Available online at www.sciencedirect.com

SCIENCE DIRECT

Journal of Nutritional Biochemistry 16 (2005) 547–553

Journal of Nutritional Biochemistry

Protective effect of polyphenol-containing azuki bean (*Vigna angularis*) seed coats on the renal cortex in streptozotocin-induced diabetic rats

Shin Sato[a,*], Jyoji Yamate[b], Yuuka Hori[c], Asako Hatai[c], Megumi Nozawa[a], Masaru Sagai[a]

[a]*Department of Life Sciences, Graduate School of Health Sciences, Aomori University of Health and Welfare, Aomori 030-8505, Japan*
[b]*Laboratory of Veterinary Pathology, Graduate School of Agriculture and Biological Sciences, Osaka Prefecture University, Osaka 599-8531, Japan*
[c]*Department of Food and Nutrition, Hakodate Junior College, Hakodate 042-0955, Japan*

Received 12 January 2005; received in revised form 2 February 2005; accepted 2 February 2005

Abstract

This study was undertaken to investigate the effect of azuki bean (*Vigna angularis*) seed coats (ABSC), which contain polyphenols, on the infiltration of macrophages and the progression of diabetic nephropathy in streptozotocin (STZ)-induced diabetic rats. The diabetic rats were divided into three groups with 0% (commercial diet), 0.1% and 1.0% ABSC diets. The vehicle-injected controls were given a commercial diet. At 10 weeks, the macrophage kinetics, the degree of fibrosis in glomeruli and mRNA expression for monocyte chemoattractant protein-1 (MCP-1) were examined. There was no difference in plasma glucose levels between diabetic rats treated with and without ABSC. The plasma levels of malondialdehyde (MDA) in the ABSC-treated diabetic rats were significantly lower than those in the untreated diabetic rats. Histopathologically, the percentage of the fibrotic areas stained by Sirius red stain in the glomeruli in the ABSC-treated diabetic rats was lower than in the untreated diabetic rats. ED1-positive macrophages in the glomeruli and tubulointerstitium in the untreated diabetic rats showed a significant increase in number compared with the controls. In contrast, the number of macrophages in the ABSC-treated diabetic rats was smaller than that in untreated diabetic rats. MCP-1 mRNA expression, estimated by real-time quantitative RT-PCR, was increased 2.5-fold in the untreated diabetic rat kidney, while a lower level was observed in the ABSC-treated diabetic rats. In conclusion, our results suggest that ABSC treatments suppress the increased number of infiltrating macrophages and MCP-1 mRNA expression, and attenuated the glomerular expansion in STZ-induced rat diabetic nephropathy.

Keywords: Azuki bean; Polyphenols; Macrophage; Monocyte chemoattractant protein-1; Diabetic nephropathy; Rats

1. Introduction

The azuki bean (*Vigna angularis*) has long been widely cultivated in Japan, China, South Korea and Taiwan, and is one of the most important crops in these areas. Azuki beans contain proanthocyanidins, which are a group of polyphenolic bioflavonoids with remarkable radical scavenging activities in vitro [1,2]. An aroma extract from azuki beans inhibits malonaldehyde formation in vitro [3]. Generally, proanthocyanidins are known to be natural antioxidants that possess protective properties against oxidative stress. For example, grape seed proanthocyanidins extract has been reported to have beneficial effects on inflammation [4–6], cancer [7,8] and diabetes [9,10]. Recently, we demonstrated that polyphenol-containing azuki bean seed coats attenuated the increase of infiltrating macrophages and tubulointerstitial fibrosis in kidneys of rats induced by cisplatin, which has been used as a chemotherapeutic agent against malignant carcinoma [11].

Diabetic nephropathy is known to be the leading cause of end-stage renal disease and the most frequent cause of mortality in patients with diabetes. Oxidative stress has been thought to be a potential factor in the progression of diabetic complications [12,13]. Thus far, attenuation of diabetic nephropathy has been demonstrated by using many antioxidants such as vitamin E, α-lipoic acid and flavonoids [14–16]. On the other hand, in the pathogenesis of diabetic nephropathy, infiltration of macrophages in the glomeruli and tubulointerstitium is one of the characteristic features, in addition to accumulation of extracellular matrix protein, which results in mesangial expansion and tubulointerstitial fibrosis [17–20]. In addition, the expression of monocyte chemoattractant protein-1 (MCP-1), involved in infiltrating

* Corresponding author. Tel.: +81 17 765 4184; fax: +81 17 765 4184.
E-mail address: s_sato3@auhw.ac.jp (S. Sato).

0955-2863/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jnutbio.2005.02.003

and activating macrophages, has been thought to play an important role in the fibrogenic process of diabetic nephropathy [13,21]. However, little is known about whether a diet of azuki bean seed coats (ABSC), which contain proanthocyanidins, has any beneficial effects on diabetic nephropathy.

