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Polyphenol-containing azuki bean (*Vigna angularis*) seed coats attenuate vascular oxidative stress and inflammation in spontaneously hypertensive rats

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Abstract

We investigated the effects of azuki bean (*Vigna angularis*) seed coats (ABSC), which contain polyphenols, on the vascular oxidative stress and inflammation associated with hypertension. Spontaneously hypertensive rats (SHR) and control normotensive Wistar-Kyoto (WKY) rats were divided into 2 groups each. One group was fed 0% ABSC; the other, a 1.0% ABSC-containing diet. Tail systolic blood pressure (SBP) was examined throughout ABSC treatment. At 8 weeks, vascular superoxide (O_2^-) production was measured by lucigenin-enhanced chemiluminescence. mRNA expressions of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits, macrophage chemoattractant protein-1 (MCP-1) and its receptor C-C chemokine receptor 2 (CCR2) in the aorta were analyzed by reverse transcriptase-polymerase chain reaction. Protein expressions of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2) were determined by western blotting. Polyphenol-containing ABSC suppressed the elevation of SBP throughout the treatment period. The NADPH-stimulated O_2^- level decreased significantly in the aorta of ABSC-treated SHR compared with the level of untreated SHR. The p47phox and Nox4 mRNA expression increased significantly in untreated SHR compared with that in WKY rats. Conversely, the level of p47phox mRNA was significantly lower in ABSC-treated SHR than in untreated SHR. The protein abundance of both iNOS and COX-2 was significantly decreased in the aorta of the ABSC-treated SHR compared with this abundance in untreated SHR. The MCP-1 and CCR2 mRNA expressions increased in untreated SHR, and these levels were significantly lower in ABSC-treated SHR. In conclusion, our results suggested that polyphenol-containing ABSC could attenuate vascular oxidative stress and inflammation during the progression of hypertension, and this may lead to an improvement in hypertension.

Keywords: Superoxide production; NADPH oxidase; iNOS; MCP-1; Azuki bean seed coats; Spontaneously hypertensive rats

1. Introduction

Hypertension is one of the most important risk factors associated with the development of atherosclerosis. Evidence of increased oxidative stress and inflammatory actions has been shown in several models of experimental hypertension [1–6]. Excessive production of reactive oxygen species (ROS), such as superoxide (O_2^-) and hydroperoxide, causes oxidative stress in the renal and vascular tissues [7–9]. O_2^- is produced by various enzymes, particularly by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, in the endothelium and smooth muscle cells of the arteries. Excess O_2^- forms peroxynitrite ($ONOO^-$), interacting with inducible nitric oxide synthase (iNOS)-derived nitric oxide (NO), and induces endothelial dysfunction and change in vascular response [10,11].

It is known that several inflammatory events occur with the development of hypertension; for example, the expressions of proinflammatory enzymes and cytokines are up-regulated, and the infiltration of immune cells increases, mainly in the kidney and vessels [10,12]. Several studies have reported the accumulation of macrophages, neutrophils, and lymphocytes in accordance with the overexpression of macrophage chemoattractant protein-1 (MCP-1) and its receptor C-C chemokine receptor 2 (CCR2), iNOS and cyclooxygenase-2 (COX-2) in models of experimental hypertension [13–16].

So far, vitamins and plant polyphenols such as quercetin and red wine polyphenols have been known to decrease vascular oxidative stress, mediated by the improvement in vascular reactivity [5,6,17]. Recently, it was reported that plant polyphenols could suppress the vascular expression of NADPH oxidase subunits [6,17]. However, to the best of our knowledge, little is known about the beneficial effects of plant polyphenols on O_2^- production by NADPH oxidase and infiltration of immunocompetent cells into vessels associated with hypertension.

Azuki bean (*Vigna angularis*) is indigenous to the tropical regions of Asia; currently, it is one of the most important crops in Japan, China and South Korea. The seed coats of azuki beans are rich in polyphenols such as proanthocyanidins and quercetin glycoside [18,19]. Proanthocyanidins are natural antioxidants that exert their antioxidant effects

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in pathological conditions such as cardiovascular diseases, inflammation, and diabetes [20–22]. Recently, we showed that the 80% ethanol-extracted azuki beans with antioxidant activity attenuated the elevation of blood pressure in an animal model of hypertension [23]. However, it remains unclear whether azuki beans would attenuate the proinflammation and/or the oxidative stress associated with the progression of hypertension.

