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Protective effect of Hachimi-jio-gan against renal failure in a subtotal nephrectomy rat model

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Abstract

The protective effect of Hachimi-jio-gan extract against chronic renal failure in a subtotal nephrectomy rat model was investigated. The level of serum urea nitrogen by nephrectomy was increased over 15 weeks, but the administration of Hachimi-jio-gan at 50 and 200 mg led to the decrease. In addition, the levels of creatinine (Cr), urinary methylguanidine (MG) and MG/Cr were increased, whereas Cr clearance dramatically decreased in nephrectomized rats. However, oral administration of Hachimi-jio-gan extract prevented the elevation of these uremic toxins in serum and urine, and the production of hydroxyl radical. Moreover, nephrectomy led to a significant decline in superoxide dismutase (SOD) and catalase activities, but increased glutathione peroxidase activity compared with normal levels, indicating an abnormal antioxidative system. The increased activity of both SOD and catalase by the oral administration of Hachimi-jio-gan suggested that these enzymes are associated with the protective role of Hachimi-jio-gan extract against oxidative stress by nephrectomy. Moreover, the decrease in serum albumin in nephrectomized control rats was increased and proteinuria was ameliorated by the administration of Hachimi-jio-gan with improved glomerular hyalinosis, interstitial fibrosis and inflammation, suggesting the beneficial effect of Hachimi-jio-gan to prevent glomerular sclerosis and progressive renal fibrosis. This study suggests that Hachimi-jio-gan plays a protective role in the progression of chronic renal failure through the decline in uremic toxins, elevation of antioxidative enzyme activity such as SOD and catalase, and amelioration of histopathological lesions in the kidney.

Introduction

The number of patients with renal failure, especially those undergoing dialysis and with end-stage renal diseases, is growing worldwide, although angiotensin-converting enzyme inhibitors, protein restriction, dialysis and transplantation are effectively employed for the management of renal disease (Chertow et al 1996). In addition, prolonged medical therapy is a great mental and physical burden on patients, and social problems, including financial issues, have risen with the increased number of patients with renal failure. The high morbidity and mortality from renal failure suggest the need for therapeutic advances and new approaches to prevent and effectively treat the associated complications as well as renal diseases. Clinical and experimental evidence has indicated that chronic renal failure is related to increased oxidative stress induced by the production of guanidine compounds such as uremic toxins (Nagase et al 1986; Nakamura et al 1991; De Deyn et al 2003). Since oxidative stress leads to histological lesions in the kidney, such as glomerular sclerosis, tubulointerstitial changes and mesangial matrix expansion under renal failure, its associated pathological conditions could be improved by the amelioration of oxidative stress. We therefore focused on the search for agents with antioxidative activity as free radical scavengers that can play crucial roles in an effective defence against renal failure.

Traditional medicines, including Kampo prescriptions, have attracted attention due to their distinctive biological activities without toxicity and/or side-effects. Hachimi-jio-gan has long been widely used to treat several chronic diseases, including nephritis, steritoty, diabetes and vegetative ataxia (Goto et al 1989; Yamada 1992; Huang 1997; Furuya et al 1999), although scientific evidence supporting the pharmacological basis for its therapeutic effects has not yet been well established. In particular, it has been widely used to treat renal dysfunction in human subjects with glomerulonephritis, hypertension and nephritic syn-

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drome (Hijikata et al 1994; Mitsuma 1996; Hikiami et al 2000). In addition, it has exhibited an antihypertensive effect associated with the partial resolution of renal injury in salt-induced hypertension (Hirawa et al 1996). Moreover, our previous studies demonstrated that Hachimi-jio-gan alleviated the pathological conditions of diabetic nephropathy in an in-vivo model (Nakagawa et al 2001; Yokozawa et al 2004). However, the protective effect of Hachimi-jio-gan against chronic renal failure from oxidative stress has not yet been investigated. With a subtotal nephrectomy rat model of chronic renal failure, we investigated the effect of Hachimi-jio-gan on chronic renal failure, particularly focusing on its antioxidative activities.

