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ORIGINAL ARTICLE

Safflower seed polyphenols (*N*-(*p*-coumaroyl)serotonin and *N*-feruloylserotonin) ameliorate atherosclerosis and distensibility of the aortic wall in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits

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Pulse wave velocity (PWV) has been used clinically as a direct measure of arterial stiffness. We investigated the inhibitory effects of defatted safflower seed extract (SSE) and serotonin derivatives (*N*-(*p*-coumaroyl)serotonin, *N*-feruloylserotonin; CS+FS), which are the active components in SSE, on hypercholesterolemia and atherosclerosis, using PWV in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits. SSE and CS+FS were supplemented with a commercial diet containing 0.5% cholesterol for 8 weeks in male KHC rabbits, aged 2 months. Pulse waves were recorded at different aortic positions using two catheters with micromanometers under pentobarbital anesthesia. The atherosclerotic lesioned area in the aorta was significantly reduced in the SSE and CS+FS groups, without significant changes in serum cholesterol and triglyceride levels among the three groups after supplementation. Local PWV (LPWV) in the middle thoracic and distal abdominal aortas was significantly smaller in the SSE and CS+FS groups than in the control group. PWV in the entire aorta was also significantly lower in the SSE and CS+FS groups, compared with that in the control group. Pressure–strain elastic modulus, an index of wall distensibility, was significantly lower in the middle thoracic and middle abdominal aortas in the SSE and CS+FS groups than in the control group. Wall thickness was also significantly smaller in the middle thoracic aorta in the SSE and CS+FS groups compared with that in the control group. Serotonin derivatives inhibited the progress of atherosclerosis and ameliorated wall distensibility, which contributed, in part, to the lowering of LPWV. Serotonin derivatives may be beneficial in improving vascular distensibility and in reducing cardiovascular risk.
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Keywords: elastic modulus; pulse wave velocity; safflower seed extract; serotonin derivatives

INTRODUCTION

Many epidemiological and clinical studies have suggested that antioxidants have a preventive role in atherogenesis. Animal studies have provided evidence that antioxidants prevent lesion formation; however, it is difficult to directly show the preventive effects of antioxidants in clinical studies. To bridge the gap between the results from animal and human studies, it seems important to evaluate the effects of antioxidants on arterial stiffness using a commonly measured index in both humans and experimental animals; for example, pulse wave velocity (PWV).

PWV has been shown to directly reflect arterial stiffness[1,2] with indices such as the abdominal aortic calcification[3] and coronary artery calcification scores.[4] PWV is measured automatically and noninvasively with high validity and reproducibility;[5] thus, PWV is used as a marker of cardiovascular risk.[6] However, there are a limited number of reports in which PWV has been used to estimate arterial stiffness in experimental animals.

The Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbit is a heritable animal model for hypercholesterolemia that is caused by a deficiency of the LDL-receptor. Atherosclerosis develops at the aortic arch and around the orifice of the main branch arteries within 3 months of birth and progresses to the peripheral aortic region over time.[7] The KHC rabbit also shows mild hypertension,[8,9] whereas the Watanabe heritable hyperlipidemic rabbit is almost normotensive,[10,11] or shows only a mild increase in systolic, but not in diastolic, pressures.[12,13] Moreover, although basic information on PWV is

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available on the KHC (described below), little information is known about the Watanabe heritable hyperlipidemic rabbit. Therefore, we chose the KHC rabbit for this study.

It is desirable to measure PWV as locally as possible in the aorta to analyze the effect of antiatherosclerotic agents on aortic stiffness more precisely, because atheromatous plaques do not spread diffusely in the aorta, but concentrate focally.[7,8] We showed previously in KHC rabbits that local pulse wave velocity (LPWV) in different aortic segments was a good reflection of the extent and severity of atherosclerotic lesions in which pulse waves traveled.[8] Detailed information obtained by LPWV may provide a better understanding of the beneficial effects of antiatherogenic agents on cardiovascular hemodynamics.

