J. Trad. Med. 20, 156－164, 2003

Amelioration of kidney damage in spontaneously diabetic WBN/Kob rats after treatment with Keishi-bukuryo-gan

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(Received February 21, 2003. Accepted April 17, 2003.)

Abstract

In this study, we investigated whether Keishi-bukuryo-gan can retard the occurrence and progression of diabetic nephropathy in spontaneously diabetic WBN/Kob rats. Administration of Keishi-bukuryo-gan did not affect body weight loss or blood glucose levels but effectively lowered urinary protein excretion and serum creatinine levels, and ameliorated glomerular, vascular and tubulointerstitial lesions. In addition, treatment of the diabetic rats with Keishi-bukuryo-gan reduced renal levels of thiobarbituric acid reactive substances and advanced glycation end products significantly and elevated renal superoxide dismutase activity significantly. These results suggest that Keishi-bukuryo-gan exerts antioxidant effects in the kidneys of diabetics and may prove that the herbal medicine is useful for inhibiting the progression of diabetic kidney disease.

Key words Keishi-bukuryo-gan, WBN/Kob rat, diabetes mellitus, kidney, oxidative stress, AGEs.

Abbreviations AGEs, advanced glycation end products; Cr, creatinine; HE, hematoxylin-eosin; O_2^-, superoxide; PAM, periodic acid-methenamine silver; PAS, periodic acid-Schiff reagent; PTAH, phosphotungstic acid-hematoxylin; SOD, superoxide dismutase; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substances.

Introduction

Prevention of diabetic complications is a serious medical matter, responsible for the marked increase of patients with diabetic mellitus. One of most life-threatening complications may be diabetic nephropathy because of higher morbidity and mortality in diabetic patients. It is clinically noticed that renal function is deteriorated more rapidly in diabetic nephropathy than in other types of chronic renal failure and diabetic nephropathy causes an earlier introduction of dialysis therapy. Although clinical trials have shown that antihypertensive treatment using angiotensin-converting enzyme inhibitors can attenuate the progression of cardiac and renal impairments related to diabetes and to reduce the risk of death in diabetic patients,[1-3] large numbers of patients remain suffering from diabetic nephropathy in many countries. As shown by the Diabetes Control and Complication Trial,[4] hyperglycemia plays a central role in diabetic complications. Recently, it was proposed that generation of oxygen free radicals due to hyperglycemia contributes to pathological conditions of diabetes and develops various complications such as nephropathy, retinopathy, and neuropathy.[5-7] Excess reactive oxygen accelerates oxidative damage to lipids, proteins and DNA, resulting in cellular and tissue injury. Indeed, increased levels of end products of lipid peroxidation induced by free radicals such as malondialdehyde, advanced glycation end products (AGEs) and 8-hydroxy-2'-deoxyguanosine were observed in serum, kidney and urine of diabetes.[8-10] Therefore, reduction of oxidative stress may improve the pathological conditions and further prevent the develop-

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ment of diabetic complications.

Traditional herbal medicines were often used for diabetic mellitus to cure several symptoms. It was suggested that the therapy combined with such medicines was effective for diabetic microangiopathy, from the results of 141 patients with non-insulin dependent diabetes mellitus prescribed them.[11] In addition, case reports indicating that traditional herbal medicine, including Keishi-bukuryo-gan, improved the quality of life of patients with diabetic nephropathy have been published,[12] suggesting the possibility that treatment with traditional herbal medicines prolongs the pre-dialysis stage of diabetic nephropathy. On the other hand, we previously found that, in rats with diabetic nephropathy induced by subtotal nephrectomy and injection of streptozotocine (STZ), Keishi-bukuryo-gan can delay the progression of renal damage, and indicated that the beneficial effects of the herbal medicine are due to its ability to improve metabolic abnormalities associated with diabetes.[13-15] These results suggested that Keishi-bukuryo-gan might be a therapeutic agent for diabetic nephropathy. More detailed studies are required to prove the therapeutic usefulness of Keishi-bukuryo-gan in diabetic nephropathy. Therefore, we conducted a long-term experiment on the preventive effect of Keishi-bukuryo-gan on diabetic nephropathy using spontaneously diabetic WBN/Kob rats.