Materials and Methods

Preparation of Hachimi-jio-gan extract

The Hachimi-jio-gan extract used in this experiment was produced by Tsumura Juntendo, Inc., Tokyo, Japan, and its composition was as follows: Rehmanniae Radix (*Rehmannia glutinosa* LIBOSCH. *var*. *purpurea* MAKINO) 6 g; Corni Fructus (*Cornus officinalis* SIEB. et ZUCC.) 3 g; Dioscoreae Rhizoma (*Dioscorea japonica* THUNB.) 3 g; Alismatis Rhizoma (*Alisma orientale* JUZEP.) 3 g; Hoelen (*Poria cocos* WOLF) 3 g; Moutan Cortex (*Paeonia suffruticosa* ANDREWS) 2.5 g; Cinnamomi Cortex (*Cinnamomum cassia* BLUME) 1.0 g; and Aconiti Tuber (*Aconitum carmichaeli* DEBX) 0.5 g. These eight crude drugs were boiled gently in 10 times each volume of water for 60 min, filtered and the filtrate was spray-dried to obtain the extract at a yield of about 10% by weight of the original preparation. To analyse the components of Hachimi-jio-gan, the aqueous extract (0.5 g) was extracted with 20 mL methanol under ultrasonication for 30 min. The solution was filtered through a membrane filter (0.45 μm) and then subjected to HPLC analysis using a TSK-GEL ODS-80TS column ($\varphi 4.6 \times 250$ mm, TOSOH, Japan) with an LC $10AV_{vp}$ pump and SPD-$M10A_{vp}$ absorbance detector. The elution solvents were (A) 0.05 M AcOH–$AcONH_4$ and (B) CH_3CN, and the column was eluted with a linear gradient of, by volume, 90% A and 10% B, changing over 60 min to 100% B. The flow rate was 1.0 mL min^{-1} and the effluent from the column was monitored and processed into three-dimensional data using an SPD-M10A array detector. All assigned peaks were identified by comparing their UV spectral data with those of co-injected authentic samples using CLASS LC-10 Ver. 1.62 software (Shimadzu, Japan). The three-dimensional HPLC profile of the Hachimi-jio-gan extract is shown in Figure 1. Morroniside and loganin obtained from Corni Fructus and paeoniflorin obtained from Moutan Cortex were detected as the major compounds of Hachimi-jio-gan, and penta-*O*-galloylglucose, benzoylmesaconine, cinnamic acid, benzoylpaeoniflorin, cinnamaldehyde and 16-ketoalisol A were also observed.

Animals and treatment

The Guidelines for Animal Experimentation, approved by Toyama Medical and Pharmaceutical University, were followed in these experiments. Male Wistar rats of 120–130 g were purchased from Japan SLC Inc. (Hamamatsu, Japan). They were kept in wire-bottomed cages under a conventional lighting regimen with a dark night. The room temperature (about 25°C) and humidity (about 60%) were controlled automatically. Laboratory pellet chow (CE-2, CLEA Japan Inc., Tokyo, Japan, comprising 24.0% protein, 3.5% lipid and 60.5% carbohydrate) and water were given ad libitum. Following several days of adaptation, the rats underwent resection of half the left kidney and total excision of the right kidney at a 10- to 14-day interval, while one group of five rats underwent sham treatment as the normal group (Platt et al 1952; Morrison 1966). Serum urea nitrogen levels were determined after recovery from the operation, and rats were divided into four groups ($n = 7$ per group), including one control and three Hachimi-jio-gan extract-administered groups, avoiding any significant difference in the levels of serum urea nitrogen among the four groups. Over the 15-week experimental period, the normal and control groups were fed water and the other three surgical groups were fed a solution of Hachimi-jio-gan extract orally at a dose of 50, 100 or 200 mg (kg body $weight^{-1}$) day^{-1} by a stomach tube. The oral doses were determined by the preliminary study that demonstrated biological activity without toxicity (Yokozawa et al 1997, 2004). Every 3 weeks, blood samples were obtained from the tail veins to determine urea nitrogen levels. At the end of the experimental period the rats were moved to metabolic cages for individual 24-h urine collection, and blood samples were obtained by cardiac puncture. The blood samples were immediately separated by centrifugation (3000 rpm, 15 min, 4°C). After renal perfusion with ice-cold physiological saline through the renal artery, the remnant kidneys were removed from each rat and then one part was immersed in formalin for histological findings and the rest kept at −80°C until analysis.

Determination of blood and urine components

Serum levels of urea nitrogen, total protein and albumin were determined using commercial reagents (BUN Kainos obtained from Kainos Laboratories, Inc., Tokyo, Japan; A/G B-Test Wako obtained from Wako Pure Chemical Industries, Ltd, Osaka, Japan). Urinary protein excretion was assayed by the sulfosalicylic acid method (Sakagisi 1968). For creatinine (Cr) and methylguanidine (MG), the serum or urine was deproteinized by the addition of trichloroacetic acid (final concentration 10% v/v). After centrifugation at 3000 rpm for 15 min, the supernatant was filtered through a 0.2-μm membrane filter and the filtrate was analysed using a Japan Spectroscopic liquid chromatograph employing a step-gradient system according to the method of Higashidate et al (1984). A fluorescence spectrometer, model FP-210 (excitation 365 nm, emission 495 nm; Japan Spectroscopic Co., Tokyo, Japan) was used to detect Cr and MG on the column. Creatinine clearance (C_{Cr}) was calculated on the basis

