Effect of Shenkang granules on the progression of chronic renal failure in 5/6 nephrectomized rats

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Abstract. Shenkang granules (SKGs) are a Chinese herbal medicinal formula, consisting of rhubarb (*Rheum palmatum* L.), *Salvia miltiorrhiza*, milkvetch root [*Astragalus membranaceus* (Fisch.) Bunge] and safflower (*Carthamus tinctorius* L.). The aim of the present study was to investigate the effect of SKG on chronic renal failure (CRF) in 5/6 nephrectomized (5/6 Nx) rats. The rats were randomly divided into seven groups (n=10 per group) as follows: (i) 5/6 Nx (model group; 2.25 ml/kg/day normal saline); (ii) SKGL (low dose; 5/6 Nx treated with 2 g crude drug/kg/day SKG); (iii) SKGM (moderate dose; 5/6 Nx treated with 4 g crude drug/kg/day SKG); (iv) SKGH (high dose; 5/6 Nx treated with 8 g crude drug/kg/day SKG); (v) benazepril treatment group (5/6 Nx treated with 5 mg/kg/day benazepril); (vi) Shenkang injection (SKI) group (5/6 Nx with 13.3 ml/kg/day SKI); and (vii) sham-operated group (2.25 ml/kg/day normal saline). After 30 days, the levels of microalbumin, total protein, serum creatinine, blood urea nitrogen and serum lipid were found to be significantly decreased in the SKGL and SKGM rats, showing histological improvement compared with the untreated 5/6 Nx rats, as determined by hematoxylin and eosin, and Masson's trichrome staining. In addition, SKG was found to significantly improve the levels of glutathione peroxidase and reduce the damage caused by free radicals to the kidney tissues. Furthermore, SKG prevented the accumulation of extracellular matrix by decreasing the expression of collagen I and III and inhibiting the expression of matrix metalloproteinases-2 and -9 in the renal tissue, as determined by western blot analysis. SKG was also shown to decrease the concentrations of serum transforming growth factor-β_1, as determined by ELISA, and kidney angiotensin II, as determined using a radioimmunoassay kit. In conclusion, SKG was demonstrated to ameliorate renal injury in a 5/6 Nx rat model of CRF. Thus, SKG may exert a good therapeutic effect on CRF.

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Introduction

Chronic renal failure (CRF) is chronic progressive renal parenchyma damage caused by a variety of chronic kidney and kidney systemic diseases. In CRF, the kidneys are unable to discharge metabolic waste and regulate the water-salt and acid-base balance, hormone secretion, hormone metabolism and other functions, resulting in nitrogen qualitative hematic disease, metabolic disorders and a series of clinical syndromes (1-4). A previous study revealed that the number of potential CRF patients is increasing worldwide (5). With the exception of renal replacement therapy and kidney transplantation, no other satisfactory treatments exist for CRF (6-9). Alternative therapies, including hemodialysis and peritoneal dialysis, are unable to improve the pathological damage of kidney tissues. In addition, renal transplantation is limited by the lack of kidney resources, the expense and high risk of transplant rejection. Therefore, the development of new medications that inhibit renal failure progression and reduce the morbidity and mortality rates of CRF is essential. Traditional Chinese medicine (TCM) is widely used to treat various chronic diseases in China and Southeast Asia (10), and the effective prescription of TCM treatment for CRF has been increasingly investigated.

Shenkang granules (SKGs) comprise a Chinese herbal medicinal formula of rhubarb (*Rheum palmatum* L.), *Salvia miltiorrhiza*, milkvetch root [*Astragalus membranaceus* (Fisch.) Bunge] and safflower (*Carthamus tinctorius* L.). SKG has the same components as Shenkang injection (SKI), which is a commercially available treatment for CRF in China. Previous pharmacological studies have revealed that SKI administration alleviates the renal pathological lesions of 5/6 nephrectomized (5/6 Nx) rats and delays CRF progression (11,12). Clinical studies have also demonstrated that SKI reduces the levels of microalbumin (mALB), total protein (U-TP), serum creatinine (Scr) and blood urea nitrogen (BUN), and increases the nitric oxide (NO) level, creatinine clearance rate (Ccr) and serum albumin (Alb) level in CRF patients. In addition, SKI was shown to improve clinical symptoms and delay the progress of CRF (13,14). However, the injection is inconvenient and painful, and the production process is complex and expensive. In addition, the direct injection of

SKI into the blood results in higher toxicity and the risk of side-effects. Thus, oral medication is considered to be more convenient and safe compared with the injections.

The beneficial role of angiotensin-converting enzyme inhibitor (ACEI) drugs has been validated in human and experimental models of renal failure (15,16). In the present study, the effect of SKG on CRF was compared with that of benazepril, which is a commonly used ACEI drug with a proven efficacy (17). Therefore, the inhibitory effect of SKG in 5/6 Nx rats suffering from CRF was investigated using SKI and benazepril as the positive controls.

Materials and methods

Drugs. SKG and SKI were obtained from Xi'an Century Shengkang Pharmaceutical Industry Co., Ltd. (Xi'an, China). Benazepril was purchased from Beijing Novartis Pharma Co., Ltd. (Beijing, China).