Materials and Methods

Preparation of Keishi-bukuryo-gan extract : Keishi-bukuryo-gan is composed of equal parts, by weight, of the following five crude drugs: Cinnamomi Cortex (*Cinnamomum cassia* BL.), Hoelen (*Poria cocos* WOLF), Paeoniae Radix (*Paeonia lactiflora* PALL.), Moutan Cortex (*Paeonia suffruticosa* ANDR.) and Persicae Semen (*Prunus persica* BATSCH). These crude drugs were obtained from Tochimoto Tenkaidou Co. Ltd. (Osaka, Japan). The extract was obtained by boiling 100 g crude mixture (20 g each) gently in 500 ml water for 50 min. The insoluble portion was removed by filtration, then the filtrate was concentrated under reduced pressure and lyophilized, yielding a brown residue, which represented 9.68%, by weight, of the original materials.

Animals and treatment : Male Wistar strain WBN/Kob rats were purchased from Japan SLC Inc. (Hamamatsu, Japan) and kept in an automatically controlled room (temperature about 23 ℃ and humidity about 60%) with a conventional dark/light cycle. They were fed a diet (LABO MR-DBT) obtained from Nosan Corporation (Yokohama, Japan). Nearly 100% of the male WBN/Kob rats were diagnosed with diabetes by continuously monitoring blood glucose levels > 200 mg/dl and then they were divided into three groups (one control and two treatment groups) at 45 weeks of age by the way of avoiding intergroup differences in blood glucose level. Each group consisted of ten rats. Keishi-bukuryo-gan supplemented in the diet at the content of 1 or 3%, by weight, was administered for 30 weeks. During this experimental period, all rats were given the diet in a pair-feeding manner and Keishi-bukuryo-gan intakes of the 1% and 3% Keishi-bukuryo-gan-treated groups were estimated to be about 0.33 and 1.1 g/rat, respectively. The body weights and blood glucose levels were monitored every two weeks over the 30-week experimental period and at 15 and 30 weeks, 24-h urine samples were collected in metabolic cages. At the end of the experimental period, the rats were sacrificed, blood samples were obtained from the heart and then the kidneys were excised from each rat. One part of each kidney was immersed in formalin for histological examination and the other was kept at -80℃ until analysis. Age-matched male Wistar rats (n=4) were used as normal.

Determination of blood and urine component levels: Blood glucose and serum Cr were determined using commercial reagents (Glucose CII-Test Wako obtained from Wako Pure Chemical Industries Ltd., Osaka, Japan; CRE-EN Kainos obtained from Kainos Laboratories Inc., Tokyo, Japan). Urinary protein excretion was determined by the sulfosalicylic acid method.[16]

Determination of thiobarbituric acid reactive substances (TBARS) levels : Serum TBARS was measured using the method of Naito and Yamanaka[17] and renal TBARS was assayed according to the method of Mihara and Uchiyama.[18]

AGEs assays : Serum AGEs was measured by the ELISA method of Ono *et al.*[19] and renal AGEs was measured by the fluorescence method of Nakayama *et al.*[20]

Superoxide dismutase (SOD) activity assay : Kidney tissue was homogenized in 9 volumes of ice-cold

physiological saline. The SOD activity of the homogenate was measured using the nitrous acid method described by Elstner and Heupel[21] and Oyanagui,[22] which is based on inhibition of nitrite formation from hydroxylamine in the presence of superoxide (O_2^-) generators.