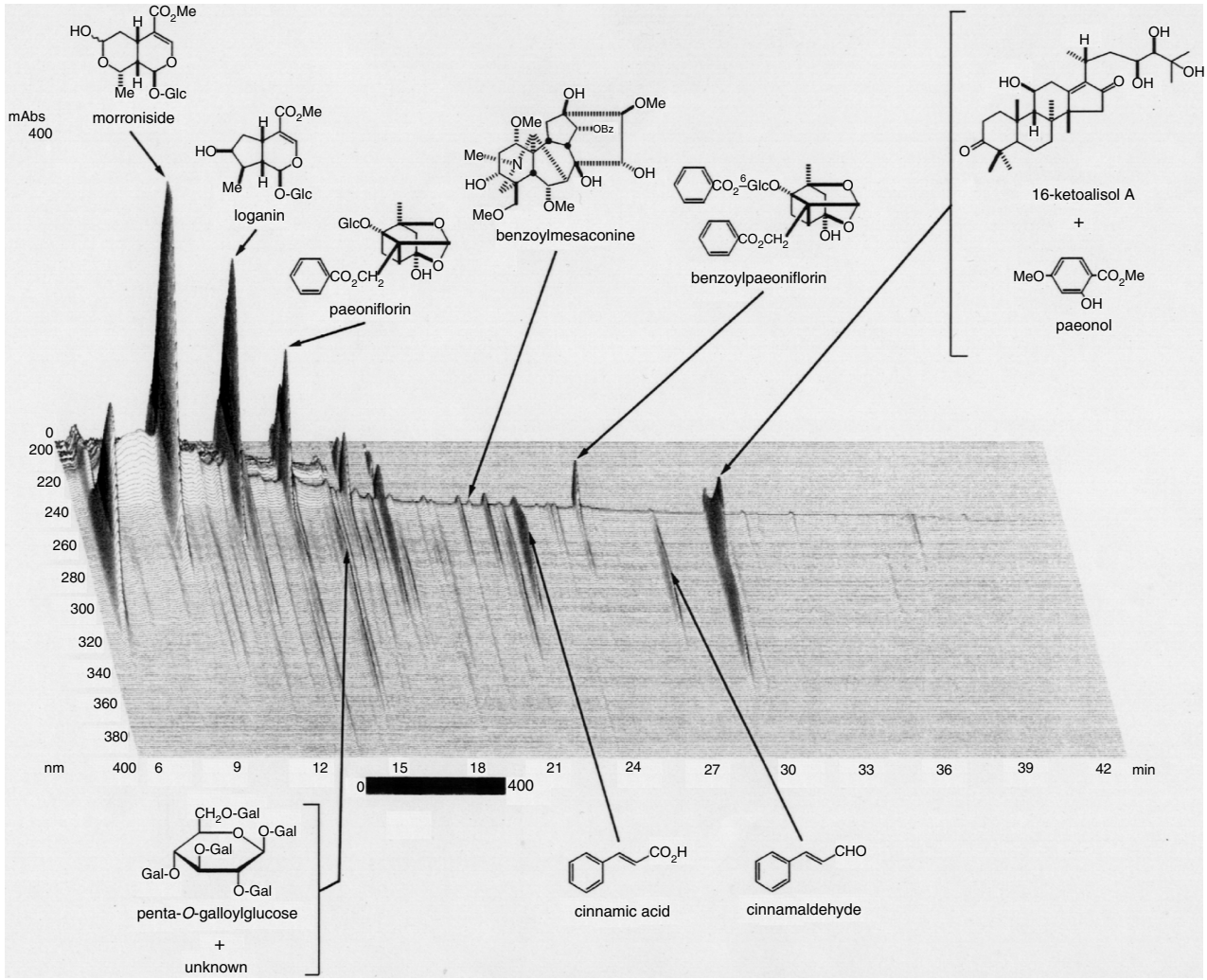

Figure 1 Three-dimensional HPLC profile of Hachimi-jio-gan extract.

of urinary Cr, serum Cr, urine volume and body weight using the equation:

$$C_{Cr}\,(\text{mL}\,(\text{kg body weight}^{-1})\,\text{min}^{-1}) = (\text{urinary Cr}\,(\text{mg dL}^{-1}) \times \text{urine volume}\,(\text{mL})/\text{serum Cr}\,(\text{mg dL}^{-1})) \times (1000/\text{body weight}\,(\text{g})) \times (1/1440\,(\text{min}))$$

Enzyme assay

The kidney was homogenized with a nine-fold volume of ice-cold physiological saline and the enzyme activities in the homogenate were determined. The superoxide dismutase (SOD) activity was measured according to the nitrous acid method, which is based on the inhibition of nitrite formation from hydroxylamine in the presence of superoxide (O_2^-) generators (Elstner & Heupel 1976; Oyanagui 1984). Catalase activity was measured by following the decomposition of hydrogen peroxide (H_2O_2). The difference in the extinction (ΔE_{240}) per unit time by the decomposition of H_2O_2 was used as the measurement of catalase activity (Aebi 1974). Glutathione peroxidase (GSH-Px) activity was obtained by colorimetry of 2-nitro-5-thiobenzoic acid, a compound produced through a reaction of glutathione and 5,5′-dithio-bis (2-nitrobenzoic acid) (Hafeman et al 1974). Protein levels were determined by the method of Itzhaki & Gill (1964) with bovine serum albumin as the standard.