The purpose of the present study was to determine whether seed coats of azuki beans could play a role in the progression of the diabetic nephropathy in streptozotocin (STZ)-induced diabetic rats. In this study, we demonstrate that the ABSC treatments suppress the increased number of infiltrating macrophages and MCP-1 mRNA expression, and attenuate the glomerular expansion in STZ-induced rat diabetic nephropathy.

2. Materials and methods

2.1. Plant materials

Japanese azuki beans harvested in Tokachi, Hokkaido, Japan, in 1998 and 1999 were kindly supplied by Dr. Y. Nagaoka (Biotech, Obihiro, Japan). The ABSC were collected from azuki beans according to the method described previously [11]. In brief, the azuki beans were immersed in distilled water overnight at 25°C. The ABSC were collected, dried, ground in a Waring blender and kept at −80°C. Normally, approximately 80 g of the dried seed coats was collected from 1 kg of azuki beans. 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, crude fiber (carbohydrate) 73.3 g/100 g, polyphenols 7632 mg and proanthocyanidins 2040 mg/100 g of total ABSC materials [11]. They were mixed with a standard commercial laboratory diet (CE-2 diet obtained from CLEA Japan, Tokyo, Japan), the composition of which was moisture 8.6%, crude protein 24.9%, crude fat 4.6%, crude ash 6.7%, crude fiber 3.7%, nitrogen-free extract (including carbohydrates) 51.4 %, minerals (Ca, 1.03 g; P, 0.97 g; Mg, 0.33 g; Na, 0.32 g; K, 1.05 g/100g of total minerals, Mn, 11.26 mg; Fe, 34.35 mg; Cu, 0.79 mg; Zn, 5.35 mg/100 g of total minerals) and vitamins (retinol, 0.96 mg; B_1, 1.70 mg; B_2, 1.33 mg; B_6, 1.25 mg; B_{12}, 6.3 μg; C, 13 mg; E, 6.2 mg; pantothenic acid, 2.90 mg; niacin, 16.9 mg; folic acid, 0.28 mg; choline, 0.21 g; biotin, 41.6 μg; inositol, 616 mg/100 g of total vitamins), according to the description of the manufacturer.

2.2. Animal treatments and sample collections

All procedures were performed according to the regulations of the Guidelines for Animal Experimentation, Aomori University of Health and Welfare. Male Wistar rats (CLEA Japan) weighing 179–202 g were used. They were maintained at a temperature of 23±1°C under a 12-h light–dark cycle starting at 8:00 a.m. The animals received a single intraperitoneal injection of 65 mg/kg body weight of STZ (Sigma, St. Louis, MO, USA) in 0.5 ml of 0.05 mol/L citrate buffer (pH 4.6) to induce diabetes mellitus. The blood glucose concentration was determined 48 h after STZ injection, and it was confirmed that in all diabetic animals it was over 300 mg/dl before starting this study. The diabetic rats were divided into three groups: a control (n=6), 0.1% (n=6) and 1.0% ABSC (n=7) diets. The vehicle-injected control animals (n=7) were given CE-2 diet only. The rats were given each diet and tap water ad libitum. Body weights were measured during the ABSC treatments. Before the animals were killed at week 10, all animals were fasted overnight, then weighed, and blood samples were collected under ether anesthesia. Kidneys were quickly removed, rapidly rinsed, weighed and fixed in 4% paraformaldehyde phosphate buffer solution for histopathology and immunohistochemistry. Portions of the kidneys were immediately frozen in liquid nitrogen and stored at −80°C until used in experiments.

2.3. Blood chemistry

Plasma samples were obtained after centrifugation (3000 rpm for 15 min) and were tested for glucose and blood urea nitrogen (BUN) by using an autoanalyzer for blood chemistry (Dry-Chem 3500 V, Fuji Film, Tokyo, Japan). The amount of lipid peroxidation was determined by measuring the accumulation of thiobarbituric acid reactive substances (TBARS) in plasma using a commercially available kit (Wako, Osaka, Japan) and was expressed as malondialdehyde (MDA) content.

2.4. Histopathology and immunohistochemistry

For histologic examination, the paraformaldehyde-fixed kidney tissues were embedded in paraffin, and sections 4 μm in thickness were stained with hematoxylin–eosin (HE). To assess the degree of fibrosis, staining of collagen fibrils by Sirius red F3BA was carried out according to a previous method [22]. For expression of the ED1 antibody (Chemicon International, Temecula, CA, USA), the avidin–biotin complex method (LSAB 2kit; Dako, Carpinteria, CA, USA) was employed using deparaffinized sections according to a previous method [23]. ED1 is a mouse monoclonal antibody to rat macrophages, labeling blood monocytes and exudate macrophages [24,25]. Briefly, deparaffinized sections were pretreated with 0.1% trypsin solution in phosphate-buffered saline (PBS), pH 7.4, for 15 min at 37°C and 3% H_2O_2 for 10 min at room temperature. Sections were then preincubated with 1.5% skim milk in PBS for 30 min at room temperature and incubated for 14 h at 4°C with the ED1 antibody (1:200), which was diluted in PBS including 1% bovine serum albumin. Thereafter, they were incubated with an antimouse immunoglobulin for 10 min. Final incubation was carried out for 10 min with streptavidin conjugated to horseradish peroxidase, and positive reactions were visualized with 3,3′-diaminobenzidine tetrahydrochloride in 50 mmol/L Tris–HCl buffer, pH 7.4, containing 3% H_2O_2. Sections were counterstained lightly with hematoxylin. Cells showing a distinct immunoreaction for ED1 were counted in 50 randomly selected glomeruli and five