This study was designed to examine whether the treatment of spontaneously hypertensive rats (SHR) with azuki bean seed coats (ABSC), which contain polyphenols, could attenuate vascular oxidative stress and inflammation; for this purpose, we measured O_2^- production and the expression of the NADPH oxidase subunits, iNOS, COX-2, and MCP-1/CCR2.

2. Methods and materials

2.1. Plant materials

Seed coats were collected from azuki beans, which were harvested in Japan, according to the method reported previously [24]. Briefly, azuki beans were immersed overnight in distilled water at 25°C. The seed coats were slipped off, collected, dried, and ground in a Waring blender. Approximately 80 g of ABSC was collected from 1 kg of azuki beans. ABSC was mixed with a standard commercial laboratory diet (MF diet; Oriental Yeast, Tokyo, Japan).

Nutrients in the ABSC were analyzed according to the standard procedure; moisture was measured by the air oven method, protein by the Kjeldahl method, fat by the acid hydrolysis method, ash by the ignition method (at 550°C), and dietary fiber by the enzymatic-gravimetric method. Carbohydrate was calculated by the formula of Notification No. 176 (2003), standard for nutrition labeling, Ministry of Health, Labor and Welfare of Japan (http://www.fukushihoken.metro.tokyo.jp/anzen/hoei/image/kkijyun.pdf). The composition of ABSC was as follows: moisture, 10.2 g; crude protein, 6.5 g; crude fat, 0.9 g; crude ash, 7.2 g and crude fiber (carbohydrate), 73.3 g/100 g of total ABSC material. The total phenolic compounds measured by the Folin-Ciocalteu method [25] were found to be 103 mg of *d*-catechin equivalent per gram of ABSC. The catechin, procyanidins B1 and rutin contents were measured by high-performance liquid chromatography or liquid chromatography/mass spectrometry (LC/MS). The ABSC contained 0.30 mg/g catechin, 0.22 mg/g procyanidins B1 and 0.51 mg/g rutin.

ABSC was mixed with a standard commercial laboratory diet (MF diet; Oriental Yeast). According to the information provided by the manufacturer, this diet contained 7.7% moisture, 23.6% crude protein, 5.3% crude fat, 6.1% crude ash, 2.9% crude fiber, 54.4% nitrogen-free extract (including carbohydrates), minerals (Ca, 1.12 g; P, 0.90 g; Mg, 0.26 g; Na, 0.21 g; K, 0.99 g; Mn, 5.89 mg; Fe, 10.8 mg; Cu, 0.82 mg and Zn, 5.28 mg/100 g of the diet), and vitamins (retinol, 2,160 IU; B_1, 2.12 mg; B_2, 1.24 mg; B_6, 0.87 mg; B_{12}, 5.3 μg; C, 4 mg; E, 11.0 mg; pantothenic acid, 2.73 mg; niacin, 10.4 mg; folic acid, 0.20 mg; choline, 0.22 g; biotin, 23.2 μg; and inositol, 578 mg/100 g of the diet).

2.2. Animals and experimental procedure

All experimental procedures were performed in accordance with the Guidelines for Animal Experimentation provided by Aomori University of Health and Welfare. Four-week-old male SHR/Izm and control normotensive Wistar-Kyoto (WKY/Izm) rats were purchased from Japan SLC (Shizuoka, Japan). They were maintained at a temperature of 23±1°C under a 12-h light/dark cycle and provided tap water ad libitum. At 18 weeks of age, the SHR and age-matched WKY rats were randomly divided into two groups each. One group was fed 0% ABSC (MF diet alone); the other, a 1.0% ABSC-containing diet (n=8). The rats were administered the diet for 8 weeks, and systolic blood pressure (SBP) and heart rate of unanesthetized, prewarmed, restrained rats were measured once every 2 weeks by the tail-cuff plethysmography method (model MK-1100; Muromachi Kikai, Tokyo, Japan). Before sacrificing at Week 26, the animals were fasted overnight and then weighed, and blood samples were collected under ether anesthesia. The heart, kidney, liver, aorta and carotid artery were immediately removed, rinsed rapidly and weighed. The aorta tissues were sectioned and kept in saline at 4°C for measurement of O_2^- production, or stored at −80°C for evaluation of mRNA and protein expression.