Electrophoretic patterns of proteinuria

Equal amounts (0.5 μg) of urinary protein were loaded onto 10% acrylamide gel, subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the protein bands were stained with CBB R-250. The molecular masses of the urinary protein bands were estimated by comparing with the low range bands of standard proteins (Bio-Rad).

Histological findings

Renal tissues were immediately fixed in 10% neutral-buffered formalin and embedded in paraffin. The tissues were

then cut into semithin sections (2 μm thick), mounted on silane-coated glass slides, and stained with hematoxylin–eosin, periodic acid–Schiff, periodic acid–methenamine silver and phosphotungstic acid–hematoxylin. About 200 glomeruli from each sample were examined by light microscopy and the severity of the histological lesions was evaluated.

Statistical analysis

The results are presented as the mean ± s.e. The effect of Hachimi-jio-gan on each parameter was examined using one-way analysis of variance. Individual differences among groups were analysed by Dunnett's test and significance was accepted at $P < 0.05$.

Results

Body and kidney weights

Table 1 shows the weight changes of the body and kidney during the 15-week experimental period by subtotal nephrectomy. The nephrectomized control rats showed significantly decreased body weight gain and increased kidney weight compared with normal rats. On the other hand, the oral administration of Hachimi-jio-gan extract at 50, 100 and 200 mg doses decreased the kidney weight with no significant body weight changes. The food intake (about 18 g day^{-1}) of the Hachimi-jio-gan-treated groups did not differ from that of the nephrectomized control rats.

The change in serum urea nitrogen level

The change in the serum urea nitrogen level during the 15-week experimental period is shown in Figure 2. Normal rats maintained 18.4 mg dL^{-1} serum urea nitrogen over the period, while nephrectomized control rats were about 1.8-fold and 3.3-fold higher at 9 and 15 weeks, respectively. However, the administration of Hachimi-jio-gan resulted in a decreased level in a dose- and administration period-dependent manner. The administration of Hachimi-jio-gan at 200 mg (kg body weight^{-1}) day^{-1} for 15 weeks showed a decrease to 35.1 from 60.6 mg dL^{-1} (42% decrease).

Urinary and serum parameters

Table 2 represents the effect of Hachimi-jio-gan on the renal functional parameters of urine and serum by nephrectomy. The urinary excretion of MG increased by nephrectomy to 17.3 μg day^{-1} from the normal value of 13.9 μg day^{-1}, while it was reduced by the administration of 50, 100 and 200 mg of Hachimi-jio-gan to 14.0, 12.4 and 9.5 μg day^{-1}, respectively. In addition, the urinary MG/Cr ratio of nephrectomized control rats was also significantly increased relative to the normal value (from 6.97×10^{-3} to 8.29×10^{-3}). In contrast, the administration of Hachimi-jio-gan showed a significant decrease in urinary MG/Cr in a dose-dependent manner. Moreover, the higher level of serum Cr in nephrectomized control rats compared with normal rats decreased with the administration of Hachimi-jio-gan. C_{Cr} in nephrectomized control rats was significantly lower than in normal rats, from 6.63 to 2.39 mL (kg body weight^{-1}) min^{-1}, while the administration of Hachimi-jio-gan at 200 mg (kg body weight^{-1}) day^{-1} elevated the value to 3.18 mL (kg body weight^{-1}) min^{-1}. Furthermore, the urinary protein excretion of nephrectomized control rats was 6-fold higher than that of normal rats. With the administration of Hachimi-jio-gan, urinary protein was reduced significantly and dose-dependently by 33 and 43% in rats given 100 and 200 mg, respectively. The subtotal nephrectomy procedure resulted in a significant decrease in serum total protein with no significant effect by Hachimi-jio-gan administration. On the other hand, the decrease in serum albumin in nephrectomized control rats was increased with the administration of Hachimi-jio-gan at doses of 100 and 200 mg.