Animal model and experimental groups. In total, 105 male Sprague-Dawley rats (weight, 180-240 g) were purchased from the Animal Center of Xi'an Jiaotong University (Xi'an, China). The rats were housed at room temperature, with a 12-h light/dark cycle and free access to tap water and standard rat chow. The rats were cared for in accordance with the principles of the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). All the experiments were approved by the Institutional Animal Investigation Committee of Xi'an Jiaotong University. The rats were fed a normal diet one week prior to the surgery in order to acclimatize to the laboratory environment. CRF was induced in the rats by a 5/6 nephrectomy that was performed under aseptic conditions. All surgical procedures were carried out under 10% chloral hydrate anesthesia (3.5 ml/kg; intraperitoneal). A left flank incision was made to expose the left kidney. The renal artery was temporarily occluded, and the upper and lower thirds of the kidney were ligated and resected. Bleeding was controlled by compression. Thus, one-third of the left kidney mass remained. The muscle and skin incisions were sutured with a polypropylene suture. In total, 15 rats were randomly selected as the sham-operated group that underwent the same procedure, but without a nephrectomy. The animals were returned to the cages for recovery and were injected daily with penicillin G (8,000,000 units; Harbin Pharmaceutical Group Co., Ltd., Harbin, China) for five days. After two weeks, a right flank incision was made, the renal vessels were tied, and the right kidney was excised. The sham-operated group underwent the same surgery, without excision of the kidney (15). Following suture of the muscle and skin, the rats were returned to the cages and were injected with penicillin daily for five days.

Four weeks after surgery, 2-ml blood samples were collected from the eyeball venous plexus of the rats. The sera were separated by 10-min centrifugation at 1,500 x g and 4˚C. The levels of BUN and Scr were detected using the Hitachi 7600 automated biochemistry analyzer (Hitachi, Ltd., Tokyo, Japan) at the Laboratory of the First Affiliated Hospital (School of Medicine, Xi'an Jiaotong University). According to the levels of BUN and Scr, the rats were randomly divided into seven groups (n=10 per group): (i) 5/6 Nx (model group; intragastrically treated with SKG vehicle; 2.25 ml/kg/day normal saline); (ii) SKGL (low dose; 2 g crude drug/kg/day SKG); (iii) SKGM (moderate dose; 4 g crude drug/kg/day SKG); (iv) SKGH (high dose; 8 g crude drug/kg/day SKG); (v) benazepril treatment group (5 mg/kg/day benazepril); (vi) SKI group (13.3 ml/kg/day SKI); and (vii) sham-operated group (2.25 ml/kg/day normal saline). Drugs and vehicle were administered by gastrogavage once per day for 30 days, and the rats were weighed once per week.

mALB and U-TP. Prior to and following the treatment period, the rats were placed in metabolic cages (Shanghai Kangway Medical Science and Technology Development Co., Ltd., Shanghai, China) for 24 h urine collection. The urine samples were centrifuged at 1,000 x g for 5 min and filtrated through a 2.2-μm membrane (Chekiang Haining Hengtai Filter Equipment Factory, Chekiang, China). The concentrations of mALB and U-TP in the urine samples were detected at the Laboratory of the First Affiliated Hospital (School of Medicine, Xi'an Jiaotong University).

Determination of Scr, BUN, total cholesterol (CH), triglyceride (TG), low-density lipoprotein (LDL) and glutathione peroxidase (GSH-PX) levels. At the end of the treatment period, the rats were anesthetized by an injection of 10% chloral hydrate (3.5 ml/kg), and blood samples were collected from the aorta ventralis. Sera were obtained following 10-min centrifugation at 1,500 x g and 4˚C, and were frozen at -80˚C until required for further analysis. Scr, BUN, CH, TG and LDL levels were detected at the Laboratory of the First Affiliated Hospital (School of Medicine, Xi'an Jiaotong University). GSH-PX levels were detected using a GSH-PX biochemical assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Serum transforming growth factor-β_1 (TGF-β_1) and angiotensin II (Ang II). A rat TGF-β_1 ELISA kit (Shanghai Westang Bio-Tech Co., Ltd., Shanghai, China) was used to determine the TGF-β_1 level in the rat serum. In a glass tissue grinder, the rat kidneys were homogenized for 30 sec in prechilled methanol. The homogenate was centrifuged at 4˚C for 20 min at 13,000 x g, and the supernatant was obtained. Serum and kidney levels of Ang II were detected using an Ang II radioimmunoassay kit (Beijing Sino-UK Institute of Biological Technology, Beijing, China).

Body weight, kidney weight/body weight ratio (KW/BW) and heart weight index (HWI). During the treatment period, the rats were weighed once per week. At the end of the treatment period, the rats were weighed and anesthetized using the aforementioned procedure. Subsequently, the remnant left kidney and the heart were excised and weighed, and the KW/BW and HWI were calculated.

Histology. To observe the renal lesions the paraformaldehyde-fixed kidneys were embedded in paraffin and cut into 4-μm sections, the sectioned kidney samples were then stained with hematoxylin and eosin. In addition, the kidney sections were stained with Masson's trichrome to measure the fibrotic areas. The images were captured and viewed using an image analysis system (Leica Q550CW; Leica Microsystems GmbH, Wetzlar, Germany).