2.3. Measurement of O_2^- production

O_2^- production was determined using a lucigenin-enhanced chemiluminescence technique. A ring segment of thoracic aorta (2–3 mm) was incubated in 1 ml lucigenin (5 μM) phosphate buffer (pH 7.8) at room temperature to determine the response to the addition of 100 μM NADPH for 3 min; then, chemiluminescence was measured for 30 s by using a sensitive luminometer (TD-20/20; Turner Designs, Sunnyvale, CA, USA). The results were reported as relative light units per minute.

2.4. Real-time polymerase chain reaction

Total RNA in the thoracic aorta was extracted using the SV Total RNA Isolation System (Promega, Madison, WI, USA) according to the manufacturer's instructions, and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). Vascular mRNA levels of Nox4, p47phox, p22phox, MCP-1, and CCR2 were respectively analyzed using the following inventoried primers: TaqMan gene expression assays Rn00580555_m1, Rn00586945_m1, Rn00577357_m1, Rn00585380_m1, and Rn00573193_s1 (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA was also analyzed using the TaqMan Rodent GAPDH Control Reagent Kit (P/N 4308313, Applied Biosystems) as an endogenous control. Real-time polymerase chain reaction (PCR) was performed using the Universal PCR Master Mix (Applied Biosystems) on an Applied Biosystems 7000 sequence detection system, according to the manufacturer's instructions. The gene expression levels were reported as fold of the levels obtained for untreated WKY rats, after adjusting for GAPDH by the 2-(-delta delta C (T)) (CT) method.

2.5. Western blot analysis

Tissues of abdominal aorta were homogenized in 6 times its volume of a homogenization buffer [50 mM *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulphonic acid (HEPES), 150 mM NaCl, 1 mM dithiothreitol, 0.5% (v/v) Tween-20, pH 7.4] containing Protease Inhibitor Cocktail Tablets (Roche Applied Science, Indianapolis, IN, USA). The homogenates were centrifuged at 5000×*g* for 10 min at 4°C. Supernatants were collected, and protein concentration was determined using BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Homogenate proteins were resolved on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electrotransferred onto polyvinylidene difluoride (PVDF) membranes. The membranes were probed with anti-COX-2 (1:200; Cayman Chemical, Ann Arbor, MI, USA) or anti-iNOS antibodies (1:200; BD Biosciences, San Jose, CA, USA). The specifically bound primary antibodies were detected with horseradish peroxidase-conjugated rabbit anti-mouse IgG or swine anti-rabbit IgG antibodies (1:1000; DAKO, Glostrup, DK), and enhanced with ECL Western Blotting Detection Reagents (GE Healthcare UK, Buckinghamshire, UK) on Hyperfilm (GE Healthcare UK). Quantitative analysis of the specific band density was performed using ATTO densitometry software (ATTO, Tokyo, Japan). Protein levels were normalized to the β-actin expression from the same sample.

2.6. Enzyme-linked immunosorbent assay for MCP-1 protein

Rat carotid artery was crushed into powder and resuspended in 100 μl of lysis buffer (20 mM HEPES, 0.4 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EGTA, 1 mM EDTA, 1% Triton X-100 and 20% glycerol) containing Protease Inhibitor Cocktail Tablets (Roche Applied Science). The homogenates were centrifuged at 13,000×*g* for 30 min at 4°C. MCP-1 in the supernatant was quantified using an ELISA kit (Biosource, Invitrogen, Carlsbad, CA, USA). The values were corrected by protein concentrations measured by BCA Protein Assay Kit (Pierce, Rockford, IL, USA).

2.7. Blood chemistry

Plasma samples were separated by centrifugation at 800×*g* for 10 min at 4°C, and tested for sodium, potassium, and glucose by using an autoanalyzer for blood chemistry (Dry-Chem 3500 V; Fuji Film, Tokyo, Japan).

2.8. Statistical analysis

Statistical analyses were performed using one-way analysis of variance, followed by Bonferroni-Dunn test. Each value was expressed as mean±S.E. P<.05 was considered statistically significant.

3. Results

3.1. Effect of ABSC treatment on body and tissue weights, and blood chemistry

No mortality was observed in both the ABSC-treated and untreated SHR and WKY rats. There was no effect of ABSC treatment on the body weights of SHR and WKY rats (Table 1). Although no significant difference was found, the absolute and relative kidney weights of ABSC-treated SHR tended to be lower than those of untreated SHR. There was no significant difference in the plasma sodium and potassium levels between the ABSC-treated and untreated SHR groups. On the other hand, the plasma glucose in ABSC-treated SHR was significantly lower than that in untreated SHR (Table 1).