Enzyme activities

Table 3 shows the effect of Hachimi-jio-gan on the activity of reactive oxygen species-scavenging enzymes in a subtotal nephrectomy rat model. In nephrecto-

Table 1 Body and kidney weights in nephrectomized rats treated with Hachimi-jio-gan for 15 weeks

Group	Dose (mg (kg body weight^{-1}) day^{-1})	Body weight			Kidney weight (g (100 g body weight^{-1}))
		Initial (g)	Final (g)	Gain (g per 15 weeks)	
Normal rats	–	256.2 ± 4.5	463.2 ± 4.9	207.0 ± 4.7	0.304 ± 0.013
Nephrectomized rats					
Control	–	242.6 ± 3.7[a]	396.8 ± 4.3[a]	156.0 ± 6.7[a]	0.615 ± 0.070[a]
Hachimi-jio-gan	50	242.1 ± 4.0[a]	407.3 ± 4.9[a]	162.2 ± 6.2[a]	0.506 ± 0.057[a,b]
Hachimi-jio-gan	100	243.3 ± 3.1[a]	401.7 ± 12.9[a]	157.0 ± 10.4[a]	0.500 ± 0.042[a,b]
Hachimi-jio-gan	200	241.1 ± 4.7[a]	402.3 ± 16.3[a]	161.1 ± 12.3[a]	0.459 ± 0.015[a,c]

[a]$P < 0.001$ vs normal rats; [b]$P < 0.01$, [c]$P < 0.001$ vs nephrectomized control rats.

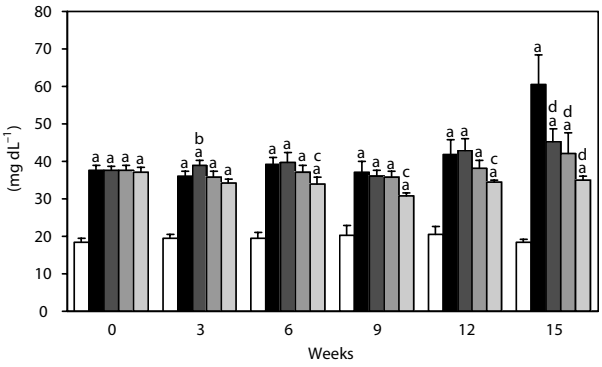

Figure 2 Serum urea nitrogen in normal rats (□) and in nephrectomized rats treated with Hachimi-jio-gan 50 mg (kg body weight^{-1}) day^{-1} (■), 100 mg (kg body weight^{-1}) day^{-1} (▩), 200 mg (kg body weight^{-1}) day^{-1} (▨) or water (control, ■) for 15 weeks. $^{a}P < 0.001$ vs normal rats; $^{b}P < 0.05$, $^{c}P < 0.01$, $^{d}P < 0.001$ vs each nephrectomized control rat.

mized control rats, SOD and catalase activities were significantly lower than in normal rats: 35% lower for SOD activity, 41% lower for catalase activity. In contrast, the administration of Hachimi-jio-gan at 100 and 200 mg (kg body weight^{-1}) day^{-1} significantly increased the activities of SOD and catalase, whereas nephrectomized control and Hachimi-jio-gan-administered groups led to increased GSH-Px activity compared with normal rats.

Electrophoretic patterns of proteinuria

Figure 3 shows the effect of Hachimi-jio-gan on proteinuria induced by subtotal nephrectomy in rats. The bands were divided into low and high molecular weight proteins relative to the marker albumin (91 kDa). The control rats subjected to subtotal nephrectomy showed several low molecular weight protein bands, but normal groups did not; however, rats administered Hachimi-jio-gan had improved proteinuria with lower intensity low molecular weight protein bands.

Histological findings

Table 4 shows the effect of Hachimi-jio-gan extract on renal lesions induced by subtotal nephrectomy. The renal tissue of the nephrectomized control indicated typical morphological changes in glomerular, tubular and interstitial lesions due to the resection of three-quarters of their

Table 2 Urinary and serum parameters in nephrectomized rats treated with Hachimi-jio-gan for 15 weeks

Item	Normal rats	Nephrectomized rats			
		Control	Hachimi-jio-gan (50 mg (kg body weight^{-1}) day^{-1})	Hachimi-jio-gan (100 mg (kg body weight^{-1}) day^{-1})	Hachimi-jio-gan (200 mg (kg body weight^{-1}) day^{-1})
u-MG (μg day^{-1})	13.9 ± 0.7	17.3 ± 1.7^{b}	14.0 ± 0.5^{f}	12.4 ± 0.5^{f}	$9.5 \pm 1.4^{c,f}$
u-MG/Cr ($\times 10^{-3}$)	6.97 ± 0.14	8.29 ± 0.82^{b}	7.23 ± 0.46^{d}	6.38 ± 0.17^{f}	$4.93 \pm 0.62^{c,f}$
s-Cr (mg dL^{-1})	0.45 ± 0.02	0.94 ± 0.16^{c}	$0.71 \pm 0.07^{b,e}$	$0.69 \pm 0.07^{b,e}$	$0.69 \pm 0.05^{b,e}$
C_{Cr} (mL (kg body weight^{-1}) min^{-1})	6.63 ± 0.41	2.39 ± 0.31^{c}	$3.11 \pm 0.28^{c,d}$	$3.13 \pm 0.40^{c,e}$	$3.18 \pm 0.23^{c,e}$
u-Protein (mg day^{-1})	17.1 ± 1.4	102.2 ± 27.2^{c}	84.6 ± 18.6^{c}	$68.7 \pm 8.8^{b,d}$	$58.4 \pm 10.8^{a,e}$
s-Total protein (g dL^{-1})	4.97 ± 0.05	4.61 ± 0.08^{c}	4.62 ± 0.07^{c}	4.62 ± 0.07^{c}	4.65 ± 0.07^{c}
s-Albumin (g dL^{-1})	3.36 ± 0.04	2.85 ± 0.08^{c}	2.98 ± 0.08^{c}	$2.99 \pm 0.05^{c,d}$	$3.11 \pm 0.08^{c,f}$

