Protective Effects of Shichimotsu-koka-To on Irreversible Thy-1 Nephritis

Takahiko Ono, Kohei Kamikado, and Tatsuya Morimoto*

1Division of Nephrology, Department of Internal Medicine, Atami Hospital, Intenational University of Health and Welfare; 13–1 Higashikai-gan-cho, Atami 413–0012, Japan; and 2Department of Molecular Medicine, School of Pharmaceutical Sciences, University of Shizuoka; 52–1 Yada, Suruga-ku, Shizuoka 422–8526, Japan.

Received May 27, 2012; accepted October 8, 2012; advance publication released online November 7, 2012

The number of patients with end-stage renal failure undergoing dialysis is increasing worldwide. Recently, it has been accepted that patients with chronic kidney disease (CKD) also develop cardiovascular diseases, with increased risks of death, cardiovascular events, and hospitalization. Improvement of the clinical outcomes of CKD patients is therefore important. In Japan, chronic glomerulonephritis and nephrosclerosis are major causes of renal failure after diabetic nephropathy, which is the most common cause. Hypertension is an important symptom which influences the outcome of CKD, and angiotensin II receptor blockers (ARBs) are beneficial to protect against renal injury in CKD.

The herbal formula Shichimotsu-koka-To (SKT; Table 1) has occasionally been prescribed to treat hypertensive patients. SKT was used for non-obesity hypertensive patients, and those complaining of the sensation of a rush of blood to the head, shoulder stiffness, tinnitus, and dull headache; however, in the last two or three decades, abundant new strong anti-hypertensive drugs have become available, and SKT has been used less. Recently, reevaluation of SKT treatment in combination with ACE-Is/ARBs drugs in CKD has begun. In basic studies, SKT had an anti-hypertensive effect in Dahl strain rats. Enhanced serum NO levels, and anti-oxidant activity through increasing superoxide dismutase (SOD)-like activity in the kidney by SKT were reported. Previously, in a rat model of nephrosclerosis induced by 5/6 nephrectomy, Bai et al. observed that SKT had suppressive effects on hypertension through the inhibition of increased plasma levels of asymmetric dimethylarginine, and the recovery of dimethylarginine dimethylaminohydrolase-2 levels in the kidney. However, 5/6 nephrectomized model is chronic renal failure through nephrosclerosis without inflammation of glomerulonephritis, and mimicking human hypertensive nephrosclerosis. Because chronic glomerulonephritis is the second cause of end-stage renal failure, we need to clarify whether SKT is effective on advanced glomerulonephritis model. Therefore, in this study, we adopted an experimental model of irreversible mesangiproliferative glomerulonephritis (MsPGN), using uninephrectomized rats with anti-Thy-1 nephritis, and we investigated the preventive effects of SKT in the peritubular capillary (PTC) networks.

Oxidative stress and peritubular capillary (PTC) injury are involved in the progression of chronic kidney disease (CKD). We investigated protective effects of Shichimotsu-koka-To (SKT), a Japanese traditional Kampo prescription, against nephrosclerosis and hypertension on a CKD model due to irreversible nephritis. Six-week-old male Wistar rats were subjected to uninephrectomy, and to injection of rabbit anti-thymocyte serum. SKT treatment was continued for 15 weeks, blood pressure was measured, and then renal specimens were collected. PTC networks were detected by immunostaining for CD-31. And superoxide dismutase (SOD)-like activity in the tissue was evaluated. Blood pressure in the SKT group, as well as sham group, was significantly lower than with the vehicle. SKT markedly ameliorated renal function, which was evaluated with urea nitrogen clearance. Compared with the vehicle, SKT treatment lowered both the glomerular enlargement and hyper-cellularity by 80%, and decreased the extracellular matrix area by 75%. SKT treatment also suppressed tubular injury, and maintained PTC networks. Furthermore, SKT recovered SOD-like activity to the basal levels. These results suggest that SKT may be useful for the treatment of CKD during the progression to nephrosclerosis, through the mechanisms of anti-oxidative activity and maintenance of PTC networks.