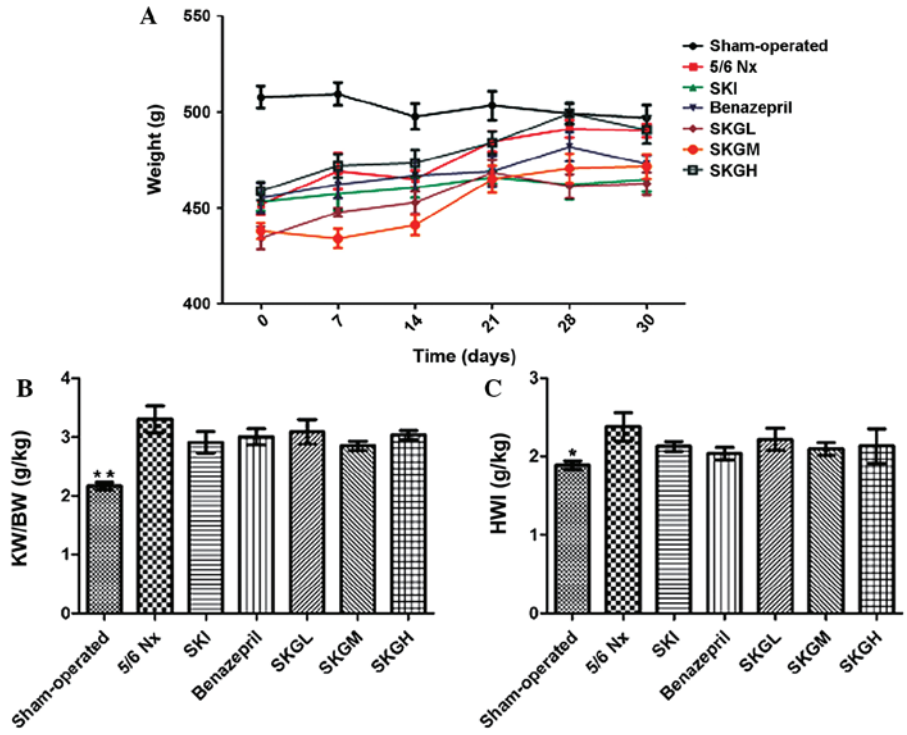

Figure 1. Effect of SKG on the (A) body weight, (B) KW/BW and (C) HWI. Values are presented as the mean ± standard error of mean (n=10 per group). *P<0.05 and **P<0.01, vs. 5/6 Nx group. SKG, Shenkang granules; KW/BW, kidney weight/body weight ratio; HWI, heart weight index; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG.

Western blot analysis. For western blot analysis, the rat kidney samples were lysed in radioimmunoprecipitation assay lysis buffer, containing 0.1 M phenylmethylsulfonyl fluoride, and centrifuged at 13,000 x g for 20 min. The total protein concentrations of the supernatants were determined using a BCA Protein Assay kit (Applygen Technologies, Inc., Beijing, China). Extracts were boiled at a 1:1 ratio with a loading buffer containing Tris (125 mmol/l; pH 6.8), 4% w/v sodium dodecyl sulphate (SDS), 10% v/v glycerol, 4% v/v 2-mercaptoethanol and 2 mg/ml bromophenol blue. Equal amounts of protein were separated using 10% SDS-polyacrylamide gel electrophoresis (18), and the separated protein was transferred to a nitrocellulose membrane. Subsequently, the membrane was blocked with 5% non-fat milk to avoid non-specific protein binding (19). Next, the membrane was incubated at 4˚C overnight with the following primary antibodies: Rabbit anti-collagen I (1:100; cat. no. bs-7158R, Beijing Biosynthesis Biotechnology Co., Ltd., Beijing, China), rabbit anti-collagen III (1:100; cat. no. bs-0948R, Beijing Biosynthesis Biotechnology Co., Ltd.), rabbit anti-matrix metalloproteinase (MMP)-2 (1:500; cat. no. ab124294, Abcam, Hangzhou, China), rabbit anti-MMP-9 (1:500; cat. no. ab7299, Abcam) and GAPDH (1:500; cat. no. ab181602, Abcam; serving as an internal control). The membrane was incubated with a horseradish peroxidase-conjugated anti-rabbit secondary antibody (1:20,000; cat. no. 111-035-00, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA) for 2 h at room temperature, after which the membranes were visualized using a Fujifilm LAS-1000 Luminescent Image Analyzer (Fujifilm Medical Systems USA, Stamford, CT, USA) and the band intensity was quantified by ImageJ software (http://rsb.info.nih.gov/ij/).

Statistical analysis. One-way analysis of variance, followed by Tukey's multiple comparison test, were performed to detect the statistically significant differences among three or more groups. Statistical analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA). Results are presented as the mean ± standard error of mean. P<0.05 was considered to indicate a statistically significant difference.

Results

Effect of SKG on body weight, KW/BW and HWI. Sham-operated rats were found to have a higher body weight compared with the 5/6 Nx animals prior to the treatment period; however, no statistically significant difference was observed (P>0.05; Fig. 1A). As shown in Fig. 1A, the body weight of the rats increased slowly during the treatment period, with the exception of the sham-operated rats. However, no statistically significantly differences were detected among the groups (P>0.05). The left KW/BW of the sham-operated group was found to be significantly lower compared with the model group and the other treatment groups (P<0.01; Fig. 1B), indicating that the remnant kidney of the 5/6 Nx rats exhibited hypertrophy. The HWI of the 5/6 Nx group was found to be significantly higher compared with the sham-operated group (P<0.05; Fig. 1C), whereas no statistically significant differences were observed between the sham-operated group and treatment groups (P>0.05). These results indicated that SKG may prevent hypertrophy of the heart.