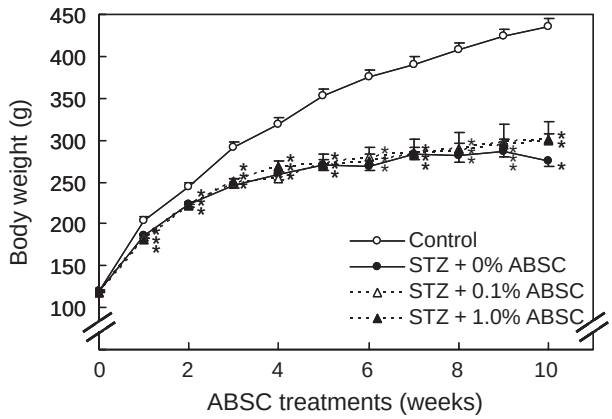

Fig. 1. Effects of ABSC treatments on body weights in control and STZ-induced diabetic rats. Values are expressed as mean±S.E. (n=6–7). *Significantly different from control, P<.01.

randomly selected areas (0.0625 mm^2 each) in the cortico-medullary junctions of sections for all animals.

2.5. Computer-aided morphometrical analysis

To evaluate fibrosis in the glomeruli, the area stained red by Sirius red was measured using a Color Image Analyzer (Mac SCOPE, Mitani, Fukui, Japan) in five randomly selected glomeruli of sections for all animals. The percentage of the fibrotic area per unit of glomerulus was calculated.

2.6. mRNA Analysis for MCP-1

Total RNA was isolated from each kidney using a spin-vacuum (SV) total RNA isolation system (Promega, Madison, WI, USA) according to the manufacturer's protocol. Transcripts from 1 μg of total RNA were reverse-transcribed with predeveloped TaqMan assay reagents (Applied Biosystems, Foster City, CA, USA) using oligo (deoxythymidine)$_{16}$ primer. Elongation was performed for 30 min at 48°C in a GeneAmp PCR system 9600 (Perkin-Elmer, Norwalk, CT, USA), and the enzyme was inactivated for 5 min at 95°C. The resultant complementary DNA was amplified by real-time quantitative reverse transcription–polymerase chain reaction (RT-PCR) using a TaqMan Universal PCR Master Mix (Applied Biosystems). The PCR reaction was directly monitored by the ABI Prism 7000 sequence detection system. To control for variation in the amount of DNA available for RT-PCR in the different samples, gene expression of the target sequences was normalized in relation to the expression of an endogenous control, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA (TaqMan Rodent GAPDH Control Reagent kit, Applied Biosystems). The nucleotide sequences of PCR primers and TaqMan probe were as follows: the forward primers were 5′-TCTCTTCTTCCACCACTA-TGCA- 3′, and the reverse primers were 5′-GGCTGAGAC-AGCACGTGGAT-3′. The TaqMan probe was 5′-FAM-TCACGCTTCTGGGCCTGTTGTTCA-TAMRA-3′. The RT-PCR conditions were one cycle of 50°C for 2 min, 95°C for 10 min, followed by 50 cycles of 95°C for 15 s and 58°C for 1 min, according to a previous report [26]. The amount of amplified cDNA was normalized by the amount of amplified GAPDH cDNA to correct for variations in the initial RNA content.

2.7. Statistical analysis

Each value is expressed as mean±S.E. Statistical analyses were performed by one-way analysis of variance (ANOVA), followed by the Tukey test.

3. Results

3.1. Effects of ABSC treatments on the STZ-induced diabetic rat kidney

There was no mortality in the STZ-induced diabetic rats treated with or without ABSC. The body weights of the STZ-induced diabetic rats were significantly lower than those of control rats (Fig. 1). There was no difference between body weights of ABSC-treated and -untreated diabetic rats. The absolute weights of left and right kidneys in the diabetic rats were heavier than those of controls (Table 1). When expressed as a function of body weight, the relative weights of the left and right kidneys in the diabetic rats were significantly higher than those in controls. On the other hand, a tendency toward lower levels in 1.0% ABSC-treated diabetic rats was observed compared with the untreated diabetic rats. The plasma glucose levels of the diabetic rats were significantly higher than those of control rats (Table 1); there was no difference in the levels between diabetic rats treated with and without ABSC, indicating irreversible damage of pancreatic islet cells by STZ. The

Table 1
Body and kidney weights, and plasma parameters in control and STZ-induced diabetic rats