Table 1
Morphological characteristics of ABSC-treated and untreated SHR and age-matched WKY rats

ABSC	WKY rats		SHR	
	0%	1.0%	0%	1.0%
Body weight at sacrifice (g)	461±6	465±7	400±7*	393±3*
Heart weight (g)	1.19±0.02	1.20±0.02	1.49±0.04*	1.45±0.02*
Kidney weight (g)	3.13±0.04	3.12±0.05	3.09±0.06	2.97±0.03
Liver weight (g)	11.5±0.24	11.2±0.31	13.5±0.38*	12.8±0.15*
Relative heart weight (g/kg)	2.59±0.03	2.58±0.04	3.72±0.05*	3.68±0.04*
Relative kidney weight (g/kg)	6.79±0.10	6.75±0.10	7.74±0.08*	7.54±0.05*
Relative liver weight (g/kg)	25.0±0.34	24.0±0.38	33.8±0.40*	32.6±0.29*
pNa (mEq/l)	144.6±0.8	144.7±0.6	146.8±0.3	147.0±0.4*
pK (mEq/l)	5.1±0.2	4.4±0.2	3.7±0.1*	4.0±0.1*
pGLU (mg/dl)	164.3±2.2	150.9±4.5	235.1±11.3*	214.9±7.4*,#

Values are expressed as mean±S.E. (n=7–8).
pNa, plasma sodium; pK, plasma potassium; and pGLU, plasma glucose.
* P<.05 compared with WKY rats+0% ABSC.
# P<.05 compared with SHR+0% ABSC.

3.2. Effect of ABSC treatment on blood pressure elevation and heart rates

As expected, the SBP of the SHR groups was elevated (Fig. 1). From 2 weeks of ABSC treatment, the SBP of ABSC-treated SHR significantly decreased as compared with that of untreated SHR. These data indicated that ABSC treatment attenuated the elevation of SBP in SHR. There was no difference in the heart rates between the ABSC-treated and untreated SHR (data not shown).

3.3. Effect of ABSC treatment on O_2^- production in the aorta

The NADPH-stimulated O_2^- levels in untreated SHR were significantly higher than those in untreated WKY rats (Fig. 2). Conversely, O_2^- levels in ABSC-treated SHR decreased significantly compared with those in untreated SHR, indicating that ABSC treatment suppressed O_2^- production. There was no difference between the ABSC-treated and untreated WKY rats.

3.4. Effect of ABSC treatment on the mRNA expression of NADPH oxidase subunits

Since ABSC treatment suppressed O_2^- production, we determined the mRNA expression of NADPH oxidase subunits in the thoracic aorta by RT-PCR (Fig. 3). The levels of p47phox mRNA in untreated SHR

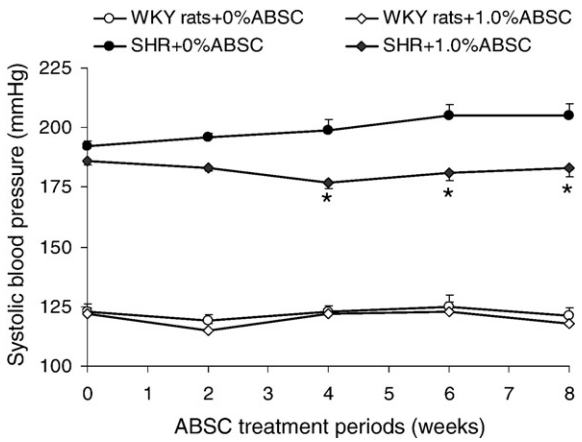

Fig. 1. Effect of ABSC treatment on the elevation of systolic blood pressure. Values are expressed as mean±S.E. (n=8). *P<.05 compared with untreated SHR.