Effect of SKG on mALB and U-TP. As shown in Fig. 2A and B, the mALB and U-TP concentrations in the 5/6 Nx rats were higher compared with the sham-operated animals

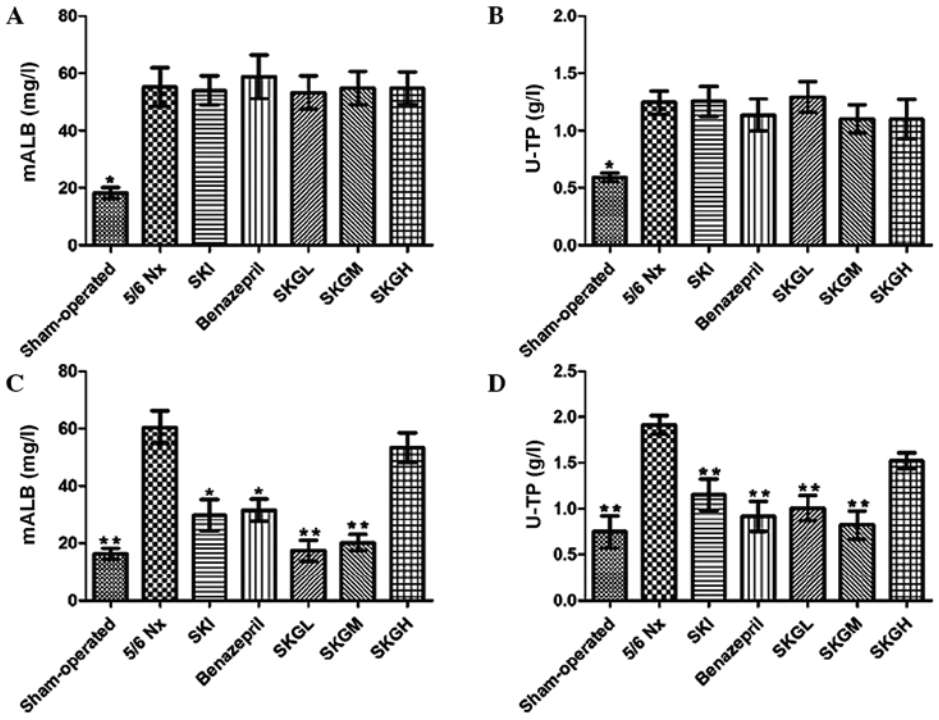

Figure 2. Effect of SKG on the concentration of (A) mALB and (B) U-TP prior to the treatment and the concentration of (C) mALB and (D) U-TP following the treatment. Values are presented as the mean ± standard error of mean (n=10 per group). *P<0.05 and **P<0.01, vs. 5/6 Nx group. SKG, Shenkang granules; mALB, microalbumin; U-TP, total protein; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG.

prior to treatment (P<0.01), while no statistically significant differences were observed among the treatment groups. At the end of the treatment period, the levels of mALB and U-TP in the 5/6 Nx group (60.43±5.15 mg/l and 1.91±0.09 g/l, respectively) were significantly higher compared with the sham-operated group (16.20±1.39 mg/l and 0.75±0.15 g/l, respectively; P<0.01). By contrast, treatment with SKGL and SKGM decreased the level of mALB (17.35±3.15 and 20.17±2.46 mg/l, respectively; P<0.01, vs. 5/6 Nx group) and U-TP (1.01±0.11 and 0.82±0.14 g/l, respectively; P<0.01, vs. 5/6 Nx group). However, treatment with SKGH did not reduce the levels of mALB and U-TP (Fig. 2C and D). In addition, SKI and benazepril were found to reduce the mALB and U-TP levels significantly (P<0.05 and P<0.01, vs. 5/6 Nx group, respectively).

Effect of SKG on renal function, serum lipid levels and GSH-PX. Scr and BUN concentrations were found to be higher in the 5/6 Nx group (73.03±3.31 μmol/l and 16.04±0.83 mmol/l, respectively) when compared with the sham-operated group (40.66±3.31 μmol/l and 6.87±0.52 mmol/l, respectively; P<0.01; Fig. 3A and B). As shown in Fig. 3A and B, SKG, SKI and benazepril lowered the increased Scr and BUN levels following 5/6 Nx surgery (P<0.05 or P<0.01, vs. 5/6 Nx group); however, the levels did not reach those of the sham-operated group. In addition, the levels of CH, TG and LDL in the treatment groups decreased significantly compared with the 5/6 Nx group (P<0.05 or P<0.01; Fig. 3C-E). The serum concentration of GSH-PX was found to be significantly increased in the SKGL and SKGM groups when compared with the 5/6 Nx group; however, the GSH-PX concentrations remained lower than those in the sham-operated rats (Fig. 3F; P<0.05, vs. 5/6 Nx group).