Histological examination : Renal tissues were fixed in 10% neutral buffered formalin solution, embedded in paraffin and cut into semi-thin sections of 2 μm in thickness. The sections were stained with hematoxylin-eosin (HE), periodic acid-Schiff reagent (PAS), periodic acid-methenamine silver (PAM) and phosphotungstic acid-hematoxylin (PTAH). Two hundred or fewer glomeruli in each sample were examined by light microscopy. The proportion of glomeruli having sclerosis relative to total glomeruli was rated as grade 0-4: no change = 0, <10% = 1, 10-30% = 2, 30-50% = 3 or >50% = 4. The degree of vascular change and tubulointerstitial damage was also assessed according to the five grades described above.

Statistics : Values are presented as means ± S.E. Differences between groups were statistically analyzed by Dunnett's test and those at $p<0.05$ were accepted as significant.

Results

Body and kidney weights

As shown in Table I, final body weight of WBN/Kob rats was significantly lower than that of age-matched Wistar rats. The 3% Keishi-bukuryo-gan-treated group had significantly higher values than that of the untreated control group. During the 30-week experimental period, the body weight of WBN/Kob rats decreased time-dependently (Fig. 1-(A)). The gain from 0-week to 30-week was -80.0 g in the untreated control group and -65.3 and -79.9 g in the 1% and 3% Keishi-bukuryo-gan-treated groups, respectively (Table I). There were no significant differences in body weight between the untreated control and Keishi-bukuryo-gan-treated groups. On the other hand, kidney weight was significantly increased in WBN/Kob rats, reaching 0.958 g/100 g body weight, as shown in Table I. The increased kidney

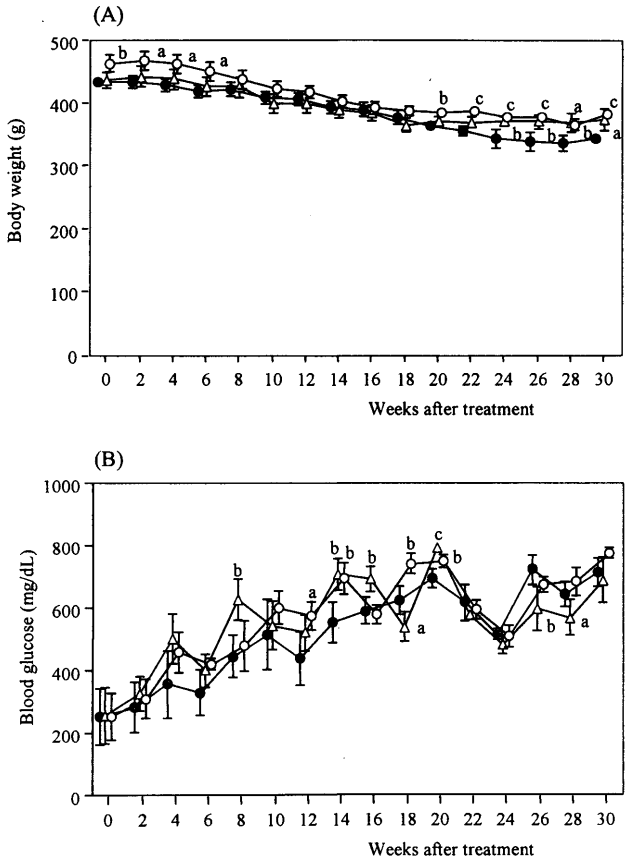

Fig. 1 Effects of Keishi-bukuryo-gan on body weight and blood glucose levels (control, ●; 1% Keishi-bukuryo-gan, △; 3% Keishi-bukuryo-gan, ○). Statistical significance: $^a p<0.05$, $^b p<0.01$, $^c p<0.001$ vs. WBN/Kob control rats.

Table I Body and kidney weights.