	Control	STZ+ABSC treatments		
		0%	0.1%	1.0%
BW (g)	421±8	245±8[a]	270±3[a]	266±7[a]
RK (g)	1.40±0.04	1.65±0.08[a]	1.60±0.04[a]	1.53±0.04
LK (g)	1.32±0.07	1.63±0.11[a]	1.59±0.09	1.48±0.03
RK/BW (g/kg)	3.13±0.13	6.70±0.50[a]	6.15±0.55[a]	5.62±0.21[a]
LK/BW (g/kg)	3.32±0.07	6.66±0.38[a]	6.17±0.51[a]	5.81±0.27[a]
Plasma glucose (mg/dl)	128±9	537±29[a]	450±57[a]	520±15[a]
Plasma urea (mg/dl)	15.8±09	45.7±2.8[a]	33.4±3.4[a,b]	33.1±1.8[a,b]
Plasma MDA (nmol/ml)	22.4±3.7	80.5±12.2[a]	20.1±3.6[b]	29.6±6.7[b]

BW, body weight at sacrifice; RK, right kidney; LK, left kidney. Values are expressed as mean±S.E. (n=6–7).
[a] Significantly different from control, P<.05.
[b] Significantly different from ABSC-untreated diabetic rats, P<.05.

plasma levels of BUN in the untreated diabetic rats were significantly higher than those of controls and were lower in the diabetic rats with 0.1% and 1.0% ABSC treatments. The plasma MDA levels of the untreated diabetic rats were significantly higher than those of controls. Compared with the untreated diabetic rats, the levels of MDA in the ABSC-treated diabetic rats significantly decreased.

3.2. Histopathological changes and ED1-positive macrophages in the STZ-induced diabetic rat kidney

Histopathologically, glomerular expansion due to thickening of the basement membrane and mesangial matrix was observed in the diabetic rat kidney; in the corticomedullary junction, tubular cell dropout and atrophy, as well as focal areas of interstitial inflammatory cell infiltration and slight fibrosis, were occasionally seen (data not shown). When the renal damage in the ABSC-treated and -untreated groups was compared, the ABSC-treated rats were considerably ameliorated. There were no pathological changes in controls.

Representative Sirius red stains of renal cortical sections from rats in control, ABSC-untreated and 1.0% ABSC-treated groups are shown in Fig. 2. The basement membrane and mesangial matrix areas in the glomeruli of the ABSC-untreated diabetic rats were expanded compared with controls (Fig. 2A and C). On the other hand, a reduced extent and magnitude of the expansion in the basement membrane and mesangial matrix areas were observed in 1.0% ABSC-treated diabetic rats (Fig. 2E). To estimate the effect of ABSC on the glomerular fibrosis, the area stained red by Sirius red stain in the glomeruli was measured using an image analyzer. The percentage of the fibrous area per glomerulus increased in the ABSC-untreated diabetic rat kidney, showing a significant difference from the controls (Fig. 3). In the diabetic rats treated with ABSC, the percentage of the fibrotic areas was significantly lower than in the ABSC-untreated diabetic rats. These results suggested that the ABSC treatments attenuated the mesangial expansion in the diabetic rat kidney.