Safflower (*Carthamus tinctorius L.*) seeds have been used as a traditional herbal medicine in Korea and other Asian countries.[14] Serotonin derivatives, *N*-(*p*-Coumaroyl)serotonin (CS) and *N*-Feruloylserotonin (FS), have been identified as major and unique phenolics in defatted safflower seed extract (SSE).[15–17] It has been documented that serotonin derivatives or SSE show potent antioxidative properties,[16,17] cholesterol-lowering activity[16] and protective effects on postischemic myocardial dysfunction.[18] Koyama *et al.*[17] showed that SSE and serotonin derivatives inhibited LDL and plasma oxidation *in vitro* and *ex vivo*, respectively, and reduced atherosclerotic lesion formation in apolipoprotein E-deficient mice. In addition to animal studies, improvement in several antioxidative and proinflammatory markers by SSE supplementation has recently been reported in healthy volunteers by the same authors.[19] It is expected that SSE/serotonin derivatives could suppress the progression of atherosclerosis and improve the distensibility of the arterial wall and cardiovascular hemodynamics.

In this study, we evaluated the preventive effects of SSE or serotonin derivatives on the extent and severity of atherosclerosis using LPWV in different aortic segments in cholesterol-fed KHC rabbits.

METHODS

Preparation of safflower seed extract

Defatted safflower seeds were provided by Oilseeds International (San Francisco, CA, USA). A total of 100 kg of defatted safflower seeds was washed with water (2000 l) at 30 °C for 30 min while stirring, followed by centrifugation. The residue was extracted with 60% (v/v) ethanol (1500 l) at 60 °C for 60 min with stirring. This procedure was repeated two more times, and the extracts obtained were combined, filtered and concentrated in vacuo at 50–60 °C after the addition of an equal amount of γ-cyclodextrin (γ-CD, CAVAMAX W8 Food; CycloChem, Kobe, Japan) to dry solids in the filtrate to facilitate concentration (as a result, this SSE preparation comprised 50% (w/w) γ-CD). The concentrated defatted safflower seed extract was heat sterilized at 88 °C for 60 min, air-dried at 60 °C for 15 h and then pulverized. A total of 6 kg of powdered SSE was finally obtained. CS and FS were synthesized from 5-HT·HCl, *p*-coumaric acid and *trans*-ferulic acid.[17] The purity of CS (96.4%) and FS (99.5%) was determined using HPLC.

Animals

Twenty-six male, 2-month-old KHC rabbits weighing 2.0 ± 0.2 kg (mean ± s.d.) were purchased from Japan Laboratory Animals (Tokyo, Japan). The rabbits were divided into control (n=9), SSE (n=8) and CS+FS (n=9) groups to equalize the mean and s.d. of serum cholesterol levels. Control group rabbits were fed a commercial diet (RC-4, Oriental Yeast, Tokyo, Japan) containing 0.5% cholesterol at 100 g day^{-1} for 8 weeks to accelerate atherosclerosis. SSE and CS+FS group rabbits were given the same rabbit food containing 4% SSE and 0.32% CS+0.23% FS, respectively. Values of 0.32% CS and 0.23% FS were almost equal to the amounts of CS and FS in 4% SSE. As the SSE preparation was composed of 50% (w/w) γ-CD, the control and CS+FS chow were also supplemented with γ-CD at a final concentration of 2% (by weight). The animals were housed in an air-conditioned room at 22–25 °C, with a relative humidity of about 50% and were provided with tap water *ad libitum*. Food intake and body weight were measured every week for three successive days. All experiments were approved by the Experimental Animal Committee of Fukushima Medical University and were performed in line with the Guidelines of the US National Institutes of Health.

Serum biochemistry

Blood was sampled from the ear auricle artery after overnight fasting before and after the administration of SSE and CS+FS for 8 weeks and centrifuged at 3000 r.p.m. for 10 min to obtain serum or plasma. Serum total (T-Chol), low-density lipoprotein (LDL-Chol) and high-density lipoprotein (HDL-Chol) cholesterol and triglyceride (TG) levels were analyzed by enzymatic methods using an automatic analyzer (AU-5232, Olympus Corporation, Tokyo, Japan). Concentrations of thiobarbituric acid reactive substances (TBARS) in the heart, liver, lung and kidney were determined using the methods described by Ohkawa *et al.*[20]