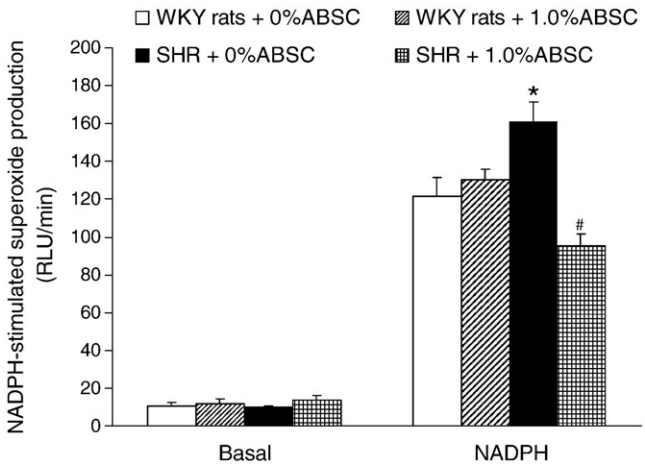

Fig. 2. Effect of ABSC treatment on NADPH-stimulated superoxide production in the aorta of SHR. Values are expressed as mean±S.E. (n=8). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

increased significantly compared with those in the WKY rats. Conversely, the levels of p47phox mRNA in ABSC-treated SHR were significantly lower than those in untreated SHR. The level of Nox4 mRNA expression in untreated SHR was significantly higher than that in WKY rats; however, there was no significant difference between ABSC-treated and untreated SHR. There was no difference between the p22phox mRNA levels of all groups.

3.5. Effect of ABSC treatment on the expression of iNOS and COX-2 proteins

Since iNOS and COX-2 are known to be proinflammatory enzymes [10,14], to examine the effect of ABSC treatment on inflammation, we

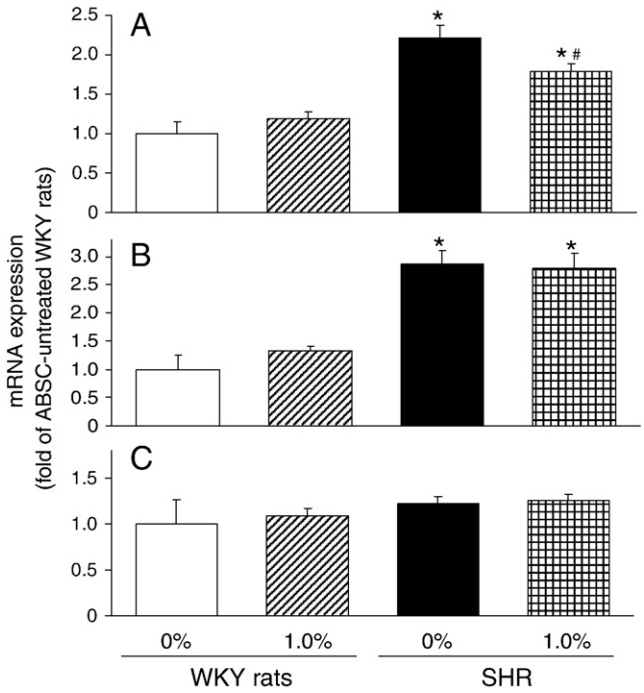

Fig. 3. Effect of ABSC treatment on the mRNA expression of the p47phox (A), Nox4 (B), and p22phox (C), NADPH oxidase subunits in the aorta of SHR. The gene expression levels were quantified as fold of the levels observed in untreated WKY rats, after adjusting for GAPDH. Values are expressed as mean±S.E. (n=5–7). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

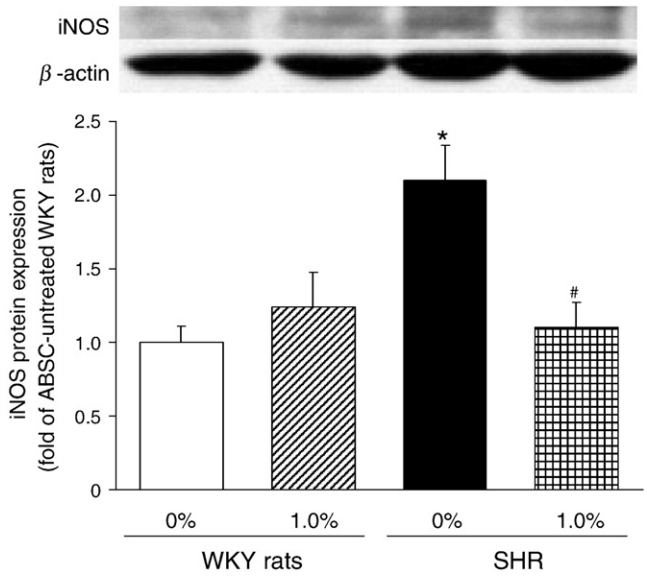

Fig. 4. Effect of ABSC treatment on iNOS protein expression in the aorta of SHR. The protein expression levels were quantified as fold of the levels observed in untreated WKY rats, after adjusting for endogenous β-actin. Values are expressed as mean±S.E. (n=7–8). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