Group	Body weight		Kidney weight (g/100g B.W.)
	Final (g)	Gain (g)	
Wistar rats	566.4 ± 6.9	52.7 ± 10.2	0.520 ± 0.014
WBN/Kob rats			
Control	354.0 ± 7.6*	-80.0 ± 13.3*	0.958 ± 0.027*
1 % Keishi-bukuryo-gan	373.1 ± 19.1*	-65.3 ± 27.8*	0.888 ± 0.045*,a
3 % Keishi-bukuryo-gan	385.7 ± 8.9*,b	-79.9 ± 14.2*	0.869 ± 0.031*,b

Statistical significance: *$p<0.001$ vs. Wistar rats; $^a p<0.05$, $^b p<0.01$ vs. WBN/Kob control rats.

weight was reduced by treatment with Keishi-bukuryo-gan, as shown in Table I.

Blood glucose and serum Cr levels

Figure 1-(B) shows the changes in blood glucose levels of WBN/Kob rats during the 30-week experimental period. The blood glucose level increased as time progressed. At the end of the experiment, it reached 720 mg/dl in the control group and 687 and 777 mg/dl in the 1% and 3% Keishi-bukuryo-gan-treated groups, respectively, as shown in Table II. In comparison with age-matched Wistar rats, higher blood glucose levels was observed in WBN/Kob rats, whereas there were no significant differences between the untreated control and Keishi-bukuryo-gan-treated groups (Table II).

As shown in Table II, the serum Cr levels of Wistar rats and the untreated WBN/Kob rats were 0.397 and 0.357 mg/dl, respectively. After administration of Keishi-bukuryo-gan for 30 weeks, the serum Cr level declined to 0.325 mg/dl in the 1% treated group and declined further to 0.278 mg/dl in the 3% treated group.

Urinary protein excretion

As shown in Fig. 2, urinary protein excretion at 15 weeks of Wistar rats was about 10 mg/day and there were no significant differences between Wistar rats and WBN/Kob rats. After 30 weeks, the urinary protein excretion was about 2-fold higher than that found after 15 weeks in the control diabetic rats, reflecting the progression of diabetic kidney damage. However, Keishi-bukuryo-gan treatment inhibited this increase more effectively in the 3% treated group than the 1% treated group (Fig. 2).

Serum and renal TBARS and AGEs levels

Tables III and IV show the effect of Keishi-bukuryo-gan on TBARS and AGEs levels in the serum and kidney, respectively. These parameters were higher in the untreated control WBN/Kob rats than those of age-matched Wistar rats. After treatment with Keishi-bukuryo-gan for 30 weeks, the serum TBARS levels decreased slightly and the serum AGEs levels did not change (Table III), whereas both the TBARS and AGEs

Table II Effects of Keishi-bukuryo-gan on blood glucose and serum Cr levels.

Group	Blood glucose (mg/dl)	Serum Cr (mg/dl)
Wistar rats	153 ± 23	0.397 ± 0.015
WBN/Kob rats		
Control	720 ± 45**	0.357 ± 0.011*
1% Keishi-bukuryo-gan	687 ± 74**	0.325 ± 0.014**,a
3% Keishi-bukuryo-gan	777 ± 16**	0.278 ± 0.013**,b

Statistical significance: *$p<0.01$, **$p<0.001$ vs. Wistar rats; [a]$p<0.01$, [b]$p<0.001$ vs. WBN/Kob control rats.

Table III Effects of Keishi-bukuryo-gan on serum TBARS and AGEs levels.

Group	TBARS (nM)	AGEs (mU/ml)
Wistar rats	2.18 ± 0.17	0.70 ± 0.01
WBN/Kob rats		
Control	2.82 ± 0.10**	1.26 ± 0.12**
1% Keishi-bukuryo-gan	2.80 ± 0.09**	1.26 ± 0.15**
3% Keishi-bukuryo-gan	2.54 ± 0.12*,a	1.22 ± 0.09**

Statistical significance: *$p<0.01$, **$p<0.001$ vs. Wistar rats; [a]$p<0.01$ vs. WBN/Kob control rats.