u-MG, urinary methylguanidine; s-Cr, serum creatinine; C_{Cr}, creatinine clearance. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs normal rats; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs nephrectomized control rats.

Table 3 Activities of reactive oxygen species-scavenging enzymes in nephrectomized rats treated with Hachimi-jio-gan for 15 weeks

Group	Dose (mg (kg body weight^{-1}) day^{-1})	SOD (U mg^{-1} protein)	Catalase (U mg^{-1} protein)	GSH-Px (U mg^{-1} protein)
Normal rats	–	34.89 ± 0.86	107.64 ± 4.88	183.7 ± 2.5
Nephrectomized rats				
Control	–	22.81 ± 2.96^{c}	63.82 ± 8.67^{c}	199.9 ± 6.7^{c}
Hachimi-jio-gan	50	29.31 ± 3.43	69.16 ± 10.86^{c}	209.3 ± 7.3^{c}
Hachimi-jio-gan	100	36.38 ± 4.39^{f}	81.29 ± 14.80^{b}	$218.3 \pm 8.5^{c,e}$
Hachimi-jio-gan	200	30.28 ± 2.98^{d}	82.28 ± 8.10^{a}	195.2 ± 7.2^{c}

SOD, superoxide dismutase; GSH-Px, glutathione peroxidase. $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ vs normal rats; $^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs nephrectomized control rats.

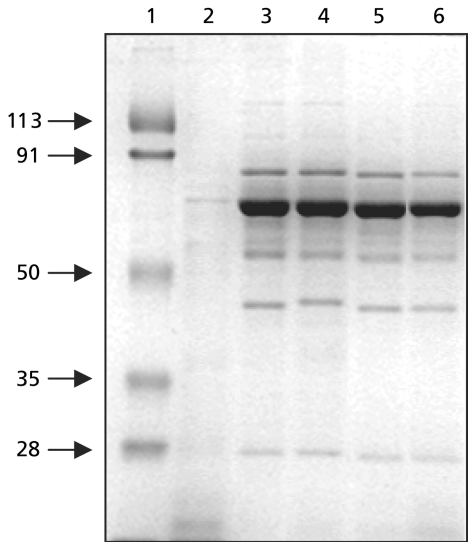

Figure 3 SDS-PAGE pattern of proteinuria in normal rats (2) and in nephrectomized rats treated with Hachimi-jio-gan 50 mg (kg body weight^{-1}) day^{-1} (4), 100 mg (kg body weight^{-1}) day^{-1} (5), 200 mg (kg body weight^{-1}) day^{-1} (6) or water (control, 3) for 15 weeks. Lane 1 shows the marker (113 kDa, phosphorylase B; 91 kDa, bovine serum albumin; 50 kDa, ovalbumin; 35 kDa, carbonic anhydrase; 28 kDa, soybean trypsin inhibitor).

kidney volume. The mesangial proliferation and glomerular segmental hyalinosis in the nephrectomized control group were significantly higher than in the normal group, from 136 to 207 nm and from 0 to 43.8%, respectively. However, in the 50, 100 and 200 mg Hachimi-jio-gan-treated groups there was a remarkable reduction in glomerular segmental hyalinosis by 45.7, 50.5 and 65.8%, respectively, but no significant change was observed in mesangial proliferation. In addition, tubular cortex thickness, and tubular expansion and hypertrophy were enlarged by nephrectomy from 3.00 to 4.50 mm and from 50 to 263 nm, respectively, but the administration of Hachimi-jio-gan did not exert any significant effect. Moreover, the severity of interstitial fibrosis and inflammation was markedly increased in nephrectomized rats compared with the normal group. In contrast, rats administered Hachimi-jio-gan had significantly reduced interstitial fibrosis and inflammation.