Effect of SKG on TGF-β_1 and Ang II. Serum TGF-β_1 concentrations were significantly higher in the 5/6 Nx group (56.14±3.14 ng/ml) when compared with the sham-operated group (39.74±1.80 ng/ml; P<0.05; Fig. 4A). Treatment with low-, moderate- and high-dose SKG was found to reduce the serum TGF-β_1 level compared with the 5/6 Nx group (P<0.01, P<0.05 and P<0.01, respectively; Fig. 4A). However, no statistically significant difference was observed when comparing the concentration in the SKI group and the 5/6 Nx group. To further clarify the possible mechanisms of the aforementioned effects of SKG on CRF, the serum and kidney levels of Ang II were measured. As shown in Fig. 4B, the content of serum Ang II was significantly increased in the 5/6 Nx group compared with the sham-operated group (P<0.01). SKGL treatment reduced the serum Ang II level (P<0.05, vs. 5/6 Nx group), while the remaining treatment groups had no effect on the serum Ang II level. By contrast, the kidney Ang II concentration was found to be significantly decreased in the treatment groups (P<0.01 or P<0.05, vs. 5/6 Nx group; Fig. 4C), with the exception of the SKGH group.

Histology. Sham-operated rats were found to have a normal kidney structure (Fig. 5). By contrast, the kidney histology of the 5/6 Nx group revealed extensive fibrosis, various grades of sclerosis and fibrosis in the glomeruli, mesangial cell hyperplasia and mesangial matrix proliferation. In addition, atrophy and necrosis of renal tubules, as well as detachment of epithelial cells, were observed. Tubulointerstitial analysis

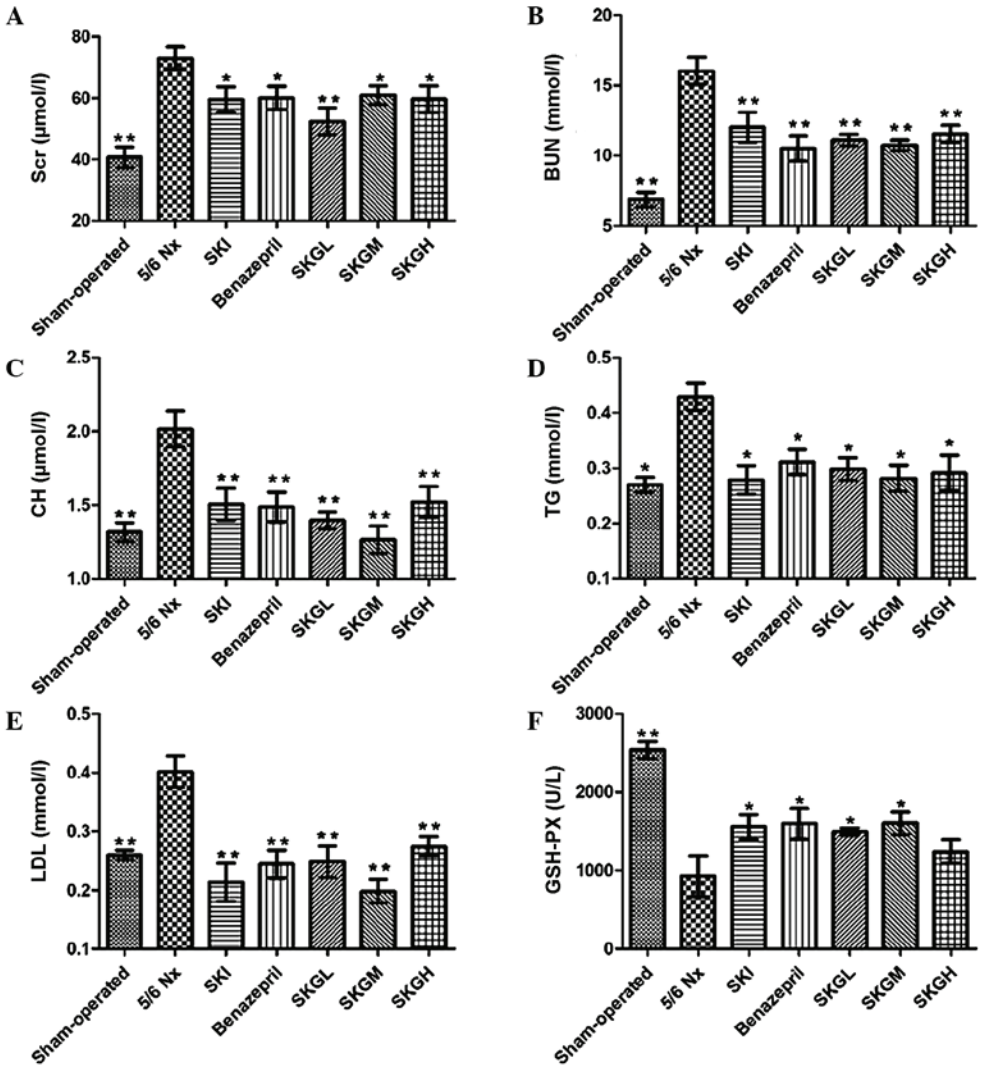

Figure 3. Effect of SKG on the levels of (A) Scr, (B) BUN, (C) CH, (D) TG, (E) LDL and (F) GSH-PX in 5/6 Nx rats at day 30 following surgery. Values are presented as the mean ± standard error of mean (n=10 per group). *P<0.05 and **P<0.01, vs. 5/6 Nx group. SKG, Shenkang granules; Scr, serum creatinine; BUN, blood urea nitrogen; CH, total cholesterol; TG, triglyceride; LDL, low-density lipoprotein; GSH-PX, glutathione peroxidase; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG.

revealed evident fibrosis and inflammation of the infiltrate. Renal damage was significantly improved in the treatment groups, particularly in the SKGL and SKGM groups; however, the renal damage was not modulated effectively in the SKGH group.