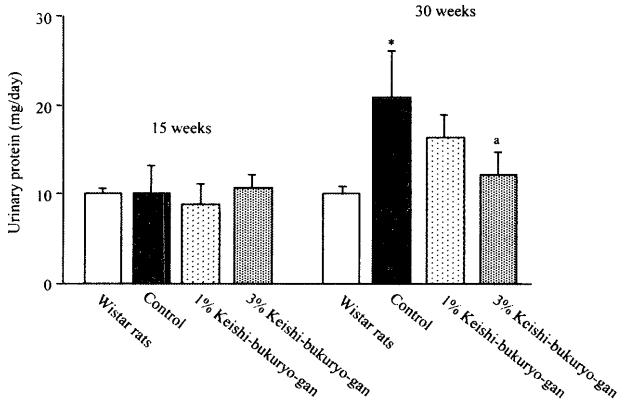

Fig. 2 Effect of Keishi-bukuryo-gan on urinary excretion of protein. Statistical significance: *$p<0.01$ vs. Wistar rats; [a]$p<0.01$ vs. WBN/Kob control rats.

Table IV Effects of Keishi-bukuryo-gan on renal TBARS and AGEs levels.

Group	TBARS (nmol/mg protein)	AGEs (AU)
Wistar rats	0.571 ± 0.035	0.822 ± 0.025
WBN/Kob rats		
Control	0.713 ± 0.007**	1.166 ± 0.040**
1% Keishi-bukuryo-gan	0.649 ± 0.055*,a	1.041 ± 0.022**,b
3% Keishi-bukuryo-gan	0.534 ± 0.034[b]	0.939 ± 0.019**,b

Statistical significance: *$p<0.05$, **$p<0.001$ vs. Wistar rats; [a]$p<0.05$, [b]$p<0.001$ vs. WBN/Kob control rats.

levels of the kidney decreased significantly, as shown in Table IV.

Renal SOD activity

As shown in Table V, the renal SOD activity of the untreated control WBN/Kob rats was significantly lower than those of Wistar rats. The renal SOD activity was increased significantly by 30-week treatment with Keishi-bukuryo-gan; to the 1% and 3% Keishi-bukuryo-gan-treated groups had 42.1 and 43.7 U/mg protein, respectively (Table V).

Histological findings

The results of histopathological evaluation of the kidney is summarized in Table VI. The degree of renal damage was evaluated by assigning lesion scores on glomerular, vascular and tubulointerstitial changes, as described in the methods. Compared with the severity of glomerular lesion, vascular and tubulointerstitial lesions showed fewer changes in WBN/Kob rats at 75 weeks of age. Glomerular sclerosis including mesangial expansion and capillary wall thickness of the untreated control group was scored as grade 4. On the other hand, the administration of Keishi-bukuryo-gan lowered the proportion of glomeruli having sclerosis. Vascular lesion, which mainly refers to interlobular arterial lesion, and tubulointerstitial damage such as tubular atrophy, dilatation and inflammatory cell infiltration were observed and graded as mild to moderate in the untreated control group. The degree of these lesions was also significantly decreased by Keishi-bukuryo-gan treatment. These results were reflected by the reduction of the total score, which expressed as the sum of the scores of the 3 lesions, showing that Keishi-bukuryo-gan protected the renal morphological changes.

Representive photomicrographs of the glomeruli obtained from each group are shown in Fig. 3. Widening of the mesangial areas with increased PAS staining of the mesangial matrix was observed in the glomeruli of diabetic WBN/Kob rats. The Keishi-bukuryo-gan-treated groups showed less marked glomerular lesions compared with the untreated control group, as shown in Fig. 3.

Table V Effects of Keishi-bukuryo-gan on renal SOD activity.

Group	SOD (U/mg protein)
Wistar rats	40.8 ± 1.3
WBN/Kob rats	
Control	37.2 ± 0.6**
1% Keishi-bukuryo-gan	42.1 ± 1.6[a]
3% Keishi-bukuryo-gan	43.7 ± 1.1*,[a]

Statistical significance: *$p<0.05$, **$p<0.001$ vs. Wistar rats; [a]$p<0.001$ vs. WBN/Kob control rats.