Discussion

Among the several theories of the pathophysiology of progressive renal failure, the most convincing one suggests that the initial reduction of nephron numbers damages the remaining nephrons, which undergo hypertrophy with the concomitant lowering of arteriolar resistance and suffer the consequences of adaptive increases in glomerular pressure and flow that precede proteinuria, glomerular sclerosis and azotemia (Hostetter et al 1981; Brenner 1985; Remuzzi et al 1997). In addition, several studies have demonstrated that nephrectomy in rats decreased the antioxidative capacity and increased oxidative stress in the remaining kidney, which aggravated chronic renal failure (Harris et al 1988; Schrier et al 1988; Sanaka et al 1991; Yokozawa et al 1997). Based on this evidence, we employed a nephrectomy rat model to investigate the protective effect of Hachimi-jio-gan extract against renal failure.

With the progression of renal failure, it is well known that guanidino compounds such as guanidine, guanidinosuccinic acid, Cr and MG are highly increased in serum, urine and tissues. In particular, Cr is converted into MG through an intermediate, 5-hydroxylcreatinine (creatol), during the Fenton reaction, which produces hydroxyl (·OH) radicals (Nagase et al 1985, 1986; Nakamura et al 1991; Marescau et al 1992). It is therefore worthwhile not only to evaluate the ratio of urinary MG/Cr as an indirect but useful indicator of the production of ·OH radicals, but also to check serum Cr and urea nitrogen levels for uremic toxins, which damage the renal function. This investigation showed elevated serum urea nitrogen in nephrectomized rats: the longer the period after nephrectomy, the higher the value of serum urea nitrogen. In addition, the levels of serum Cr, urinary MG and MG/Cr were also increased by nephrectomy, whereas C_{Cr} was dramatically decreased. However, the oral administration of Hachimi-jio-gan prevented the elevation of these uremic toxin levels in serum and urine. On the other hand, the decline in C_{Cr} by nephrectomy was increased, but the ratio of MG/Cr was significantly decreased by Hachimi-jio-gan extract,

Table 4 Histopathological evaluation of the kidney in nephrectomized rats treated with Hachimi-jio-gan for 15 weeks

Group	Dose (mg (kg body weight^{-1}) day^{-1})	Mesangial proliferation (nm)	Glomerular segmental hyalinosis (%)	Tubular cortex thickness (mm)	Tubular expansion and hypertrophy (nm)	Interstitial fibrosis (%)	Interstitial inflammation (%)
Normal rats	–	136 ± 2	0	3.00 ± 0.01	50 ± 1	5.0 ± 0.1	0
Nephrectomized rats							
Control	–	207 ± 7[c]	43.8 ± 6.9[c]	4.50 ± 0.29[c]	263 ± 31[c]	28.3 ± 4.4[c]	11.67 ± 1.67[c]
Hachimi-jio-gan	50	200 ± 4[c]	23.8 ± 6.9[c,d]	4.75 ± 0.48[c]	250 ± 41[c]	18.3 ± 1.7[c,d]	7.00 ± 1.78[c,d]
Hachimi-jio-gan	100	208 ± 8[c]	21.7 ± 1.7[c,d]	5.00 ± 0.01[c]	263 ± 38[c]	11.7 ± 1.7[b,d]	5.00 ± 0.01[c,d]
Hachimi-jio-gan	200	195 ± 10[c]	15.0 ± 0.1[c,d]	4.75 ± 0.25[c]	250 ± 29[c]	10.0 ± 0.1[a,d]	5.00 ± 0.01[c,d]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs normal rats; [d]$P < 0.001$ vs nephrectomized control rats.

suggesting that Hachimi-jio-gan inhibits the production of uremic toxins and ·OH radicals with the progression of renal failure. Hachimi-jio-gan is expected to ameliorate renal damage and failure by nephrectomy.

The remaining nephrons in nephrectomized rats work excessively to maintain renal function. As a result, the increased oxygen consumption in the remaining nephrons is attributable to both the decrease of antioxidative activities and the state of oxidative stress (Harris et al 1988; Schrier et al 1988). It is also reported that decreased antioxidant enzyme activity in the renal cortex of rats after subtotal nephrectomy and increased oxidative stress related to nephrectomy play a role in the development of renal fibrosis (Yokozawa et al 1997). The oral administration of Hachimi-jio-gan resulted in increased SOD and catalase activities; however, GSH-Px activity was not affected. SOD and catalase are considered to associate with the protective role of Hachimi-jio-gan extract against renal failure by nephrectomy. SOD is the upstream enzyme that protects against the deleterious actions of O_2^- by catalysing its dismutation to H_2O_2 plus oxygen. Catalase exclusively detoxifies H_2O_2 and requires no electron donor. Both catalase and GSH-Px mainly remove H_2O_2, whereas the function of GSH-Px is to remove H_2O_2 followed by a loss of catalase activity (Rister & Baehner 1976). GSH-Px, a selenium-dependent enzyme, plays a role in the recycling process of GSH with the detoxification of H_2O_2 to H_2O, and the oxidized form of GSH may then be reduced by a second enzyme, glutathione reductase, with NADPH as the reducing agent. In addition, GSH-Px can metabolize lipid hydroperoxides to less reactive hydroxy fatty acids. The ability of GSH-Px to reduce H_2O_2 or other hydroperoxides is therefore dependent on the activity of glutathione reductase as well as the availability of NADPH and GSH (Fantone & Ward 1982). Considering this, GSH-Px not only acts as an H_2O_2 scavenger, but also works in the oxidation–reduction system. The action of GSH-Px by the administration of Hachimi-jio-gan is different from the actions of SOD and catalase, whose behaviours are mainly controlled by radical scavenging. To verify the protective effect of Hachimi-jio-gan against GSH-Px, further investigations are needed into the oxidation and reduction systems in relation to GSH and NADPH.