Surgical procedure and measurement of LPWV

Animals were anesthetized with an intravenous administration of pentobarbital sodium at a dose of 30 mg kg^{-1}, fixed supine and intubated through tracheotomy. Procaine chloride was applied to the incising areas to reduce pain. An additional dose of pentobarbital sodium was administered at 5 mg kg^{-1} every 15–20 min. A catheter with a micromanometer at the tip (SPC-330, 3Fr, Millar Instruments, Dallas, TX, USA) was placed at the origin of the ascending aorta (AA) through the left common carotid artery. Another catheter with a three-micromanometer tip at intervals of 40 mm (SPR-892, 3Fr, Millar Instruments) was introduced into the proximal portion of the descending aorta (position 1; P.1) through the left femoral artery. Pulse waves at AA and at three adjacent descending aortic positions (P.1, P.2 and P.3; P.2, P.3 and P.4; and P.4, P.5 and P.6) were simultaneously recorded by moving the catheter at intervals of 80 mm to the peripheral region. P.1, P.2 and P.3 were located at the proximal, middle and distal thoracic aortas, and P.4, P.5 and P.6 were located at the proximal, middle and distal abdominal aortas, respectively. Pulse waves were fed into a personal computer (PowerBook G4 M9691J/A, Apple, Cupertino, CA, USA) using an analog-to-digital converter (PowerLab System/16SP, AD Instruments, Sydney, Australia). The distance between AA and P.1 was determined as $\Delta D_{AA-FA}-280$ mm, where ΔD_{AA-FA} was the length from AA to the entry site of the catheter at the left femoral artery. LPWV was calculated as $\Delta D/\Delta T$ for 50 successive waves, where ΔD and ΔT were the distance between two adjacent aortic positions and the difference in the rising time of two adjacent pulse waves, respectively. The rising point of pulse waves was the peak of the second derivatives of pulse waves, as described previously.[8]

Measurement of tensile characteristics of the aortic wall *in vivo*

After pulse waves were recorded at different aortic positions, the catheter transducer with three pressure sensors was removed, and an IVUS catheter (2.9 Fr, Eagle Eye Gold, Volcano, Cordova, CA, USA) was advanced to P.1 from the left femoral artery. Intravascular images of the aorta were recorded using the IVUS system (In-Vision Tsunami, EndoSonic, Cordova, CA, USA) at the same positions as those at which pulse waves were measured. The diameter of the aorta at systole and diastole in six successive pulse waves was carefully measured at P.2 and P.5, where the extent of sclerotic damage and LPWV were decreased in the SSE and CS+FS groups. The pressure–strain elastic modulus (E_P) was determined as E_P=PP/(D_s-D_d)/D_m,[8] where D_s, D_d and D_m were systolic, diastolic and mean ((D_s+D_d)/2) diameters, respectively. A ring sample of the wall 3.0 mm in width was excised from P.2 and P.5, where the diameter was measured *in situ* and then weighed with a precision balance. Wall thickness *in situ* was estimated as $h=W/(1.06\times\pi\times D_m\times W_d)$, where 1.06, W and W_d were specific weight,[21] sample weight and sample width, respectively.

Determination of the percent fractional lesioned area and histological section

The aorta was removed from its origin to the bifurcation of the left and right common iliac arteries after the animals were killed, cut open longitudinally and Xerox copied. The outline of the entire aorta and plaques was carefully traced. Monochromatic images were scanned and fed into a personal computer.

The percent fractional lesioned area (PFLA) was calculated using a free image analyzing software (Scion Image, Scion Corporation, Frederick, MD, USA). The lesioned and lesion-free areas within each aortic region were displayed as black and white, respectively. PFLA in each aortic region was determined as a ratio of lesioned area to total surface area in each region. The wall strips used for measuring sample weight and width were fixed in a 10% neutral-buffered formalin solution and embedded into paraffin. The strips were sliced at 5 μm and stained with Elastica-van Gieson stain.