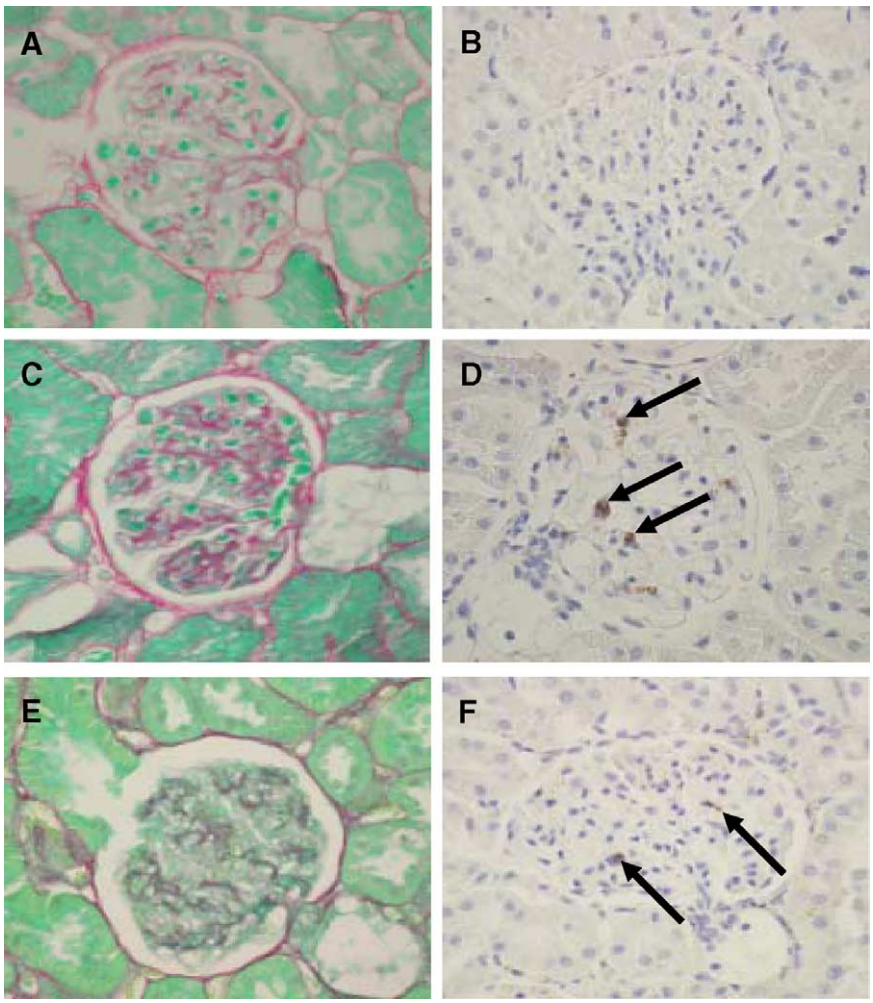

Fig. 2. Histopathology and immunohistochemical staining of rat macrophage-specific ED1 in the glomeruli. (A, B) Controls, (C, D) ABSC-untreated diabetic rats, (E, F) 1.0% ABSC-treated diabetic rats. Sirius red staining in (A), (C) and (E). ED1-positive macrophages are shown by arrows in (D) and (F). Counterstaining with hematoxylin is carried out (original magnification ×600).

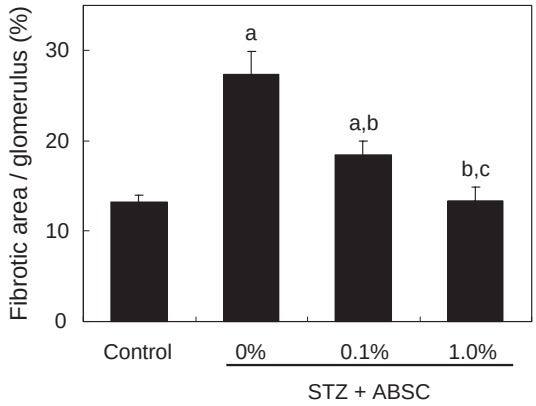

Fig. 3. Effects of ABSC treatments on fibrotic areas per unit of glomerulus in control and STZ-induced diabetic rats. Values are expressed as mean±S.E. (n=6–7). [a]Significantly different from control, P<.01. [b]Significantly different from ABSC-untreated diabetic rats, P<.01. [c]Significantly different from 0.1% ABSC-treated diabetic rats, P<.01.

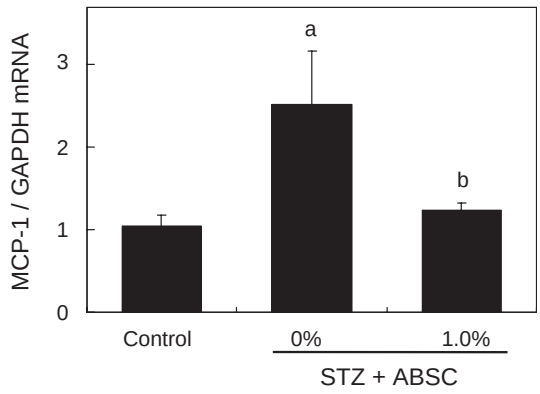

Fig. 5. Expression of MCP-1 mRNA in kidneys of control, ABSC-untreated and 1.0% ABSC-treated diabetic rats. Values are indicated as arbitrary ratios of the amount of expressed mRNA of each factor to that of GADPH. Values are expressed as mean±S.E. (n=6–7). [a]Significantly different from control, P<.05. [b]Significantly different from ABSC-untreated diabetic rats, P<.05.

Immunohistochemical analyses revealed that ED1-positive macrophages were present in the glomeruli in both the ABSC-treated and -untreated diabetic rats (Fig. 2D and F). When compared with the ABSC-untreated rats, fewer ED1-positive macrophages were seen in the glomeruli of the ABSC-treated rats (Fig. 2F). To determine the effect of ABSC on the appearance of macrophages in the diabetic rat kidney, the numbers of ED1-positive macrophages were counted in the glomeruli and tubulointerstitium of the corticomedullary junction. As shown in Fig. 4, ED1-positive macrophages in the ABSC-untreated diabetic rats showed a significant increase in number (P<.01), compared with those of control rats. On the other hand, the number of macrophages in the diabetic rats treated with 1.0% ABSC was significantly smaller than that in the ABSC-untreated diabetic rats (P<.01). These results indicated that the ABSC treatments prevented the appearance of ED1-positive macrophages in both the glomerular and tubulointerstitial areas.