Key words hypertension; glomerular hypertrophy; peritubular capillary; chronic kidney disease; oxidative stress

Materials and Methods

Materials Six-week-old male Wistar rats weighing 180 to 200 g were obtained from Japan SLC (Shizuoka, Japan). Rats were housed under specific pathogen-free conditions in the Animal Facilities of the University of Shizuoka. Animal care and experiments were performed in accordance with

<table>
<thead>
<tr>
<th>Herbal name (botanical name)</th>
<th>Quantity (dry, g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeoniae Radix (<em>Paeonia lactiflora</em>)</td>
<td>4.0</td>
</tr>
<tr>
<td>Angelicae Radix (<em>Angelica acutiloba</em>)</td>
<td>4.0</td>
</tr>
<tr>
<td>Astragali Radix (<em>Astragalus membranaceus</em>)</td>
<td>3.0</td>
</tr>
<tr>
<td>Rehmanniae Radix (<em>Rehmannia glutinosa</em>)</td>
<td>3.0</td>
</tr>
<tr>
<td>Cnidii Rhizoma (<em>Cnidium officinale</em>)</td>
<td>3.0</td>
</tr>
<tr>
<td>Uncariae Uncis cum Ramulus (<em>Uncaria rhynchophylla</em>)</td>
<td>3.0</td>
</tr>
<tr>
<td>Phellodendri Cortex (<em>Phellodendron amurense</em>)</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Table 1. Herbal Compositions of Shichimotsu-koka-To (TJ-46)
the guidelines of 2008 for the Care and Use of Laboratory Animals of the University of Shizuoka, and the Ethics Review Board of the University granted permission for this study. SKT was supplied by Tsumura (TJ-46, Lot No. 2050046010; Tokyo, Japan), and was prepared as an aqueous solution of dried powder extracts from medicinal plants, as shown in Table 1. The contents of indicator constituents per daily clinical dose were as following: berberine, 9.41 mg/4.0 g dried extract; and paenoniflorin, 42.66 mg/4.0 g dried extract.

**Experimental Design**  Irreversible anti-Thy-1 nephritis in rats was induced as described previously by Cheng et al. 10 Briefly, 5 weeks after rats had been uninephrectomized under pentobarbital sodium anesthesia, Thy-1 nephritis was triggered by the intravenous injection of rabbit anti-rat thymocyte serum (ATS) (week 0), which was prepared as described earlier, 11 and kindly provided by Nippon Shinyaku Co. (Kyoto, Japan). Three days after the ATS injection, urinary protein was evaluated to assess whether nephritis was triggered; these rats were then randomly assigned to a control vehicle group (n = 5) and SKT group (500 mg/kg/d; n = 5). A sham operation group (n = 6) without ATS injection was also prepared. All rats were sacrificed in week 16 (Fig. 1). Blood pressure was measured in week 1, 2, 4, 8, and 16 of the ATS injection. Urine was collected in week 2, 0.3, 2, and 16. Kidney tissues were collected when rats were sacrificed in weeks 16. Tissues were stained for ordinary light microscopy and immunohistochemical evaluation, and were also evaluated for SOD activity and histological evaluation, and were also evaluated for SOD activity and Western blot analysis, as described below. SKT was orally administered in drinking water ad libitum for 15 weeks after the random assignment in week 1. The amount of drinking water was checked weekly, and the dosage of SKT was controlled by adjusting the concentration.

**Measurement of Blood Pressure, Urinary Protein, and Serum Urea Nitrogen**  Systolic blood pressure was measured in the conscious state by the tail-cuff method (BP-98A; Softron, Tokyo, Japan). Urinary protein excretion was measured with a Bio-Rad Protein Assay kit (Bio-Rad, Hercules, CA, U.S.A.) by the ordinary Bradford method according to the manufacturer’s protocol. Serum and urine urea nitrogen levels were determined with a kit by the urease indophenol method (Wako Pure Chemical Industries, Osaka, Japan).