Statistics

Serum and plasma biochemical data, as well as food intake and body weight values, were tested by a repeated measures two-way analysis of variance (ANOVA). Scheffe's multicomparison test was followed by ANOVA when a significant difference ($P<0.05$) was observed. Systolic (SAP), diastolic arterial and pulse (PP) pressures, LPWV and PFLA were compared by repeated measures two-way ANOVAs among the control, SSE and CS+FS groups at different aortic positions or regions. Aortic PWV from the ascending to distal abdominal aorta and the total percent lesioned area in the entire aorta were also tested by a repeated measures two-way ANOVA. The difference in each parameter between the control and two test groups was compared at each aortic position or region using Scheffe's method, when a significant difference ($P<0.05$) was observed in the ANOVA. The TBARS concentration was tested using the repeated measures one-way ANOVA. The Holm–Sidak test was followed by a one-way ANOVA if a significant difference was observed.

RESULTS

Serum biochemical parameters

Serum total (T-Chol) and low-density lipoprotein cholesterol (LDL-Chol) and TG levels were drastically increased when fed the diet containing 0.5% cholesterol for 8 weeks in the three groups. There were no significant differences in T-Chol (64.1 ± 10.1 mmol in the control, 54.6 ± 3.2 in the SSE and 65.5 ± 7.1 in the CS+FS groups; mean ± s.d.), LDL-Chol (32.2 ± 5.7 mmol in the control, 29.9 ± 4.9 in the SSE and 30.1 ± 2.9 in the CS+FS groups) and TG (2.4 ± 1.7 mmol in the control, 2.2 ± 1.5 in the SSE and 2.8 ± 1.9 in the CS+FS groups) levels after cholesterol feeding between the control and two test groups. Food intake was almost 100 g on any day in the three groups. No significant difference in body weight was observed among the three groups before and after feeding.

Antioxidative effects of CS and FS

Antioxidative effects of CS and FS were observed in the heart of KHC rabbits. The TBARS concentration (nmol mg^{-1} protein; in mean ± s.d.) in the heart was 0.65 ± 0.20 in the control group, 0.34 ± 0.16 in the SSE group ($P<0.05$ *vs.* control) and 0.42 ± 0.14 in the CS+FS group ($P<0.05$ *vs.* control).

Arterial pressure and local pulse wave velocity

SAP was significantly lower in the SSE group than in the control group at P.1, P.2 and P.3, and in the CS+FS group than in the control group at AA, P.1 and P.2 (Figure 1). Diastolic arterial pressure showed slightly lower values at each aortic position in the SSE and CS+FS groups than in the control group, although this effect was not significant. PP was significantly smaller in the SSE and CS+FS groups than in the control group at AA, P.1 and P.2, and in the SSE group than in the control group at P.2. LPWV was significantly lower in the SSE and CS+FS groups than in the control group in the distal thoracic (P.2–P.3) ($P<0.01$ and $P<0.05$, respectively, for SSE and CS+FS groups) and distal abdominal (P.5–P.6) ($P<0.05$ for SSE and CS+FS groups) aortas (Figure 2). PWV in the entire aorta (mean ± s.d.) in the SSE (5.6 ± 0.3 m s^{-1}; $P<0.01$) and CS+FS (5.4 ± 0.2 m s^{-1}; $P<0.01$) groups showed a significant decrease compared with that in the control (6.3 ± 0.7 m s^{-1}) group.

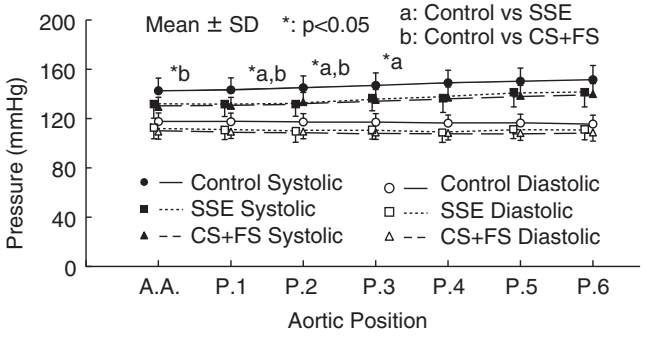

Figure 1 Systolic and diastolic pressures at different aortic positions. CS: *N*-(*p*-Coumaroyl)serotonin; FS: *N*-Feruloylserotonin; SSE: safflower seed extract. AA: ascending aorta; P.1: Position 1; P.2: Position 2; P.3: Position 3; P.4: Position 4; P.5: Position 5; P.6: Position 6. P.1, P.2 and P.3 located at proximal, middle and distal thoracic aortas, respectively. P.4, P.5 and P.6 present at proximal, middle and distal abdominal aortas, respectively.