Effect of SKG on MMP-2 and MMP-9. Renal concentrations of MMP-2 and MMP-9 in the 5/6 Nx group were significantly higher compared with the sham-operated group. As shown in Fig. 6, SKG markedly decreased the MMP-2 and MMP-9 levels (P<0.01, vs. 5/6 Nx group). The level of MMP-2 in the SKGL and SKGM groups exhibited no statistical difference with that of the sham-operated rats. In addition, the MMP-9 level in the SKGL group was lower compared than the level in the sham-operated rats, but the difference was not statistically significant.

Effect of SKG on collagen I and III. Western blot analysis was used to analyze the extracellular matrix (ECM) protein expression, which was found to be significantly increased in the 5/6 Nx group. By contrast, the expression levels of collagen I and III were reduced in the treatment groups. As shown in Fig. 7, the SKGL and SKGM groups exhibited significantly reduced expression levels of collagen I, showing similar levels to the sham-operated group. However, the SKI, benazepril and SKGH groups had no effect on the collagen I expression levels. The content of collagen III in all the treatment groups was evidently decreased when compared with the 5/6 Nx group (P<0.01, Fig. 7B); however, the collagen III level in the SKI, benazepril and SKGH groups remained higher compared with the sham-operated group.

Discussion

The aim of the present study was to investigate the beneficial effect of SKG during the progressive phase of renal injury in 5/6 Nx rats. A previous study demonstrated that in a 5/6 Nx rat model of CRF, proteinuria and renal dysfunction associated with glomerular sclerosis and tubulointerstitial fibrosis were observed (20). In the present study, SKG was shown to

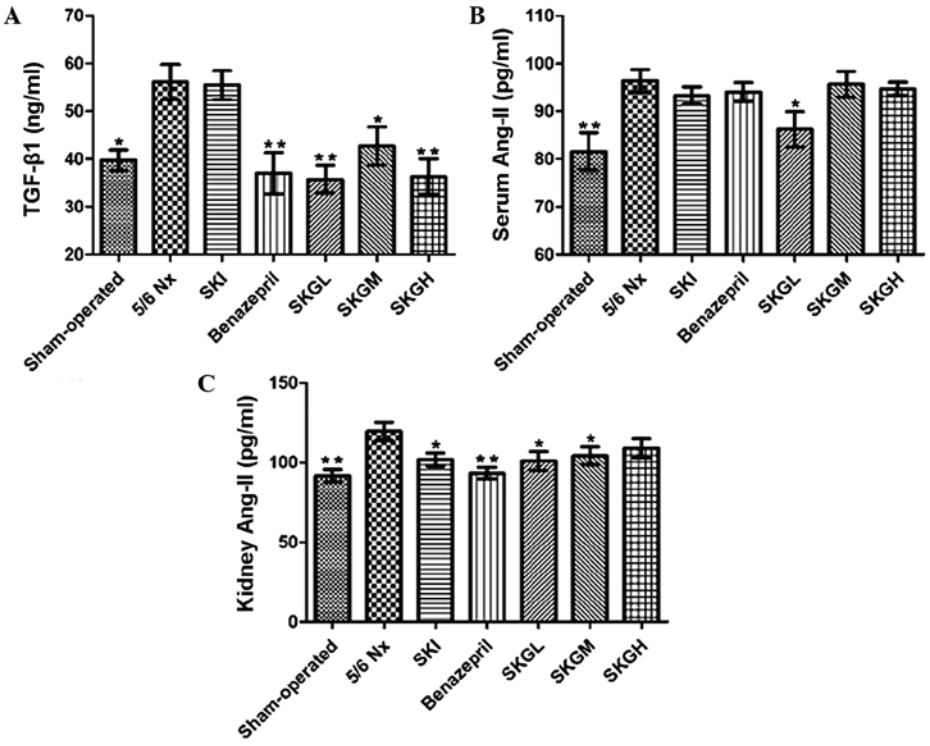

Figure 4. Effect of SKG on the serum concentration of (A) TGF-β_1 and (B) Ang II and the level of (C) Ang II in the kidney tissues. Values are presented as the mean ± standard error of mean (n=10 per group). *P<0.05 and **P<0.01, vs. 5/6 Nx group. SKG, Shenkang granules; TGF-β_1, transforming growth factor-β_1; Ang II, angiotensin II; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG.

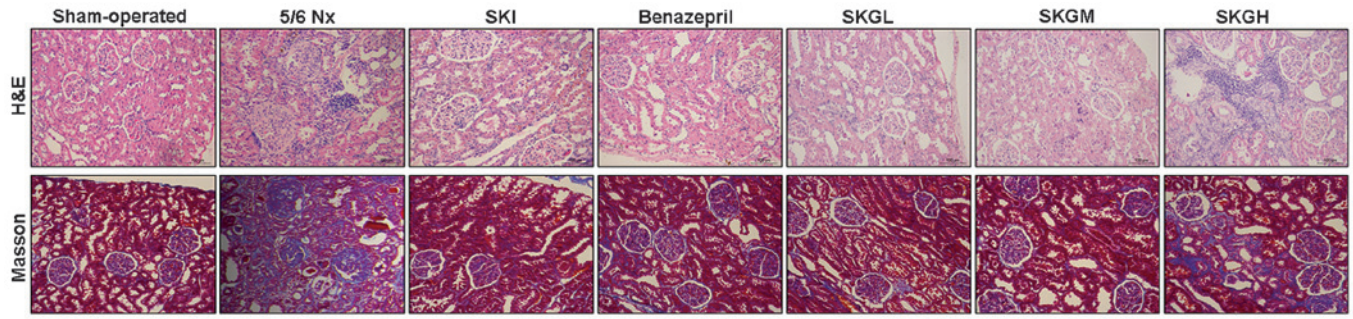

Figure 5. Renal histology with hematoxylin and eosin and Masson's trichrome staining (magnification, x200), showing the kidney histological injury in each group. 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; SKG, Shenkang granule; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG.