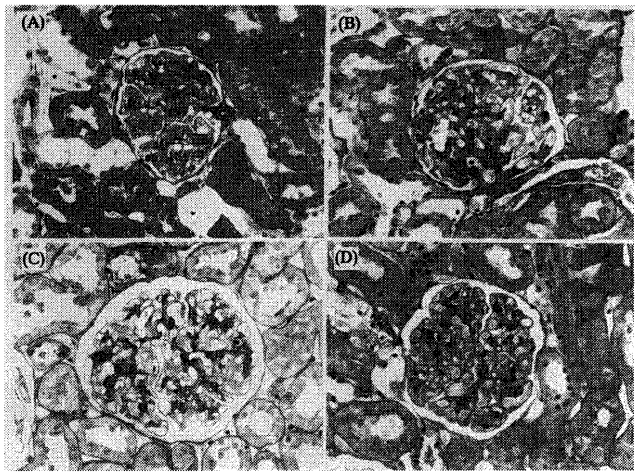

Fig. 3 Photomicrographs of the glomeruli. Wistar rat (A) and WBN/Kob rat (control, (B); 1% Keishi-bukuryo-gan, (C); 3% Keishi-bukuryo-gan, (D)). X 200.

Table VI Histopathological evaluation of the kidney.

Group	Lesion score			
	Glomerular sclerosis	Vascular change	Tubulointerstitial damage	Total
Wistar rats	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
WBN/Kob rats				
Control	4.00 ± 0.00	2.50 ± 0.22	2.50 ± 0.22	9.00 ± 0.45
1% Keishi-bukuryo-gan	3.14 ± 0.26[a]	1.00 ± 0.00[b]	1.71 ± 0.18[b]	5.86 ± 0.40[b]
3% Keishi-bukuryo-gan	2.29 ± 0.18[b]	1.00 ± 0.00[b]	1.14 ± 0.14[b]	4.43 ± 0.30[b]

Statistical significance: [a]$p<0.01$, [b]$p<0.001$ vs. WBN/Kob control rats.

Discussion

Proteinuria is a prognostically important clinical manifestation for occurrence and progression of nephropathy in diabetic patients. Mori *et al.*[23] investigated the development of renal lesions in male WBN/Kob rats and reported that urinary total protein began to increase at about 13 months of age. However, no significant increase in serum Cr levels was observed until 24 months of age.[23] In our study, WBN/Kob rats received Keishi-bukuryo-gan treatment for 30 weeks from the age of 45 weeks (about 10.5 months) to 75 weeks (about 17.5 months) in order to evaluate its effects on the development of diabetic kidney damage. After treatment with Keishi-bukuryo-gan for 15 weeks (14 months of age), urinary protein excretion was about 10 mg/day and there was no significant difference in that excretion between the control and Keishi-bukuryo-gan-treated groups. After 30 weeks of treatment (17.5 months of age), urinary protein content increased to about 20 mg/day in the untreated control group, whereas that content of age-matched male Wistar rats was about 10 mg/day. This showed that renal function had deteriorated with long-term diabetes mellitus. However, oral administration of Keishi-bukuryo-gan to diabetic WBN/Kob rats for 15 to 30 weeks inhibited the increase of proteinuria. At the end of treatment, serum Cr levels were reduced significantly in the Keishi-bukuryo-gan-treated groups. These results show that long-term treatment of Keishi-bukuryo-gan could delay the deterioration of renal function in diabetic WBN/Kob rats.