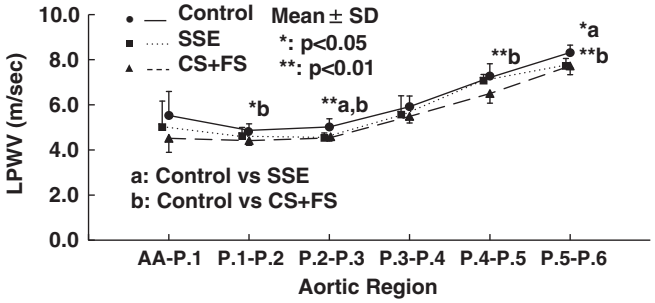

Figure 2 Changes in local pulse wave velocity in different aortic regions. The aortic positions are shown in the legend in Figure 1.

Tensile characteristics of the aortic wall

Figures 3 and 4 illustrate the values of E_p, h and D_m and E_ph/D_m at the middle thoracic (P.2) and middle abdominal (P.5) aortas. The value of E_p was significantly smaller in the SSE ($P<0.01$ and $P<0.05$, respectively, for P.2 and P.5) and CS+FS ($P<0.001$ and $P<0.01$, respectively, for P.2 and P.5) groups than in the control group in P.2 and P.5. The value of h in the SSE ($P<0.05$) and CS+FS ($P<0.01$) groups was significantly lower at P.2 compared with that in the control group, but it did not show a significant change at P.5. There was no significant difference in D_m among the three groups in P.2 and P.5. The value of E_ph/D_m, the inside item of the square root sign in the Moens–Korteweg equation,[22,23] was significantly lower in the SSE ($P<0.001$ and $P<0.05$, respectively, for P.2 and P.5) and CS+FS ($P<0.001$ and $P<0.01$, respectively, for P.2 and P.5) groups than in the control group in P.2 and P.5.

Percent fractional lesioned area

PFLA in the SSE and CS+FS groups tended to be reduced in all aortic regions compared with that in the control group (Figure 5). There were significant differences in PFLA between SSE and control groups in P.2–P.3 ($P<0.05$) and between CS+FS and control groups in AA-P.1 ($P<0.05$), P.2–P.3 ($P<0.05$) and P.3–P.4 ($P<0.05$). Total percent lesioned area in the entire aorta (mean ± s.d.) was significantly smaller in the SSE (38.9 ± 10.0%, $P<0.05$) and CS+FS (37.3 ± 12.1%, $P<0.05$) groups than in the control (53.8 ± 12.2%) group.