significantly reduce the levels of mALB, U-TP, Scr, BUN, CH, TG, LDL, TGF-β_1 and kidney Ang II. In addition, SKG increased the level of GSH-PX and attenuated the progression of pathological renal damage in the CRF model; thus, SKG was demonstrated to improve renal function.

Lipid metabolism disorder is a common complication of CRF that induces an increase in CH and TG levels and upregulates the LDL level (8,21). Hyperlipidemia may lead to biological metabolic disorders, and alter the blood- and hemodynamics of kidney tissues, aggravating proteinuria and renal pathological changes (7,22). Abnormal lipoprotein levels can result in the formation of foam cells in the kidney and promote glomerular sclerosis (23). In the current study, SKG was found to significantly reduce the levels of CH, TG and LDL, thus inhibiting abnormal lipid deposition and alleviating the lipid-induced damage of the renal tissues. Therefore, SKG may improve renal damage by reducing the serum lipid concentration. Furthermore, CRF leads to a decreased glomerular filtration rate and the accumulation of Scr, BUN, proteinuria and other metabolites, resulting in an increase in the total oxygen free radical content (24,25). GSH-PX is an important enzyme that eliminates free radicals (26,27). In the present study, the concentration of GSH-PX in the 5/6 Nx group was found to be significantly lower compared with the sham-operated group, whereas SKG was found to significantly increase the level of GSH-PX. The results indicated that SKG may increase the removal of toxicity products, remove oxygen free radicals and reduce the free radical-induced damage of kidney tissues, subsequently improving renal function.

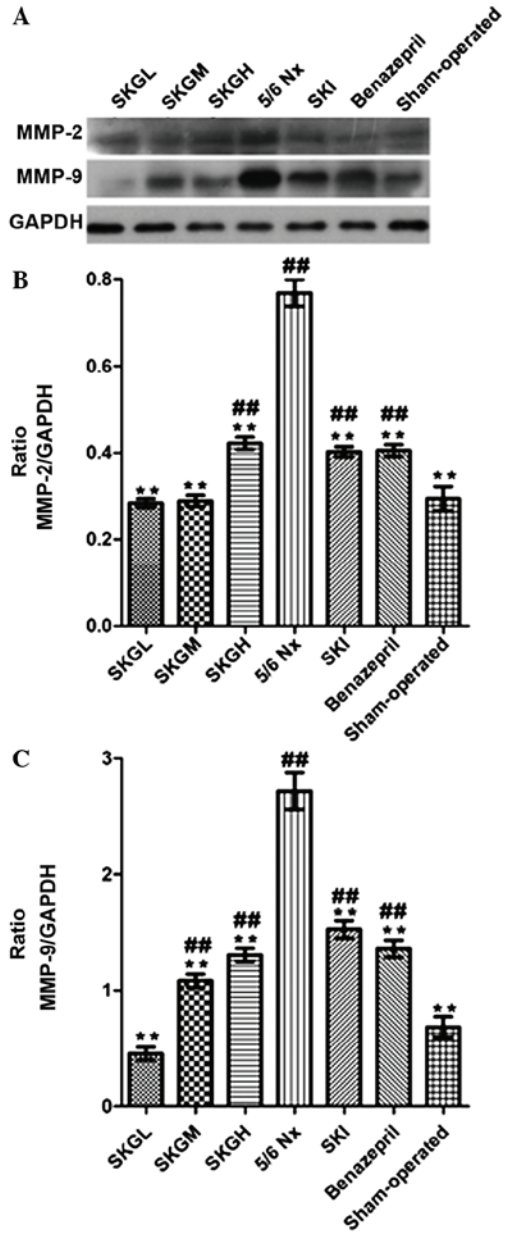

Figure 6. (A) Western blot analyses showing MMP-2 and MMP-9 expression in the renal tissue. Quantification of the expression levels of (B) MMP-2 and (C) MMP-9 in the different groups. Values are presented as the mean ± standard error of mean (n=10 per group). **P<0.01, vs. 5/6 Nx group; $^{\#\#}$P<0.01, vs. sham-operated group. MMP, matrix metalloproteinase; SKG, Shenkang granule; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

