a result of the oxidative stress-reducing (antioxidant) properties of vitamin E. We previously demonstrated that SHRSP neurons are more vulnerable than those from WKY and that antioxidants including vitamin E prevented neuronal cell death both *in vitro* (17) and *in vivo* (18). Recently, some studies showed that sesamin, a lignan from sesame oil, has potent antioxidative effects (19, 20). Moreover, Yamashita *et al.* have indicated that sesame seed lignan enhances vitamin E activity in rats (21). It has been suggested that 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanine base (22, 23). Urinary 8-OHdG is considered a new putative biomarker of the total systemic oxidative stress *in vivo*. 8-Hydroxy-2'-deoxyguanosine can be measured by an enzyme-linked immunosorbent assay (ELISA) (24, 25).

Thus, we hypothesized that vitamin E and sesamin may have preventive effects not only on hypertension but also on cerebral thrombosis in SHRSP. The purpose of this study was to determine whether sesamin and vitamin E can prevent hypertension and cerebral thrombosis in SHRSP. To this end, we measured blood pressure, urinary 8-OHdG excretion, cerebral arteriole diameter, MRBCV and thrombus formation under different conditions of diet.

Methods

Animals and Experimental Protocol

Twenty male SHRSP rats, 5 weeks of age, were purchased from the Disease Model Cooperative Research Association (Kyoto, Japan). All experiments in the present study conformed to the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences, as drawn up by the Physiological Society of Japan (26). Sesamin (sesamin content >97.0 %; not including epi-sesamin) was obtained by supercritical fluid extraction in this experiment (Fujimi-Youhou-En, Saitama, Japan). Animals were separated into four groups: (i) a control group, which was given a normal diet; (ii) a vitamin E group, given a 1,000 mg α -toco-

pherol/kg diet; (iii) a sesamin group, given a 1,000 mg sesamin/kg diet; and (iv) a vitamin E plus sesamin group, given a 1,000 mg α -tocopherol plus 1,000 mg sesamin/kg diet for 5 weeks from 5 to 10 weeks of age. Resting blood pressure was measured by the tail-cuff method (UR-1000; Ueda Seisakusho, Tokyo, Japan) once weekly.

Measurement of Oxidative DNA Damage

Twenty-four hour urine was collected with a metabolic cage (NALGENE; Nalge, New York, USA). Urine samples were filtered (Acrodisc LC13, 0.45 μ m; Gelman Science, Ann Arbor, USA) and were used for the determination of 8-OHdG with competitive enzyme-linked immunosorbent assay (ELISA) (8-OHdG check; Japan Institute for the Control of Aging, Shizuoka, Japan). It has been established that the characterization of the monoclonal antibody is specific for 8-OHdG (27, 28).

Closed Cranial Window

Cranial windows were created as described by Morii *et al.* (29). Briefly, the experimental animals were anesthetized with sodium pentobarbital (60 mg/kg) and, after tracheotomy, artificially ventilated with 25% oxygen in air. Both femoral arteries were exposed and cannulated with polyethylene tubing (PE50; Beckton Dickinson and Co., Parsippany, USA) to collect blood samples for the measurement of blood gases and pH (Blood Gas Analyzer 248; Bayer Medical, Medford, USA) and for the measurement of mean arterial blood pressure. The rate of respiration and the stroke volume of the respirator were adjusted to maintain constant blood gases and pH levels. One of the femoral veins was cannulated for the administration of Evans blue dye. Animals were immobilized in a stereotaxic frame and a craniotomy was performed using a hand drill to form a cranial window 5 mm in diameter in the center of the right parietal bone. A coverslip 14 mm in diameter was placed over the window and secured with dental resin. Artificial cerebrospinal fluid (1.3 mmol/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 2.6 mmol/l KCl, 0.9 mmol/l $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 21.0 mmol/l NaHCO_3 , 125 mmol/l NaCl, 3.5 mmol/l dextrose, pH 7.2–7.4) was continuously infused within the cranial window and the intracranial pressure was adjusted to 3–5 mmHg to avoid brain herniation. The animal in the stereotaxic frame was placed on the stage of an Olympus BH2 microscope equipped with a long working distance objective (Olympus ULWD MSPlan 20 $\times$ 20; Olympus, Tokyo, Japan) and the cerebral vessels were monitored with a CCD camera (Pulnix; Takenaka System, Kyoto, Japan).