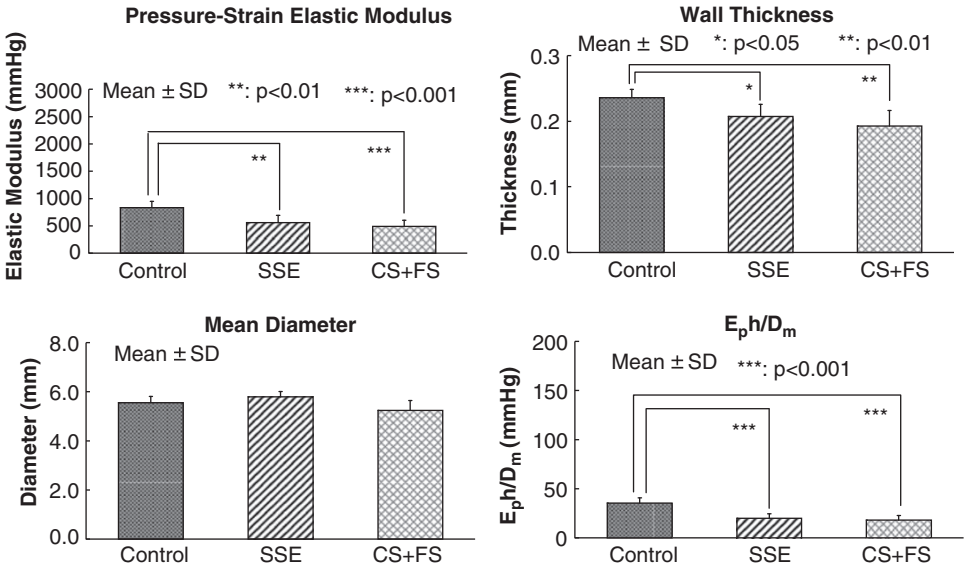

Figure 3 Changes in pressure–strain elastic modulus (E_p), wall thickness (h), mean diameter (D_m) and E_ph/D_m in the middle thoracic aorta (P.2). E_ph/D_m: inside value of the root sign in Moens–Korteweg's equation.

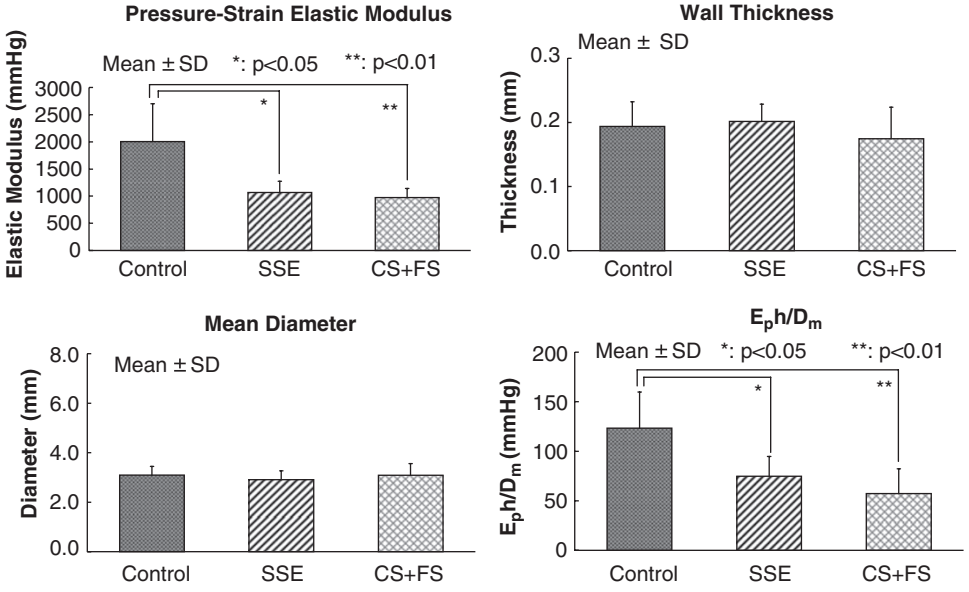

Figure 4 Changes in pressure–strain elastic modulus (E_p), wall thickness (h), mean diameter (D_m) and E_ph/D_m in the middle abdominal aorta (P.5). E_ph/D_m: inside value of the root sign in Moens–Korteweg's equation.

Histological findings

Figure 6 shows photographs of transverse histological sections in the middle thoracic aorta (P.2). Marked intimal thickening with abundant foam cells was observed in the control KHC rabbit. The internal lamina and arrangement of extracellular matrix, such as elastin and collagen fibers, were relatively well preserved in the three groups. Calcification was not observed in any histological sections tested. Severity of lesions was mild in SSE and CS+FS rabbits compared with the control rabbit.

DISCUSSION

Effect of CS and FS on serum lipid levels

In our study, SSE and CS+FS did not attenuate the remarkable increase in serum T-Chol and LDL-Chol levels, nor did it result in a significant change in the HDL-Chol level in the KHC rabbits fed a diet containing 0.5% cholesterol for 8 weeks. By contrast, Cho *et al.*[16] reported that, in ovariectomized rats fed a 0.5% cholesterol-containing diet, T-Chol and HDL-Chol levels significantly decreased and increased, respectively, with the ingestion of serotonin derivatives isolated from defatted safflower seeds for 4 weeks. They suggested that the decreased plasma T-Chol level was due, in part, to the increased formation of HDL-Chol and the excretion of cholesterol. Koyama *et al.*[17] also found in apolipoprotein E-deficient mice fed CS+FS and SSE for 15 weeks that serum T-Chol levels decreased significantly without a significant increase in the HDL-Chol level. These findings were inconsistent with our results in KHC rabbits. The discrepancy mainly arises from the difference in animal species and cholesterol metabolism. In the KHC rabbit, a heritable