determined the expression of these proteins in the abdominal aorta by western blot analysis (Figs. 4 and 5). Immunoreactive bands corresponding to iNOS and COX-2 were seen at 130 and 72 kDa, respectively, in the proteins extracted from the aorta. The protein abundance of both iNOS and COX-2 in untreated SHR increased as compared with that in untreated WKY rats. In contrast, iNOS and COX-2 protein expression was significantly decreased in the aorta of ABSC-treated SHR.

3.6. Effect of ABSC treatment on the mRNA expression of MCP-1 and CCR2

The expressions of MCP-1 mRNA significantly increased in the thoracic aorta of untreated SHR, as compared with both ABSC-treated and untreated WKY rats (Fig. 6A). Conversely, the levels of MCP-1 mRNA in ABSC-treated SHR were significantly lower than those in untreated SHR. In response to MCP-1, the expression of CCR2 mRNA in the aorta of untreated SHR was higher than that in WKY rats (Fig. 6B). In contrast, the CCR2 mRNA expression in ABSC-treated SHR was significantly decreased compared with untreated SHR, indicating that ABSC treatment suppressed the expression of MCP-1/CCR2 in the arterial walls of SHR.

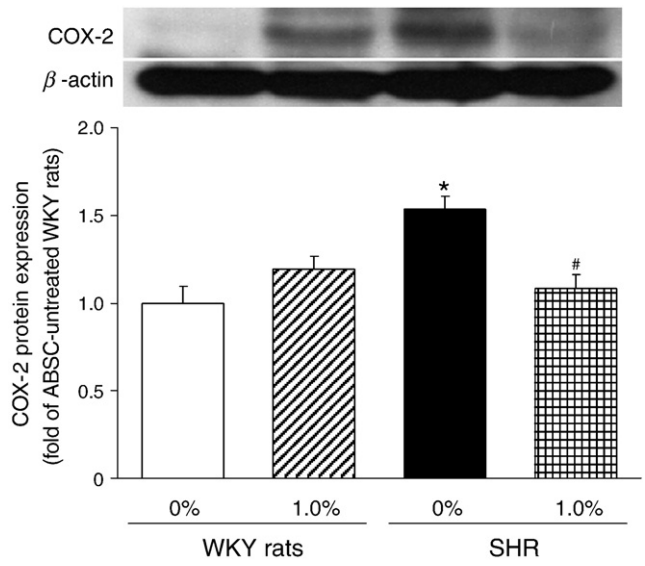

Fig. 5. Effect of ABSC treatment on COX-2 protein expression in the aorta of SHR. The protein expression levels were quantified as fold of the levels observed in untreated WKY rats, after adjusting for endogenous β-actin. Values are expressed as mean±S.E. (n=8). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

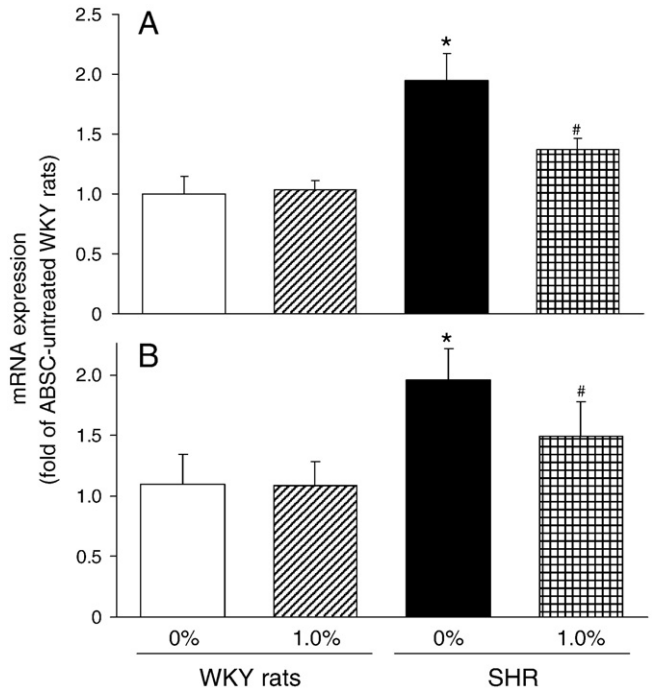

Fig. 6. Effect of ABSC treatment on the mRNA expression of the MCP-1 (A) and CCR2 (B) in the aorta of SHR. The mRNA expression levels were quantified as fold of the levels observed in untreated WKY rats, after adjusting for GAPDH. Values are expressed as mean±S.E. (n=5–7). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