Measurements of Arteriole Diameter and Mean Red Blood Cell Velocity (MRBCV)

The diameters of cerebral arterioles were determined from a montage of video images of the entire closed cranial window

captured on a personal computer. The vessels were classified according to the scheme originally devised by Ley *et al.* (30) and originally described in detail by Horton (31) and Fenton and Zweifach (32). The branches of the middle cerebral arterioles were defined in order from A1 to A4. MRBCV (mm/s) in cerebral arterioles was measured with a fiber-optic laser Doppler anemometer microscope (33, 34). Wall shear rates were calculated according to the equation $(8 \times \text{MRBCV})/(\text{inner diameter of the blood vessel})$ (35).

Thrombotic Potential by the He-Ne Laser-Induced Thrombosis Method

This method was established by Gorog and Kovacs (36, 37) and has been utilized for the measurement of thrombotic tendency and platelet reactivity (38, 39). A He-Ne laser beam, 15 μm in diameter, was focused on the center of selected blood vessels through the optical path of the microscope and thrombi were formed by repeated irradiation for 10 s at 20-s intervals at a power of 8 mW in arterioles (20–35 μm) and 13 mW in venules (25–40 μm). The number of laser pulses needed to form an occlusive thrombus was used as an index of thrombotic tendency.

Statistical Analysis

Results are expressed as the means $\pm$ SE for each experiment. Statistical evaluation was performed by analysis of variance (ANOVA), and by Fisher's post hoc test using commercially available statistical packages (StatView 5.0 and SuperANOVA; ABACUS Concepts, Inc., Berkeley, USA). Values of p less than 0.05 were considered to indicate statistical significance.

Results

Body Weight, Systolic Blood Pressure and Blood Parameters

Body weights, blood pressures, heart rates and blood parameters for each group of animals are shown in Table 1. There were no statistically significant differences in body weight or blood parameters among the four groups at 10 weeks of age. Body weight increased over time up to 10 weeks of age without statistically significant differences among the groups (Fig. 1). Systolic blood pressure in the control rats increased significantly from 6 weeks of age and reached a plateau at 8 weeks (Fig. 2). This elevation in blood pressure was significantly suppressed in rats administered vitamin E, sesamin, or vitamin E plus sesamin. The antihypertensive effect of vitamin E was stronger than that of sesamin ($p < 0.05$). Moreover, systolic blood pressure in the vitamin E plus sesamin group was significantly lower than in the vitamin E group ($p < 0.05$), indicating a synergistic effect of sesamin and vitamin E on

blood pressure at 10 weeks of age.

Effects of Vitamin E and Sesamin on Cerebral Vessel Diameter and Microcirculation

Since the rate of respiration and the stroke volume of the respirator were adjusted to maintain constant blood gases and pH, there were no statistically significant differences in PaO_2 , PaCO_2 or pH among the four groups at 10 weeks of age (Table 2). Physical changes in the cerebral vasculature were assessed by measurements of blood vessel diameters in the branches of the middle cerebral artery as shown in Fig. 3. In the sesamin, vitamin E, and vitamin E plus sesamin groups, pial arteriole diameter was significantly greater than in the control group in all branches ($p < 0.05$). Moreover, the vasodilator effects were more pronounced in smaller microvessels. MRBCV (mm/s; Fig. 4) in the sesamin (15.2 ± 0.6), vitamin E (17.7 ± 0.9) and vitamin E plus sesamin (21.1 ± 0.5) groups were significantly greater than in the control group (12.2 ± 0.3) at 10 weeks of age ($p < 0.05$). In addition, cerebral blood flow (nl/s), calculated from red cell velocity and vessel diameter for each vessel, was significantly greater in the sesamin (48.3 ± 2.5) and vitamin E (56.6 ± 5.9) groups than in the control group (25.9 ± 2.5 ; $p < 0.05$). Furthermore, a synergistic effect of vitamin E and sesamin on cerebral blood flow was observed in the vitamin E plus sesamin group (70.8 ± 4.6 ; $p < 0.05$ vs. vitamin E group, Fig. 5).