**Histological Evaluation of Renal Tissues**  Renal tissues from each animal were evaluated by light microscopy and immunohistochemistry. The tissues were fixed in 10% neutral-buffered formalin (pH 7.4) and embedded in paraffin. Sections (4 µm) were stained with periodic acid–Schiff (PAS) or trichrome, which was evaluated quantitatively by measuring the blue-stained areas in 10 selected glomerular cross sections with Image J program (NIH, Bethesda, MD, U.S.A.), and expressed as the staining area (µm2). 12 Tubular injury was evaluated according to the method and criteria of Uehara et al. 13 Briefly, tubular injury was scored as follows: 0, no lesions; 1, very mild focal dilatation of tubules; 2, larger number of dilated tubules with widening of interstitium; 3, fairly extensive dilatation of tubules with cystic formation and widening of interstitium; and 4, complete atrophy of tubules. Scores in 10 cross sections were calculated. Next, the ratio of the luminal area per total renal area was evaluated in 10 selected cross sections of the outer stripe of the outer medulla with the Image J program.

**Immunohistochemical Assay for CD31-Positive PTCs**

Deparaffinized sections were followed by microwave antigen retrieval, and then incubated with a goat anti-mouse platelet-endothelial cell adhesion molecule-1 antibody (PECAM, CD31) (1:100; Santa Cruz Biotechnology, Santa Cruz, CA, U.S.A.) overnight at 4°C, as described previously by Saito et al. 14 Sections were then incubated with biotinylated immunoglobulin G (IgG) of the secondary antibody (Vector laboratories). The sections were then reacted with avidin-DH-biotinylated horseradish peroxidase complex ( Vectastain ABC kit; Vector Laboratories). Color was then developed by incubation with a Peroxidase Substrate kit (Vector Laboratories). The sections were then counterstained with hematoxylin. The number of CD31-positive PTCs was counted in 10 positions on the outer stripe of the outer medulla by high-power-field microscopy. Negative control was made using normal serum as blocking peptide without primary antibodies for adjustment.

**Western Immunoblot Analysis**  For protein extract preparation, renal cortex tissues were homogenized in lysis buffer (50 mM Tris–HCl, 150 mM NaCl, 2 mM ethylenediaminetetraacetic acid (EDTA), 1% w/v NP-40, 0.1% w/v sodium dodecyl sulfate, 2 µg phenylmethylsulfonyl fluoride, Protease inhibitor cocktail (Roche Diagnostics Japan, Tokyo, Japan)) with a Polytron (Kinematica AG, Cincinnati, OH, U.S.A.). The protein extracts were put on ice for 30 min and spun at 13200 rpm. The supernatant was then collected. Proteins were separated by electrophoresis with NuPage 4–12% Bis Tris gels (Invitrogen, Carlsbad, CA, U.S.A.) using 15 µg protein per sample. The resultant proteins were electroblotted onto nitrocellulose membranes (GE Healthcare, Tokyo, Japan). Membranes were then incubated for 2 h at 37°C in 5% skim milk solution. The resultant blots were incubated at room temperature for 1 h with either a rabbit anti-mouse collagen type IV antibody (1:2000; LSL, Cosmo Bio, Tokyo, Japan) or a mouse monoclonal anti-β-actin antibody (1:2000; Sigma, St.

<table>
<thead>
<tr>
<th>Group name</th>
<th>Nx(a)</th>
<th>ATS(b)</th>
<th>Drug administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>–</td>
<td>–</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Vehicle</td>
<td>+</td>
<td>+</td>
<td>Vehicle</td>
</tr>
<tr>
<td>SKT</td>
<td>+</td>
<td>+</td>
<td>SKT(c): 500 mg/kg/d</td>
</tr>
</tbody>
</table>

(a) Nx, uninephrectomy; (b) ATS, anti-rat thymocyte serum; (c) SKT, Shichimotsuko-To.
January 2013 43

**Measurement of Tissue SOD-Like Activity**
Tissue SOD-like activity was measured with a SOD assay kit, WST (Dojindo Laboratories, Mashiki-machi, Kumamoto Prefecture, Japan) according to the manufacturer’s protocol. Briefly, the supernatant of the protein extracts in the renal cortex was collected. To 20 µL sample, 200 µL WST working solution and 20 µL enzyme working solution were added. Then, after incubation at 37°C for 20 min, absorbance at 450 nm was read with a microplate reader, and the SOD-like activity (inhibition rate %) was calculated using the equation in the protocol.