Oxidative stress in the kidney by nephrectomy led to elevated urinary protein excretion and decreased serum total protein and albumin levels. However, SDS-PAGE analysis showed that the major band, albumin, and other minor high and/or low bands of proteins were lowered by the administration of Hachimi-jio-gan, indicating that Hachimi-jio-gan ameliorates proteinuria. The increased load of urinary protein creates problems in the tubular cells because of an excess burden in the process of reabsorption. Since the reabsorption and clearance of protein require significant energy, tubular cells lack energy and are exposed to oxidative stress by producing active oxygen species. Arterial hypertension contributes to the acceleration of renal injury associated with enhanced traffic of plasma proteins. Himmelfarb & McMonagle (2001) demonstrated that albumin is also a major target of protein carbonyl formation in plasma, and albumin may act as an important defence for tubular cells against oxidative stress in hemodialysis patients. Hachimi-jio-gan administration led to increased serum albumin in a dose-dependent manner. The severity of proteinuria caused by nephrectomy led to the induction of oxidative stress in the kidney, whereas Hachimi-jio-gan exerted a protective effect against proteinuria through the amelioration of renal oxidative damage.

Proteinuria over a long period due to subtotal nephrectomy causes irreversible structural changes in nephrons and impairment of renal function, i.e. glomerular-capillary hypertension, proximal tubule damage, activation of nuclear signals such as nuclear factor-κB, releasing vasoactive and inflammatory substances into the interstitium, interstitial inflammatory reaction and consequent fibrosis (Remuzzi et al 1997; Remuzzi & Bertani 1998). In addition, increased glomerular function caused by renal ablation is triggered by glomerular hyperfiltration, enlargement and destruction of glomerulus, leading eventually to glomerular sclerosis. According to the hyperfiltration theory, the resulting loss of functioning glomerulus exerts a positive feedback stimulus to compensatory hyperfiltration in the less affected glomerulus, contributing in turn to glomerular destruction (Brenner et al 1982). This observation showed that nephrectomy induced the early stage of chronic renal failure with changes in the glomerulus, including increased mesangial cell proliferation, glomerular capillary endothelial damage and matrix production, as well as tubulointerstitial injury. Obvious histological lesions of the increased severity of glomerular, tubular and interstitial lesions were observed under nephrectomy, whereas in nephrectomized rats given Hachimi-jio-gan orally, the glomerular and interstitial lesions were ameliorated. Hachimi-jio-gan plays a role in improving renal pathological lesions by nephrectomy. Hachimi-jio-gan lowered the elevated levels of glomerular segmental hyalinosis, which is an index of the early stage of glomerular sclerosis, interstitial fibrosis and inflammation, which are downstream of progressive renal injury, whereas no protective effect was observed in tubular lesions such as tubular expansion and hypertrophy. Hachimi-jio-gan may therefore have a beneficial effect on the prevention of glomerular sclerosis and progressive renal fibrosis by inhibiting proteinuria.

Among the several factors for progression of chronic renal failure, blood pressure is also a monitoring factor for renal function. The effect on blood pressure and haemodynamics of Hachimi-jio-gan, including its crude drugs and main components, has not been reported yet. Whether or not the protective effect of Hachimi-jio-gan from renal failure is related to the control of blood pressure is therefore questionable and has to be investigated to verify clearly its protective role against renal failure. In addition, the further study of the protective role of its individual component drugs and main components has to be supported, even though it is cautiously proposed that the effect of Hachimi-jio-gan against renal failure would probably result from the synergistic effect of the crude drugs as well as the effect of component drugs.

In conclusion, the administration of Hachimi-jio-gan extract to subtotal nephrectomized rats exerted a favourable influence on the prevention of chronic renal failure progression. It showed the protective effect from elevation of uremic toxins and it elevated the activities of antioxidative enzymes such as SOD and catalase. In addition, it improved the pathological lesions under renal failure. Although the protective mechanisms of Hachimi-jio-gan extract from the progression of renal failure remain to be elucidated in the future, this study shows the promising potential of Hachimi-jio-gan in protecting against renal failure.

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