Effects of Vitamin E and Sesamin on Cerebral Thrombosis

The thrombotic tendency in cerebral microvessels for each group of animals, as assessed by the He-Ne laser technique, is illustrated in Fig. 6. In the sesamin, vitamin E and vitamin E plus sesamin groups (7.0 ± 0.4 , $n=3$; 7.6 ± 0.2 , $n=3$; and 9.4 ± 0.1 , $n=3$, respectively), the number of laser pulses required to induce an occlusive thrombus in cerebral arterioles was significantly higher than in the control group (4.1 ± 0.4 , $n=3$) at the age of 10 weeks ($p < 0.05$). There were no apparent differences in thrombogenicity between arterioles and venules in these four groups of rats.

Oxidative Stress

The amount of urinary 8-OHdG, adjusted for body weight, was calculated (Fig. 7). At the age of 5 weeks, there were no significant differences in the amount of urinary 8-OHdG among the four groups. In the control rats, the amount of urinary 8-OHdG increased significantly with age ($p < 0.05$). However, the amount of urinary 8-OHdG in the sesamin ($1,046 \pm 77$), vitamin E (933 ± 154) and vitamin E plus sesamin (966 ± 101) groups were significantly lower than that in the control group ($1,778 \pm 100$) at 10 weeks of age ($p < 0.05$).

Table 1. Body Weight, Blood Pressure, Heart Rate and Blood Parameters

Parameters	Control (n=5)	Sesamin (n=5)	Vitamin E (n=5)	Vitamin E plus sesamin (n=5)
Body weight (g)	232±4	229±5	212±8	229±8
Systolic blood pressure (mmHg)	261±3	241±3*	230±1*	209±7*, †, §
Mean blood pressure (mmHg)	174±3	149±5*	147±5*	120±6*, †, §
Heart rate (beat/min)	366±1	350±2*	368±9	362±3
Red blood cell ($\times 10^4/\mu\text{l}$)	938±32	893±45	914±33	942±16
White blood cell ($\times 10^2/\mu\text{l}$)	38.2±2.1	38.0±4.6	41.6±5.8	44.8±4.3
Platelet ($\times 10^4/\mu\text{l}$)	66.3±5.2	67.3±7.3	72.7±6.9	64.3±2.3
Hemoglobin (g/dl)	18.2±1.0	19.9±1.6	20.0±1.6	18.4±0.9
Hematocrit (%)	45.4±4.1	51.1±1.7	51.8±1.6	52.6±0.9

Data are the means±SE and *n* is the number of rats. *p<0.05 vs. control. †*p*<0.05 vs. sesamin. §*p*<0.05 vs. vitamin E.

Fig. 1. Changes in body weight (g) during sesamin (closed circles), vitamin E (open squares) and sesamin plus vitamin E (closed squares) intake as compared with a control group (open circles). Data shown are the means±SE (*n*=5).

Fig. 2. Changes in systolic blood pressure (SBP; mmHg) during sesamin (closed circles), vitamin E (open squares) and sesamin plus vitamin E (closed squares) intake as compared with a control group (open circles). SBP was measured by the tail-cuff method for 5 weeks in rats from 5 to 10 weeks of age. Sesamin and vitamin E intake significantly reduced SBP from 6 weeks of age onward. Data shown are the means±SE (*n*=5). *p<0.05 vs. control, †*p*<0.05 vs. sesamin, §*p*<0.05 vs. vitamin E.