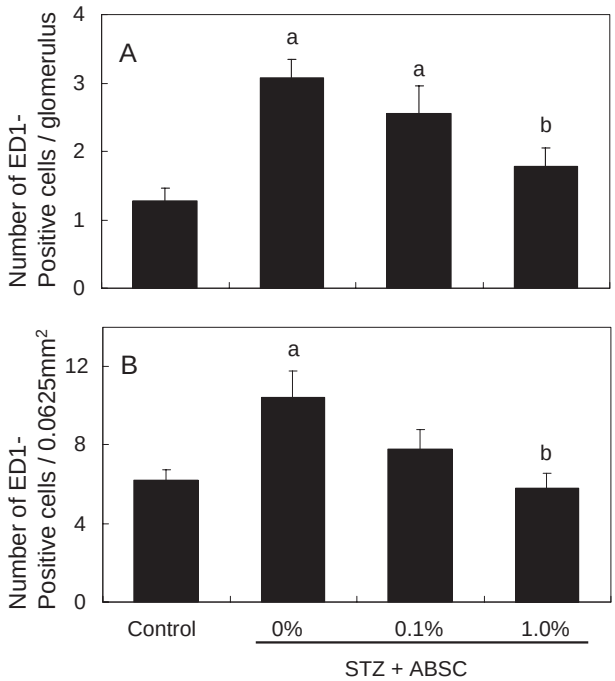

Fig. 4. Effects of ABSC treatments on the number of ED1-positive macrophages in the glomeruli (A) and in the tubulointerstitium of the corticomedullary junction areas (B) in control and STZ-induced diabetic rats. Values are expressed as mean±S.E. (n=6–7). [a]Significantly different from control, P<.01. [b]Significantly different from ABSC-untreated (0%) diabetic rats, P<.01.

3.3. Effect of ABSC treatments on the expression of MCP-1 mRNA in the STZ-induced diabetic rat kidney

MCP-1 mRNA expression determined by RT-PCR was significantly increased in the kidneys of the ABSC-untreated diabetic rats (P<.05), compared with controls (Fig. 5). On the other hand, the levels of MCP-1 mRNA expression in the ABSC-treated diabetic rats were significantly lower than in the ABSC-untreated rats (P<.05), indicating that the ABSC treatments suppressed the expression of MCP-1 mRNA in the diabetic rat kidney.

4. Discussion

The major findings of the present study were that polyphenol-containing seed coats of azuki beans attenuated the glomerular expansion in STZ-induced diabetic rats (Fig. 3), and that the ABSC treatments suppressed the increase of the number of infiltrating macrophages in the glomerular and tubulointerstitial areas (Fig. 4), compared with the ABSC-untreated diabetic rats. Moreover, the ABSC treatments suppressed the expression of MCP-1 mRNA involved in infiltrating and activating macrophages in the kidney of the diabetic rats (Fig. 5). Azuki beans are known to contain polyphenols such as proanthocyanidins with remarkable radical scavenging activities in vitro [1,2]. Proanthocyanidins have some preventive effects against oxidative damage associated with a variety of diseases,

including inflammation, cardiovascular diseases, atherosclerosis and diabetes [4–6]. However, little is known about whether a diet of seed coats of the azuki bean has beneficial effects on diabetic nephropathy.

Glomerular expansion has been reported to be a characteristic of experimental diabetic animals and humans with diabetes mellitus [18,19,27,28]. In our histologic analyses, we confirmed that the glomerular lesions consisted of the thickening of basement membrane and mesangial matrix areas in the STZ-induced diabetic rats and were regarded as an early stage of glomerular sclerosis. As compared with diabetic rats without ABSC treatments, there was markedly less staining by the Sirius red method in the glomeruli in the ABSC-treated diabetic rats (Fig. 2), and, morphometrically, the areas of fibrosis in the glomeruli of the ABSC-treated diabetic rats were significantly smaller than those of the ABSC-untreated diabetic rats (Fig. 3). In addition, in the ABSC-treated diabetic rats, a decrease in BUN levels was seen in contrast to the ABSC-untreated diabetic rats, and the kidney weights showed a tendency to recover (Table 1). These results suggested that the ABSC treatments attenuated the diabetic nephropathy in the STZ-induced rat model.

More interestingly, the number of ED1-positive macrophages in the glomerular and tubulointerstitial areas was reduced in the ABSC-treated diabetic rats, compared with the ABSC-untreated diabetic rats (Figs. 2 and 4). Previous studies have observed an increased number of glomerular macrophages in diabetic animals [17,21]. The infiltrating macrophages have been considered to be related to the progression toward glomerulosclerosis via the production of fibrogenic factors such as transforming growth factor-β and platelet-derived growth factor [17–19]. Polyphenols seem likely to affect activation and/or infiltration of macrophages, and green tea polyphenols were reported to inhibit UV-induced infiltrating monocytes/macrophages and neutrophils in the skin in mice [29]. In addition, a green tea extract including epicatechin suppressed infiltration of monocytes/macrophages after ischemia-reperfusion and prevented ischemia-reperfusion injury to the rat liver [30]. We previously reported that seed coats of azuki beans suppressed the increased number of infiltrating macrophages and tubulointerstitial fibrosis in kidneys of rats induced by cisplatin, which is often used as a chemotherapeutic agent against malignant carcinoma [11]. Thus, polyphenol-containing ABSC may be expected to suppress the infiltration of macrophages in the glomerular and tubulointerstitial areas in rat diabetic nephropathy. As infiltrating macrophages are known to play crucial roles in the progression of glomerulosclerosis [13,21,26], the reduced number of ED1-positive macrophages in the glomeruli of the ABSC-treated diabetic rats might be attributable to the decreased glomerular expansion.