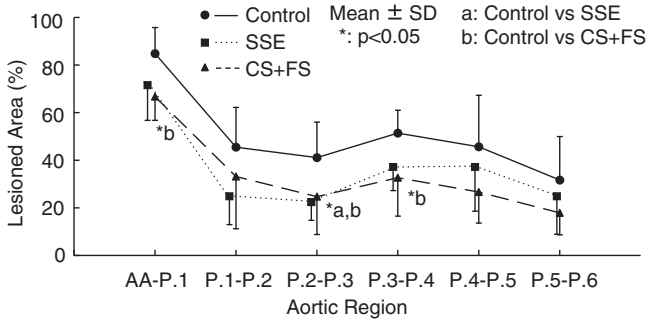

Figure 5 Change in the percent fractional lesioned area in different aortic regions. The aortic positions are shown in the legend in Figure 1.

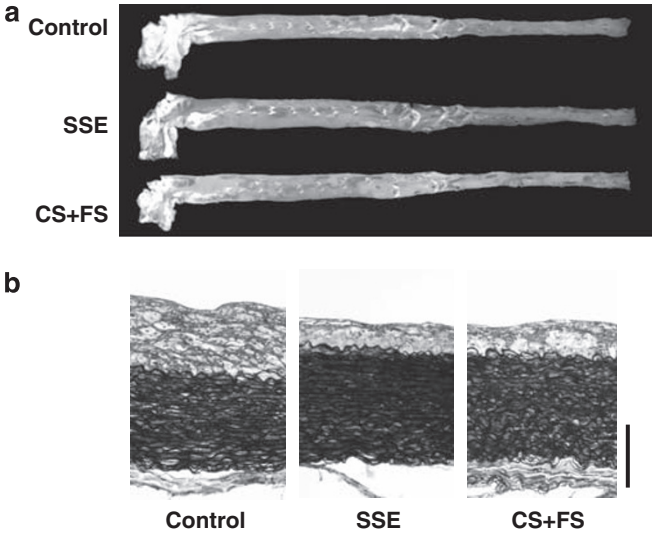

Figure 6 Photographs of the intimal surface of the aorta (**a**) and photomicrographs of transverse histological sections in the middle thoracic aorta (**b**). Elastica-van Gieson stain; perpendicular bar: 100 μm.

hypercholesterolemic animal deficient in the LDL-receptor, the recovery of cholesterol back into hepatocytes and the subsequent excretion into bile are compromised, which may be partly responsible for the small effect of serotonin derivatives on serum T-Chol and TG levels.

Inhibitory effect of CS and FS on atherosclerosis

Antioxidative dietary components, such as polyphenols, have recently been in the limelight, as they have been shown to be effective in the prevention of cardiovascular disease.[24,25] SSE contains some serotonin derivatives composed mainly of CS and FS, which have been reported to act as potent radical scavengers.[16,17] Koyama *et al.*[17] showed that serotonin derivatives (CS and FS) were detectable in both intact and conjugated forms in the plasma in apolipoprotein E-deficient mice fed an SSE-containing diet. They also found that intragastric bolus administration and long-term supplementation of serotonin derivatives inhibited $CuSO_4$-induced plasma oxidation *ex vivo* and decreased the plasma titer of autoantibodies to oxidized LDL, plasma lipid peroxides and the sclerotic lesioned area in apoE-deficient mice.[17] We observed a significant decrease in TBARS concentration in the hearts of SSE and CS+FS groups. Hence, the antioxidative effect of CS and FS may have contributed partly to the attenuation of atherosclerosis.