Discussion

In the present study, the antihypertensive and anti-thrombotic effects of sesamin and vitamin E intake were investigated in SHRSP. We observed a synergistic effect of sesamin and vitamin E on hypertension and thrombosis. Oxidative stress in cardiac and vascular myocytes describes injury caused to cells resulting from increased formation of reactive oxygen intermediate (ROI) and/or decreased antioxidant reserves. Increases in ROI generation seem to be due to impaired mitochondrial reduction of molecular oxygen to water, secretion of ROI by white blood cells, endothelial dysfunction, and auto-oxidation of catecholamines, as well as exposure to radiation or air pollution (40, 41). Vitamin E is a mixture of

compounds known as tocopherols, the most potent antioxidant of which is α -tocopherol (42). Low levels of vitamin E are related to a higher occurrence of cardiovascular disease, and increased intake of vitamin E decreases the risk of coronary heart disease (43, 44). Mehta *et al.* assessed the modulation of arterial thrombosis by vitamin E in rats. They showed that the antioxidant vitamin E has important effects on platelet aggregation, superoxide dismutase activity, superoxide generation and thrombus formation (45). In the present study, systolic blood pressure and the amount of urinary 8-OHdG became significantly elevated with age in control

Table 2. PaO₂, PaCO₂ and pH on Measurement of Cerebral Microcirculation

Parameters	Control (n=3)	Sesamin (n=3)	Vitamin E (n=3)	Vitamin E plus sesamin (n=3)
PaO ₂ (mmHg)	129±4	127±4	128±5	126±2
PaCO ₂ (mmHg)	38.4±2.0	38.1±1.4	36.9±1.3	35.8±1.8
pH	7.45±0.02	7.50±0.03	7.50±0.01	7.50±0.07

Data are the means±SE and *n* is the number of rats.

Fig. 3. Changes in vessel diameters after sesamin (hatched bars), vitamin E (shaded bars) and sesamin plus vitamin E (closed bars) intake. Branches of the middle cerebral artery were defined as sections A1–A4 and vessel diameters were measured at 10 weeks of age. Data shown are the means±SE (*n*=3 at each point). *p<0.05 vs. control (open bars), †*p*<0.05 vs. sesamin, §*p*<0.05 vs. vitamin E.

Fig. 4. Mean red blood cell velocity in control (open bar), sesamin (hatched bar), vitamin E (shaded bar) and sesamin plus vitamin E (closed bar) groups as determined by a fiber-optic laser Doppler anemometer microscope. Data shown are the means±SE (*n*=3 at each point). *p<0.05 vs. control, †*p*<0.05 vs. sesamin, §*p*<0.05 vs. vitamin E.

rats. However, the elevation in blood pressure and 8-OHdG were significantly suppressed in rats administrated vitamin E, sesamin, or vitamin E plus sesamin. We propose that the antioxidative properties of vitamin E and sesamin were responsible for the reduction of systolic blood pressure and the prevention of cerebral infarct and thrombus formation in the present study.

Some studies of the antihypertensive effects of sesamin have been carried out using deoxycorticosterone acetate (DOCA)-salt hypertensive rats (46) and salt-loaded SHRSP (47). Those reports suggested that sesamin feeding ameliorated the development of salt-induced vascular hypertrophy in both the aorta and mesenteric artery. Furthermore, Newaz *et al.* recently reported that α -tocopherol increased nitric oxide synthase activity in blood vessels of SHR (48). As sesamin and vitamin E prevented hypertension in this study, nitric oxide synthase activity may also be increased by vitamin E and sesamin. In terms of spontaneous hypertension, several authors have found a reduction in the number of cerebral arterioles in SHR, and showed that this reduction

was accompanied by structural changes of the arterioles (49). The number of arterioles perfused was decreased even after maximal dilation. The reduction in arteriole numbers was clearly an early characteristic of spontaneous hypertension in the rat, since other works have shown that the number of terminal arterioles is reduced by almost 50% compared to that in normotensive controls (50, 51). Cerebral arterioles undergo structural alterations in several models of chronic hypertension. In SHR and SHRSP, arterioles undergo hypertrophy of the vessel wall accompanied by a paradoxical increase in distensibility and at the same time, remodeling with a reduction in external diameter (52, 53). Mayhan *et al.* showed that in cerebral arterioles of SHRSP, endothelium-dependent dilation in response to acetylcholine was completely abolished (54). In a previous study, we demonstrated that both the number of terminal arterioles and arteriole diameters were decreased on aging in pial microvessels in SHRSP as compared with WKY (39). In the present study, we found that chronic ingestion of vitamin E and sesamin might prevent cerebrovascular disease in SHRSP. Genba *et al.* (55) showed