The reasons for the attenuation of STZ-induced diabetic nephropathy caused by ABSC treatments remain unclear. However, this phenomenon may be interpreted as follows: one possibility is that polyphenol-containing ABSC may be associated with the expression of MCP-1 in the kidney in STZ-induced diabetic rats. MCP-1 has been believed to play an important role in infiltration and activation of macrophages, thus leading to progressive fibrosis in diabetic nephropathy [13,21]. In our RT-PCR analyses, the ABSC-treated diabetic rats showed significantly lower level of MCP-1 mRNA expression in the kidneys, compared with the ABSC-untreated diabetic rats (Fig. 5). Therefore, we supposed that ABSC treatments, at least in part, may play a role in reducing macrophage infiltration through the suppression of up-regulation of MCP-1 expression in the glomeruli and tubulointerstitium, and that the reduction might lead to the attenuation of the nephropathy of STZ-induced diabetic rat. This hypothesis is also supported by reports that flavonoids found in berries and red wine inhibit MCP-1 expression in vitro and in vivo [31,32]. Another possibility is that ABSC may act as an antioxidant to suppress the oxidative toxicity in the diabetic rat kidney, because the plasma levels of MDA in the ABSC-treated diabetic rats were significantly lower than those in the untreated diabetic rats (Table 1). Oxidative stress is well known as an important factor in the progression of diabetic complications [12,33]. As azuki beans have been reported to contain proanthocyanidins with marked radical scavenging activities in vitro [1,2], we hypothesized that polyphenols, including the proanthocyanidins in ABSC might, at least in part, play a role in reducing oxidative injury by suppressing macrophage infiltration in the diabetic rat kidney.

In conclusion, we herein demonstrated that polyphenol-containing seed coats of azuki beans suppressed the increased number of infiltrating macrophages, mRNA of MCP-1 and glomerular expansion in diabetic nephropathy, whereas there was no difference in the plasma glucose level between diabetic rats treated with and without ABSC. Although the mechanisms by which molecules in ABSC attenuate the macrophage infiltration and glomerular expansion in STZ-induced diabetic kidneys should be investigated in further studies, the current results would be useful for investigation of a potential strategy for the preventive care of diabetic nephropathy.

Acknowledgment

This study was supported by a Grant-in-Aid (No. 14658034) for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] Ariga T, Koshiyama I, Fukushima D. Antioxidative properties of procyanidins B-1 and B-3 from azuki beans in aqueous system. Agric Biol Chem 1988;52:2717–22.

[2] Ariga T, Hamano M. Radical scavenging action and its mode in procyanidins B-1 and B-3 from azuki beans to peroxyl radicals. Agric Biol Chem 1990;54:2499–504.