3.7. Effect of ABSC treatment on the expression of MCP-1 protein

The expressions of MCP-1 protein in the carotid artery of untreated SHR increased significantly, as compared with both ABSC-

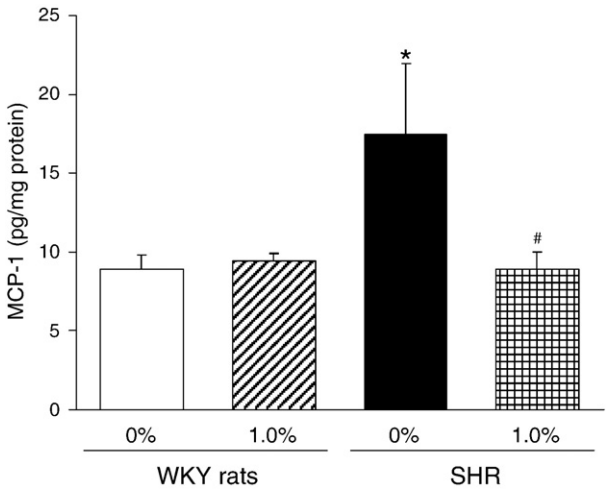

Fig. 7. Effect of ABSC treatment on MCP-1 protein expression in the carotid artery of SHR. Values are expressed as mean±S.E. (n=4–7). *P<.05 compared with untreated WKY rats. #P<.05 compared with untreated SHR.

treated and untreated WKY rats (Fig. 7). Conversely, the levels of MCP-1 protein in ABSC-treated SHR were significantly lower than those in untreated SHR. The results indicated that ABSC treatment suppressed the expression of MCP-1 protein in the carotid artery of SHR.

4. Discussion

The major findings in this study were as follows: (i) ABSC treatment decreased the vascular generation of O_2^-, and suppressed the mRNA expression of the p47phox subunit of NADPH oxidase in the aorta of SHR, and (ii) ABSC treatment suppressed the mRNA expression of MCP-1 and its receptor CCR2 and the protein expression of iNOS and COX-2 in the aorta of SHR.

In this study, we confirmed that polyphenol-containing ABSC suppressed the elevation of SBP throughout the treatment period (Fig. 1). The results were consistent with our previous report that 80% ethanol-extracted azuki beans attenuated blood pressure elevation in young models of hypertension [23]. Moreover, these findings were supported by previous reports that flavonoids such as quercetin and wine polyphenols reduced the development of hypertension [17,26].

In the pathogenesis and maintenance of hypertension, several studies have shown that ROS, especially O_2^-, are excessively produced by endothelial and vascular smooth muscle cells in hypertensive humans and animals [7–9]. In this study, NADPH-stimulated O_2^- levels increased in the aorta of untreated SHR compared with the levels in the aorta of untreated WKY rats (Fig. 2). In contrast, the NADPH-stimulated O_2^- produced in the aorta of ABSC-treated SHR was less than that produced in the aorta of untreated SHR, suggesting that ABSC suppressed excess O_2^- production in the aorta during the progression of hypertension.

One of the most important producers of O_2^- in vascular cells is NADPH oxidase [8,27]. NADPH oxidase in the arterial wall is composed of multiple subunit proteins, such as Nox4, p22phox, p47phox and Rac1 [27]. Previous studies revealed that NADPH oxidase activity was abnormally increased in the aortic wall of hypertensive rats and that this abnormality was associated with up-regulated mRNA and protein expression of NADPH oxidase subunits [28,29]. In the present study, we found that the expression of p47phox and Nox4 mRNA increased in the aorta of untreated SHR. Conversely, this up-regulated expression of p47phox mRNA was suppressed in ABSC-treated SHR (Fig. 3). Our results are supported by several studies that polyphenols, such as red wine polyphenols and quercetin, suppressed the overexpression of NADPH oxidase subunits in the aorta in some models of hypertension [6,17]. Therefore, ABSC seems likely to attenuate O_2^- production via reduction of the expression of the p47phox subunit of NADPH oxidase.