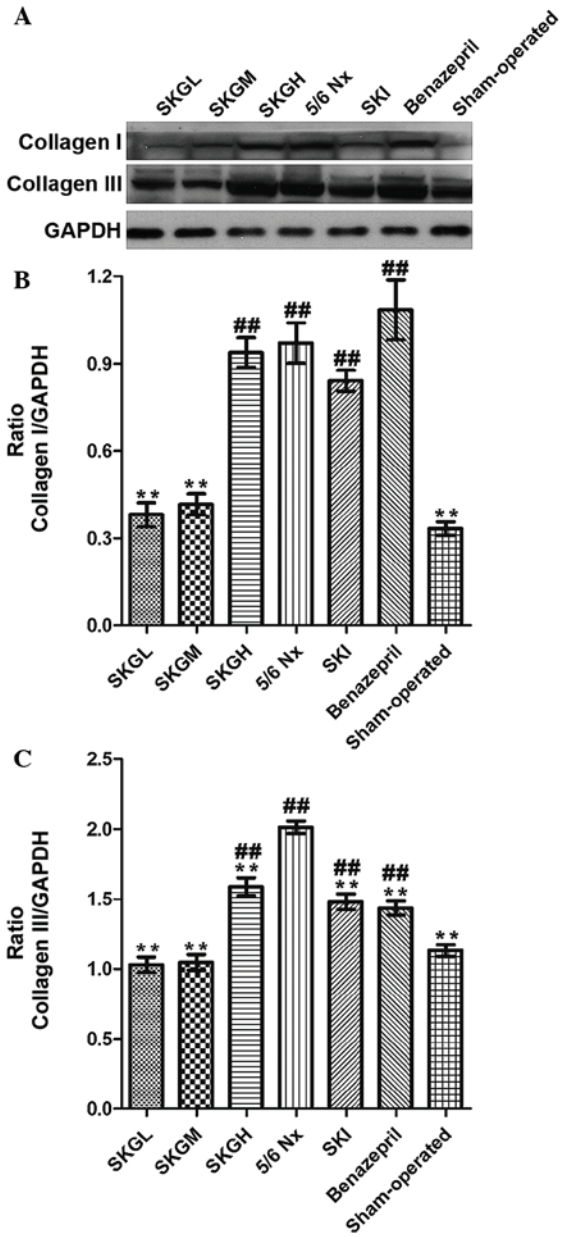

Figure 7. (A) Western blot analyses showing collagen I and collagen III expression in the renal tissue. Quantification of the expression levels of (B) collagen I and (C) collagen III in the different groups. Values are presented as the mean ± standard error of mean (n=10 per group). **P<0.01, vs. 5/6 Nx group; $^{\#\#}$P<0.01, vs. sham-operated group. SKG, Shenkang granule; SKGL, low-dose SKG; SKGM, moderate-dose SKG; SKGH, high-dose SKG; 5/6 Nx, 5/6 nephrectomized (model group); SKI, Shenkang injection; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

Previous studies have demonstrated that TGF-β_1 is a key profibrotic growth factor involved in renal fibrogenesis and an important factor among cytokines, with TGF-β_1 expression upregulated in CRF (28-31). In the present study, SKG was shown to markedly reduce the serum concentration of TGF-β_1. Ang II is an inflammatory molecule that activates TGF-β_1 (32), and Ang II and TGF-β_1 are critical mediators of renal fibrosis (33). Previous studies have indicated that Ang II is expressed in renal interstitial cells in a Nx model, paralleling the severity of renal injury (34,35). In the current study, Ang II levels increased in the serum and kidney tissues of 5/6 Nx rats, while SKG was found to markedly decrease the kidney Ang II level. However, the various treatments had no statistically significant effect on the serum Ang II level, with the exception of the low-dose SKG treatment . In addition, the level of Ang II was in accordance with the level of TGF-β_1, indicating that SKG may prevent CRF by regulating Ang II and TGF-β_1.

The progression of CRF is characterized histologically by progressive glomerulosclerosis and tubulointerstitial fibrosis (9,36). A significant observation of the present study was the marked improvement effect of SKG on renal pathological damage, through inhibition of the relative area of renal glomerulosclerosis and tubulointerstitial fibrosis,

particularly in the SKGL and SKGM groups. Since the magnitude of fibrosis has been shown to indicate the degree and progression of renal failure (37), the antifibrotic effect of SKG may be relevant to the attenuation of renal disease progression. The effect of SKG in ameliorating renal pathological damage may be directly associated with a reduction in the synthesis of major ECM components (38,39), as demonstrated by the diminished production of collagen I and III in the kidney tissues of the SKG-treated 5/6 Nx rats . As revealed by western blot analysis, SKGL and SKGM may significantly reduce the content of collagen I and III, with no statistically significant difference observed with the sham-operated group.

Matrix metalloproteinases (MMPs) are a large family of zinc-dependent proteases that regulate tissue remodeling, cell proliferation and angiogenesis through affecting ECM accumulation (40). MMPs are known to play an important role in the pathophysiology of renal diseases (41). In addition, increasing evidence indicates that MMP abnormalities are involved in the vascular changes associated with kidney failure (42). MMP-2 and MMP-9 are important factors participating in the progression of atherosclerosis in CRF patients (42). Deposition of ECM also results in an increase in MMP-2 and MMP-9 levels (43). The present study investigated the MMP-2 and MMP-9 levels in kidney tissues using western blot analysis. SKG was found to significantly reduce the levels of MMP-2 and MMP-9, as compared with the 5/6 Nx group.

In conclusion, the current study demonstrated that SKG ameliorated renal injury in a 5/6 Nx rat model of CRF, through the prevention of albuminuria, glomerulosclerosis and interstitial fibrosis, and the reduction of Scr, BUN and serum lipid levels. These effects may be associated with the impact of SKG on ECM deposition and the inhibition of TGF-β_1 and kidney Ang II. The observations demonstrate that SKG may have a good therapeutic effect on chronic renal failure. However, high-dose SKG did not have a marked renoprotective effect in 5/6 Nx rats; thus, further study is required.

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