**Statistical Analysis**
Values are represented as the means±S.E.M. Statistical differences were assessed using Kruskal–Wallis one-way analysis of variance (ANOVA) on ranks. ANOVA followed by Fisher’s protected least significant difference (PLSD) test was used to see whether urea nitrogen clearance varied significantly among groups. Differences were considered significant at $p<0.05$.

**RESULTS**

**Body Weight, Volume of Drinking Water, and Blood Pressure**
Body weight did not differ among groups (data not shown). The volume of drinking water increased along with body weight gain during the experimental period for 15 weeks from week 1 to week 16 (data not shown). The volume of drinking water with or without SKT did not differ among groups throughout the study, adjusting the concentration of the drug in relation to the volume of water drunk. Systolic blood pressure levels were elevated in the vehicle group, but were significantly suppressed by SKT to similar levels to the sham group (week 16: sham group, 125±2 mmHg; vehicle group, 138±2 mmHg, $p<0.05$ vs. sham; SKT-treated group, 127±1 mmHg, $p<0.01$ vs. vehicle) (Fig. 2).

**Urinary Protein and Renal Function**
The amount of urinary protein was increased in the vehicle group after the induction of Thy-1 nephritis, had a trend toward reduction in the SKT-treated group (week 16: sham, 2.6±0.1 mg/mg creatinine; vehicle, 19.2±3.1 mg/mg creatinine, $p<0.01$ vs. sham;
urea nitrogen clearance decreased in the vehicle group, and 0.05 ml/min; vehicle, 0.50 ±

the change in blood urea nitrogen levels. SKT, 0.74 ± significantly suppressed by 80% by SKT treatment (sham, 9.00 ±

nificantly suppressed by 80% by SKT treatment (sham, 65.6±1.8 cells; vehicle, 95.5±0.3 cells, p<0.05 vs. sham; SKT, 80.2±1.6 cells/glomerular cross section, p<0.05 vs. vehicle) (Fig. 4e). To evaluate extracellular matrix widening, trichrome-stained renal tissues were observed as shown in Figs. 5a to c. In the vehicle group, blue trichrome-stained areas in glomeruli were significantly increased compared with the sham operation group, and SKT significantly suppressed this increase by 75% (sham, 1.89±0.13×10^3 μm^2; vehicle, 3.55±0.16×10^3 μm^2, p<0.05 vs. sham; SKT, 2.63±0.15×10^3 μm^2, p<0.05 vs. vehicle) (Fig. 5d).

Figures 6a to 6c show PAS-stained tubular findings. In the vehicle group, findings of extensive dilatation of tubules with cystic formation increased compared with the sham operation group, and the tubular injury score was significantly suppressed by treatment with SKT (sham, 0.2±0.1; vehicle, 3.0±

± 0.1; vehicle, 3.05±0.16 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

SKT, 16.8±2.0 mg/mg creatinine) (Fig. 3). Blood urea nitrogen levels were increased in the vehicle group, but were significantly reduced in the SKT-treated group in week 16 (sham, 19.0±0.9 mg/dL; vehicle, 40.3±3.4 mg/dL, p<0.05 vs. sham; SKT, 27.4±1.3 mg/dL, p<0.05 vs. vehicle). On the other hand, urea nitrogen clearance decreased in the vehicle group, and significantly recovered in the SKT-treated group (sham, 1.12±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.

Effects of SKT on Histological Findings Figures 4a to 4c show PAS-stained renal tissues. A significant 70% increase in the glomerular area was observed in the vehicle group compared to the sham operation group. This increase was significantly suppressed by 80% by SKT treatment (sham, 9.00±

± 0.05 mL/min; vehicle, 0.50±0.06 mL/min, p<0.01 vs. sham; SKT, 0.74±0.06 mL/min, p<0.05 vs. vehicle), a mirror image of the change in blood urea nitrogen levels.
SKT-treated group. However, the reduction was not statistically significant due to the data dispersion. Figure 8 shows a representative image and densitometric analysis data (sham, \( n = 6 \); vehicle, \( n = 5 \); and SKT, \( n = 5 \) in each group). These findings were basically the same as the data of the blue-stained area by trichrome staining.