There are a few reports in which histopathological changes in the kidneys of WBN/Kob rats were examined. As reported by Ishizaki *et al.*,[24] thicking of the basement membrane, increase of the mesangial matrix and linear deposition of IgG in the basement membrane of the glomeruli, tubules and Bowman's capsule were observed in 19-21 month-old WBN/Kob rats. It is noticed that these renal histopathological features observed in diabetic WBN/Kob rats were similar to those observed in humans with diabetic nephropathy. Mori *et al.*[23] reported in light microscopic examinations that in diabetic WBN/Kob rats, glomeruli with segmental or global increase in the mesangial areas were noted at 17 months of age, developing glomerulosclerosis, and that fibrin-cap lesions, or exudative glomeruli, at the age of 21 months. In this study, glomerular sclerosis including the widening of mesangial areas and capillary wall thickness were observed in WBN/Kob rats at 75 weeks of age (about 17.5 months of age). Keishi-bukuryo-gan treatment inhibited the development of glomerular sclerosis, suggesting the contribution toward the reduction of kidney weight. Although the kidney of 75-week-old WBN/Kob rats had fewer vascular lesions and tubulointerstitial damage, Keishi-bukuryo-gan treatment significantly reduced the development of these renal lesions. These ameliorative effects of Keishi-bukuryo-gan on morphological changes in the kidney of diabetic WBN/Kob rats provide evidence that Keishi-bukuryo-gan protects the kidney against progressive diabetes-induced damage.

In general, natural senescence is considered to be involved in the factors which caused deterioration of renal function and morphological changes. To take this point into consideration, we employed age-matched Wistar rats in this study. From our present results of WBN/Kob rats and age-matched Wistar rats, we found that 75 week-old WBN/Kob rats had kidney damage, and that this damage relatively contributed toward the influence of diabetes rather than senescence. Thus, we concluded that oral administration of Keishi-bukuryo-gan protects the kidney against the development of diabetes-induced damage, resulting in reduction of proteinuria and serum Cr.

Excessive production of oxygen free radicals is widely recognized as having a harmful influence on the body, because they injure lipids, proteins, and nucleic acids, which leads to structural and functional impairments. In recent experimental and clinical studies, it has been extensively discussed that oxidative damage induced by oxygen free radicals significantly participates in the development of diabetic complications, including nephropathy.[5,25,26] Indeed, in a number of studies, increased plasma levels of TBARS, which is well accepted as a parameter for evaluating oxidative lipid damage, were observed in diabetes.[8,27] In this study, we measured the lipid peroxidation levels and found that the untreated WBN/Kob rats had higher lipid peroxidation levels than Wistar rats. Following the treatment of Keishi-bukuryo-gan, serum lipid peroxidation levels decreased slightly, whereas in the kidney it was decreased significantly. These results indicated that Keishi-

bukuryo-gan reduced the enhanced oxidative stress in diabetic WBN/Kob rats.

Ha and Kim[8] demonstrated that in rats with STZ-diabetes, the concentration of lipid peroxides in the blood plasma and urine was increased, and concurrently proteinuria, which is important parameter of diabetic nephropathy, was also increased. Proteinuria is caused by the damage of the filtration barrier of renal glomeruli, but the detailed mechanisms for the increase of proteinuria are not fully understood. As one of the possible mechanisms, impairments of glomerular basal membrane due to lipid peroxidation induced by enhanced reactive free radicals has been suggested.[28] In this study, it was found that there was a significant correlation between renal lipid peroxidation level and proteinuria ($r = 0.653$, $p<0.01$). These results provide information that explains, at least in part, the preventive effect of Keishi-bukuryo-gan against diabetic nephropathy.