Interestingly, we demonstrated that iNOS protein expression in the aorta of ABSC-treated SHR decreased compared with the expression in the aorta of untreated SHR (Fig. 4). iNOS is known to be a proinflammatory enzyme, and its expression increases in response to various cellular stresses [10]. Several reports have demonstrated that in hypertension, iNOS is overexpressed in the aorta and epithelial cells of the kidney [10,11,30–32]. Excess NO derived from iNOS reacts with enhanced O_2^- to generate $ONOO^-$, which is responsible for vascular dysfunction [33]. Several antioxidants, including flavonoid and tea polyphenol, have been shown to reduce endothelial dysfunction [34]. Therefore, long-term treatment with ABSC might be effective in decreasing $ONOO^-$ concentration and preventing endothelial dysfunction through the suppression iNOS expression and O_2^- production.

In addition, we demonstrated that COX-2 protein expression reduced in the aorta of ABSC-treated SHR compared with the expression in the aorta of untreated SHR (Fig. 5). Increased activity and expression of COX-2 have been described in animal models of hypertension in vivo [14,35] and in vascular cells from hypertensive rats in vitro [36]. Therefore, ABSC may, at least in part, play a role in suppressing the vascular expression of COX-2.

More interestingly, the up-regulated expression of MCP-1 and its receptor CCR2 mRNA decreased in the aorta of ABSC-treated SHR compared with the expression in the aorta of untreated SHR (Fig. 6). MCP-1 is one of the chemotactic factors produced by damaged endothelial cells during the development of atherosclerosis. MCP-1 through the activation of CCR2 can induce migration and attachment of monocytes/macrophages, lymphocytes, endothelial cells and vascular smooth muscle cells [12,13,37,38]. Recent studies have shown that plant polyphenols, such as resveratrol and those found in cava, grape seed, and green tea, attenuated MCP-1 expression in vitro, in experiments in vivo and in humans [39–42]. We have also shown that seed coats of azuki beans reduced the number of infiltrated macrophages and the expression of MCP-1 mRNA in the kidney of the streptozotocin-induced diabetic rats [24]. In addition, plant polyphenols such as cathechin and resveratrol suppressed the expression of MCP-1/CCR2 mRNA on a human monocytic cell line and apolipoprotein E-deficient mice [40,43]. Moreover, we demonstrated that the levels of MCP-1 protein in ABSC-treated SHR were significantly lower than those in untreated SHR (Fig. 7). Taken together with our results and previous studies, we hypothesized that ABSC could suppress the expression of MCP-1/CCR2 mRNA and the infiltration of macrophages during the progression of hypertension.

The reasons for the suppression of SBP in SHR treated with ABSC remain unclear. However, this phenomenon may be interpreted as follows. One possibility is that ABSC may attenuate oxidative stress in the aorta of SHR by suppressing the expression of NADPH oxidase subunits and may lead to the suppression of blood pressure elevation. This hypothesis is supported by several reports that have demonstrated that some antioxidants and flavonoids, such as quercetin and tea polyphenols, attenuate the increase in blood pressure [17,33,44]. Another possibility is that ABSC has anti-inflammatory activity. COX-2 and iNOS are known to be proinflammatory enzymes, and their activity and expression increases during the progression of hypertension [10,14]. In addition, MCP-1/CCR2 system is known to be associated with the activation, attachment and migration of monocytes/macrophages [12,15]. In this study, the up-regulated expression of iNOS, COX-2 and MCP-1/CCR2 reduced significantly in ABSC-treated SHR. Therefore, we suppose that ABSC treatment, at least in part, plays a role in the attenuation of the inflammatory response associated with hypertension. This hypothesis is also supported by several studies that have reported that plant flavonoids, such as cocoa and soy nuts, attenuated the inflammation associated with hypertension in humans and animals [45,46].

In conclusion, we demonstrated that ABSC attenuated vascular O_2^- production by down-regulation of NADPH oxidase subunits, and that ABSC suppressed the expression of iNOS, COX-2 and MCP-1/CCR2 in the aorta of SHR. On the basis of our results, polyphenol-containing ABSC could attenuate vascular oxidative stress and/or inflammation during the progression of hypertension, and this may lead to an improvement in hypertension. ABSC treatment should be useful as a preventive strategy for atherosclerosis.

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