[3] Lee KG, Mitchell AE, Shibamoto T. Determination of antioxidant properties of aroma extracts from various beans. J Agric Food Chem 2000;48:4817–20.
[4] Ray SD, Kumar MA, Bagchi D. A novel proanthocyanidins IH66 grape seed extract increases in vivo bcl-XL expression and prevents acetaminophen-induced programmed and unprogrammed cell death in mouse liver. Arch Biochem Biophys 1999;369:42–58.
[5] Bagchi D, Bagchi M, Stohs S, Ray SD, Sen CK, Preuss HG. Cellular protection with proanthocyanidins derived from grape seeds. Ann N Y Acad Sci 2002;957:260–70.
[6] Avramovic V, Vlahovic P, Mihailovic D, Stefanovic V. Protective effect of a bioflavonoid proanthocyanidin-BP1 in glycerol-induced acute renal failure in the rat: renal stereological study. Ren Fail 1999;21:627–34.
[7] Singletary KW, Meline B. Effect of grape seed proanthocyanidins on colon aberrant crypts and breast tumors in a rat dual-organ tumor model. Nutr Cancer 2001;39:252–8.
[8] Kim H, Hall P, Smith M, Kirk M, Prasain JK, Barnes S, et al. Chemoprevention by grape seed extract and genistein in carcinogen-induced mammary cancer in rats is diet dependent. J Nutr 2004;134:3445S–52S.
[9] Landrault N, Poucheret P, Azay J, Krosniak M, Gasc F, Jenin C, et al. Effect of a polyphenols-enriched chardonnay white wine in diabetic rats. J Agric Food Chem 2003;51:311–8.
[10] Facchini FS, Saylor KL. A low-iron-available, polyphenol-enriched, carbohydrate-restricted diet to slow progression of diabetic nephropathy. Diabetes 2003;52:1204–9.
[11] Sato S, Hori Y, Yamate J, Saito T, Kurasaki M, Hatai A. Protective effect of dietary azuki bean (*Vigna angularis*) seed coats on renal interstitial fibrosis of rats induced by cisplatin. Nutrition 2005 [in press].
[12] Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405–12.
[13] Jerums G, Panagiotopoulos S, Forbes J, Osicka T, Cooper M. Evolving concepts in advanced glycation, diabetic nephropathy, and diabetic vascular disease. Arch Biochem Biophys 2003;419:55–62.
[14] Koya D, Lee IK, Ishii H, Kanoh H, King GL. Prevention of glomerular dysfunction in diabetic rats by treatment with D-α-tocopherol. J Am Soc Nephrol 1997;8:426–35.
[15] Melhem MF, Craven PA, Liachenko J, DeRubertis FR. α-Lipoic acid attenuates hyperglycemia and prevents glomerular mesangial matrix expansion in diabetes. J Am Soc Nephrol 2002;13:108–16.
[16] Anjaneyulu M, Chopra K. Quercetin, an anti-oxidant bioflavonoid, attenuates diabetic nephropathy in rats. Clin Exp Pharmacol Physiol 2004;31:244–8.
[17] Young BA, Johnson RJ, Alpers CE, Eng E, Gordon K, Floege J, et al. Cellular events in the evolution of experimental diabetic nephropathy. Kidney Int 1995;47:935–44.
[18] Wolf G, Ziyadeh FN. Molecular mechanisms of diabetic renal hypertrophy. Kidney Int 1999;56:393–405.
[19] Reeves WB, Andreoli TE. Transforming growth factor-β contributes to progressive diabetic nephropathy. Proc Natl Acad Sci U S A 2000; 97:7667–9.
[20] Li JH, Huang XR, Zhu HJ, Johnson R, Lan HY. Role of TGF-β signaling in extracellular matrix production under high glucose conditions. Kidney Int 2003;63:2010–9.
[21] Sassy-Prigent C, Heudes D, Mandet C, Belair M-F, Michel O, Perdereau B, et al. Early glomerular macrophage recruitment in streptozotocin-induced diabetic rats. Diabetes 2000;49:455–75.
[22] López-De León A, Rojkind M. A simple micromethod for collagen and total protein determination in formalin-fixed paraffin-embedded sections. J Histochem Cytochem 1985;33:737–43.
[23] Sato S, Yamate J, Saito T, Hosokawa T, Saito S, Kurasaki M. Protective effect of taurine against renal interstitial fibrosis of rats induced by cisplatin. Naunyn-Schmiedeberg's Arch Pharmacol 2002;365:277–83.
[24] Dijkstra CD, Dopp EA, Joling P, Kraal G. The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 1985;54:589–99.
[25] Yamate J, Ishida A, Tsujino K, Tatsumi M, Nakatsuji S, Kuwamura M, et al. Immunohistochemical study of rat renal interstitial fibrosis induced by repeated injection of cisplatin, with special reference to the kinetics of macrophages and myofibroblasts. Toxicol Pathol 1996;24: 199–206.
[26] Ota T, Takamura T, Ando H, Nohara E, Yamashita H, Kobayashi K. Preventive effect of cerivastatin on diabetic nephropathy through suppression of glomerular macrophage recruitment in a rat model. Diabetologia 2003;46:843–51.
[27] Mauer SM, Lane P, Hattori M, Fioretto P, Steffes MW. Renal structure and function in insulin-dependent diabetes mellitus and type I membranoproliferative glomerulonephritis in humans. J Am Soc Nephrol 1992;2:S181–4.
[28] Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural–functional relationships in diabetic nephropathy. J Clin Invest 1984;74:1143–55.
[29] Katiyar SK, Challa A, McCormick TS, Cooper KD, Mukhtar H. Prevention of UVB-induced immunosuppression in mice by the green tea polyphenol (−)-epigallocatechin-3-gallate may be associated with alterations in IL-10 and IL-12 production. Carcinogenesis 1999;20: 2117–24.
[30] Zhong Z, Froh M, Connor HD, Li X, Conzelmann LO, Mason RP, et al. Prevention of hepatic ischemia-reperfusion injury by green tea extract. Am J Physiol Gastrointest Liver Physiol 2002;283: G957–64.
[31] Feng AN, Chen YL, Chen YT, Ding YZ, Lin SJ. Red wine inhibits monocyte chemotactic protein-1 expression and modestly reduces neointimal hyperplasia after balloon injury in cholesterol-fed rabbits. Circulation 1999;100:2254–9.
[32] Youdim KA, McDonald J, Kalt W, Joseph JA. Potential role of dietary flavonoids in reducing microvascular endothelium vulnerability to oxidative and inflammatory insults (small star, filled). J Nutr Biochem 2002;13:282–8.
[33] Baliga R, Ueda N, Walker PD, Shah SV. Oxidant mechanisms in toxic acute renal failure. Drug Metab Rev 1999;31:971–97.