Alterations of the activities of antioxidant enzymes are involved in the pathological conditions associated with oxidative stress. SOD, which is a scavenger of O_2^-, plays a key role in the endogeneous defense system against oxygen free radicals. Loven *et al.*[29] demonstrated decreased SOD activity in the kidney of diabetic rats, and this phenomenon was confirmed by other authors.[30,31] Our results also agreed with them, suggesting that decreased SOD activity is associated with enhanced oxidative stress in diabetes. Thus, it is suggested that elevated SOD activity in Keishi-bukuryo-gan-treated groups compared with the untreated control group causes a beneficial change to the diabetic kidney in which oxidative stress is involved. On the other hand, Kawamura *et al.*[32] revealed an increased content of the glycated form of SOD in erythrocytes of diabetic patients. Arai *et al.*[33] demonstrated that glycation of SOD in lysine residues resulted in the loss of biological activity. Thus, enhanced glycation reaction which results in AGEs accumulation induces oxidative stress due to SOD inactivation. In this study, Keishi-bukuryo-gan treatment did not change AGEs levels in the circulation, but effectively reduced renal AGEs levels. In the diabetic body, many factors, including SOD inactivation and AGEs accumulation, interdependently contribute to the enhanced oxidative stress, and further cause the development of nephropathy. We assume that Keishi-bukuryo-gan exerts antioxidant effects in the kidney of WBN/Kob rats through increased SOD activity and reduced AGEs accumulation, and that these antioxidant effects bring preservation and morphological changes.

AGEs are also associated with other toxic effects including cross-linking of long-lived proteins such as collagens and other matrix proteins, induction of transforming growth factor β genes, and synthesis of extracellular matrix proteins.[34-36] Mesangial extracellular matrix accumulation is a principal cause of glomerular sclerosis. Glomerular sclerosis affects serum Cr levels which is an important parameter of renal function associated with the ability of glomerular filtration. In patients with diabetic nephropathy, high levels of AGEs accumulate within the vascular lesions and the sclerosing glomeruli.[37,38] Chronic administration of AGEs to rats led to glomerular sclerosis and increased serum Cr levels.[39] Thus, it is considered that Keishi-bukuryo-gan treatment prevents the glomerular sclerosis, at least in part, due to the reduction of AGEs accumulation, and therefore reduced serum Cr levels.

Traditional herbal medicines have been employed for thousands of years and have contributed greatly to the prevention and treatment of various diseases, including renal disorders and diabetes. They are still valuable for human health and are receiving much attention as potential sources of new therapeutic agents, because they are composed of several crude drugs and have low toxicities. However, the mechanisms by which traditional herbal medicines mediate beneficial effects remain unclear. Our research group has been investigating the effects of traditional herbal medicines, crude drugs and their components on renal diseases in various kinds of models of chronic and acute renal failure.[40-48] Recently, we found that such medicines including Keishi-bukuryo-gan had a beneficial influence on metabolic abnormalities accompanied with diabetes such as enhancement of polyol pathway, oxidative stress and AGEs accumulation in rats receiving subtotal nephrectomy and injection of STZ.[13,15] Similar results were also observed in the present study using spontaneously diabetic WBN/Kob rats. Furthermore, Keishi-bukuryo-gan prevented proteinuria, serum Cr levels and morphological changes peculiar to diabetic nephropathy, indicating the potential therapeutic usefulness of Keishi-bukuryo-gan against the development of diabetic kidney disease. On the basis of these results, we proposed that Keishi-bukuryo-gan exerted

antioxidative activity and had an inhibitory effect on AGEs accumulation in diabetic kidneys and that these effects might be the mechanisms responsible for the beneficial action of this traditional herbal medicine. Experimental evidence supporting the therapeutic usefulness of Keishi-bukuryo-gan for diabetic nephropathy is accumulating.

和文抄録

本研究では，自然発症糖尿病 WBN/Kob ラットを用い，桂枝茯苓丸が糖尿病性腎症の発症・進展を遅延するか否かについて検討した。その結果，体重と血糖値に対しては，桂枝茯苓丸投与による変化は見られなかったが，尿蛋白排泄量と血清クレアチニンレベルが有意に低下し，病理組織所見（糸球体硬化，血管病変，間質・尿細管障害）も改善していた。一方，腎組織中の過酸化脂質と advanced glycation end products レベルは，桂枝茯苓丸投与群で有意に低下し，スーパーオキシドジスムターゼ活性は有意に上昇していた。これらの結果より，桂枝茯苓丸は糖尿病ラットの腎臓において抗酸化作用を発揮し，腎症の進展抑制に有用であることが示唆された。

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