Fig. 5. Cerebral blood flow in control (open bar), sesamin (hatched bar), vitamin E (shaded bar) and sesamin plus vitamin E (closed bar) groups were calculated according to the equation (inner diameter/2)² × (mean red blood cell velocity). Data shown are the means ± SE (n=3 at each point). *p<0.05 vs. control, †p<0.05 vs. sesamin, §p<0.05 vs. vitamin E.

Number of Laser Pulses

Fig. 6. Anti-thrombotic effects of sesamin (hatched bars), vitamin E (shaded bars) and sesamin plus vitamin E (closed bars). Thrombotic potential was measured in arterioles and venules by He-Ne laser-induced thrombosis. The number of laser pulses required to occlude the vessel completely was used as an index of thrombotic tendency. Thus, an increased number of pulses reflected a reduced tendency to thrombosis. Data shown are the means ± SE (n=3 at each point). *p<0.05 vs. control (open bars), †p<0.05 vs. sesamin, §p<0.05 vs. vitamin E.

8-OHdG (ng/kg/day)

Fig. 7. The amount of urinary 8-OHdG, adjusted for body weight, was calculated in control (open bars), sesamin (hatched bars), vitamin E (shaded bars) and sesamin plus vitamin E (closed bars) groups. Data shown are the means ± SE (n=3 at each point). *p<0.05 vs. control, †p<0.05 vs. sesamin, §p<0.05 vs. vitamin E.

that cerebral ischemia for 20 min in SHRSP induced massive efflux of glutamate, causing delayed neuronal death in the hippocampal CA1 region, whereas the mother strain of SHRSP, WKY, lacked these characteristics under the same conditions. We have demonstrated altered gene expression during hypoxia and reoxygenation in cortical neurons isolated from SHRSP (56). Our data suggested that redox regulatory functions and energy metabolism in SHRSP neurons were markedly inhibited by oxygen stimulation after hypoxia. Oxygen radical generation is believed to occur upon cerebral ischemia and reperfusion, whereby the free radicals heavily damage the neuron (57). Our findings in a previous report suggested that 24 h urinary 8-OHdG was significantly higher in SHRSP than in age-matched normotensive WKY (58). Recently, Cui *et al.* (59) reported that 8-OHdG positive cells were increased in focal cerebral ischemia and reperfusion. Worp *et al.* showed that after permanent focal cerebral ischemia, the infarct is larger in vitamin E-deficient rats than in rats raised on a diet with the usual, supraphysiological amount of vitamin E (60). Those experimental results indicate that administration of vitamin E is effective in protecting the brain against cerebral ischemia. Yamashita *et al.* indicated that sesame seed lignan enhances vitamin E activity in rats (21). Thus, vitamin E and sesamin showed preventive effects not only against hypertension but also against cerebral thrombosis in SHRSP. Similarly, Kamal-Eldin *et al.* suggested that the bioavailability of γ -tocopherol was enhanced in phenol-containing diets as compared with purified diets (61). Our present study showed synergistic effects of

sesamin and vitamin E against hypertension and thrombosis.

In conclusion, this study investigated the antihypertensive and anti-thrombotic effects of sesamin and vitamin E intake in SHRSP rats. Sesamin produced a marked enhancement of the antihypertensive and anti-thrombotic activities of vitamin E in SHRSP. These results indicate that chronic ingestion of vitamin E and sesamin reduced the elevation in blood pressure and thrombotic tendency, suggesting that these treatments might be beneficial for the prevention of hypertension and stroke.

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