Effect of CS and FS on LPWV

PWV can be easily quantified with high reproducibility to estimate arterial stiffness, and has thus been frequently measured in recent years in epidemiological studies, mass health screenings and in the diagnosis and assessment of the prognosis of cardiovascular disorders. Recent studies have revealed that PWV is an independent predictor of all-cause[26] and cardiovascular mortality in patients with hypertension,[27] type II diabetes[28] and end-stage renal failure.[29]

PWV can be theoretically explained by the Moens–Korteweg equation;[22,23] $PWV = K\sqrt{(Eh/\rho D)}$, where K, E, h, ρ and D are a constant, the elastic modulus of the wall, the thickness of the wall, the density of the blood, and internal diameter, respectively. The value of ρ was not determined in this study. Because serum cholesterol and triglyceride levels were almost the same in the two groups of rabbits, ρ might not vary between groups. Hasegawa *et al.*[30] also reported no significant differences in ρ even between normal and hyperlipidemic rabbits. There was no significant difference in D between the SSE and CS+FS groups and the control group at any aortic position tested. The significant decrease in LPWV in the distal thoracic and distal abdominal aortas is mainly due to the significant decrease in both E_p and h, and E_p, respectively.

How do CS and FS improve the distensibility of the aortic wall? One candidate mechanism is an antiatherosclerotic effect of CS and FS. The oxidation and intake into macrophages of LDL are pivotal to the development of atherosclerosis.[31,32] The antioxidative actions of CS and FS would be associated with the suppression of atheromatous plaque formation. This was thought to reflect the decreased PFLA and wall thickness in the distal thoracic aorta. Plantinga *et al.*[33] showed that short-term supplementation of the diet with antioxidative vitamins C and E in patients with essential hypertension ameliorated endothelial function and lowered PWV. However, it is difficult to illustrate that the decrease in E_p is only due to the antiatherogenic effect of serotonin derivatives, because there are some paradoxical observations in which a smaller E_p was shown in the aortic wall with early atherosclerotic lesions when compared with the lesion-free normal wall.[34,35] Abundant cholesterol-rich foam cells are considered to endow the wall with viscoelastic properties. Therefore, the wall with more abundant foam cells in the control group can be interpreted to be less rigid than that in the SSE and CS+FS groups. This is inconsistent with the smaller E_p in the SSE and CS+FS groups.

Alterations in the arrangement of the extracellular matrix, such as the presence of elastin and collagen fibers, are greatly involved in the stiffening of the wall, in addition to intimal thickening.[36,37] Medial injury, however, is relatively slight in the control, SSE and CS+FS groups. The age of rabbits (4-months old) is considered to be too early to induce severe remodeling of media, although a 0.5% cholesterol-containing diet was fed to the rabbits for 8 weeks. Medial remodeling may bear some association with a change in E_p in this study.

Another candidate mechanism of lowering E_p is associated with the amelioration of endothelial function due to CS and FS. Endothelial dysfunction has been shown to be related to the development of atherosclerosis and hypertension.[31,38,39] Oxidative stress reduces the bioavailability of nitric oxide, which could lead to suppressed endothelium-derived vasodilation.[38,39] It has been reported that supplementation of antioxidative vitamins improved the endothelium-derived vasodilating function in essential hypertensive[33] and hypercholesterolemic[40] patients. Piga *et al.*[41] recently demonstrated a protective effect of CS and FS against high glucose-induced oxidative damage in cultured human aortic endothelial cells. Therefore, antioxidative actions of CS and FS could have ameliorated endothelial function in the arterial system, which may have contributed partly to the lower

arterial pressure (AP) level and PP. The smaller E_p would be dependent, in part, on the decreased AP level and PP.

Takimoto *et al*.[42] investigated the effects of CS and FS on wall tension in the excised aortic ring of normal rat, the influx of Ca^{2+} into the vessels surrounding smooth muscle cells and the proliferation of smooth muscle cells . They demonstrated that CS and FS exerted direct vasodilatory actions by inhibiting the influx of Ca^{2+} into smooth muscle cells and suppressed the proliferation of smooth muscle cells . These mechanisms contributed, in part, to the decreased E_p in this study. In addition to the suppressing effect of CS and FS on atherosclerosis, CS and FS may exert a vasodilatory effect in normal rabbits. The precise mechanism of vasodilation remains to be elucidated.

In conclusion, safflower seed polyphenols improve atherosclerosis and wall distensibility, which is beneficial for preventing cardiovascular events and for the promotion of health.

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