**Recovery from Oxidative Stress by SKT**

SOD-like activity in the renal cortex of vehicle rats was markedly depleted to 79.3% of the level in the sham group. This depletion significantly recovered to the basal levels of the sham group by treatment with SKT (sham, \( 343 \pm 15 \) U/mg; vehicle group, \( 272 \pm 6 \) U/mg, \( p < 0.05 \) vs. sham; and SKT-treated group, \( 337 \pm 21 \) U/mg, \( p < 0.05 \) vs. vehicle group) (Fig. 9).

---

**Fig. 6. Protective Effects of SKT against Tubular Injury**

Compared with the sham (a), tubular lumina were dilated together with widening of the interstitium (b). SKT treatment lowered dilatation of tubular lumina and interstitial widening (c). Representative photos are shown. Original magnification, ×200. According to the scoring criteria and through quantitative analysis of the luminal area, tubular injury was significantly suppressed by SKT treatment (d and e, respectively). *\( p < 0.05 \) vs. sham, †\( p < 0.05 \) vs. vehicle (\( n = 6 \) sham group; and \( n = 5 \) per vehicle and SKT groups, respectively).

**Fig. 7. Suppressive Effects of SKT on CD31-Positive Peritubular Capillaries**

Immunohistochemical findings of CD31, an endothelial marker, are shown. CD31-positive capillaries were detected in the sham group (a), and were scarcely observed in the vehicle group (b). CD31-positive capillaries were again abundant in the SKT group (c). Representative photos are shown. Original magnification, ×200. After quantitative analysis, it was revealed that the number of CD31-positive capillaries was significantly recovered by SKT treatment (d). *\( p < 0.05 \) vs. sham, †\( p < 0.05 \) vs. vehicle (\( n = 6 \) sham group; and \( n = 5 \) per vehicle and SKT groups, respectively).

**Fig. 8. Immunoblotting for Collagen IV and β-Actin Expression**

Tissue lysates were analyzed by immunoblotting with antibodies against collagen IV or β-actin (a). β-actin served as a loading control. Protein levels were quantified with scanning densitometry to analyze the effect of SKT on collagen IV expression (b) (\( n = 6 \) sham group; and \( n = 5 \) per vehicle and SKT groups, respectively).

**Fig. 9. Effect of SKT on SOD-Like Activity in Renal Cortex**

SOD-like activity in the vehicle group was markedly depleted as compared with the sham group. This depletion was significantly recovered by treatment with SKT. *\( p < 0.05 \) vs. sham, †\( p < 0.05 \) vs. vehicle (\( n = 6 \) sham group; and \( n = 5 \) per vehicle and SKT groups, respectively).
In the present study, SKT prevented a blood pressure increase, and ameliorated renal function together with CD31-positive peritubular capillaries after the induction of irreversible Thy-1 nephritis. SKT also suppressed glomerular hypertrophy, extracellular matrix deposition, and tubular injury. Tissue SOD activity in the renal cortex was recovered by SKT treatment.

Traditional indications for SKT are vascular system-related complaints accompanied with the sensation of a rush of blood to the head, shoulder stiffness, tinnitus, and dull headache. SKT is now occasionally administered to patients with CKD in combination with ACE-Is/ARBs in Japan. Previously, anti-hypertensive effects, enhancement of serum NOx levels, and anti-oxidant activity by SKT were reported in basic studies. In this context, we tested the effects of SKT using a rat model of irreversible Thy-1 nephritis induced by the injection of anti-rat thymocyte serum following uninephrectomy. Since the report of a rat experimental model for chronically progressive MsPGN by Cheng et al., several common pathologic findings have been made in humans and experimental glomerulonephritis, including increased expression of transforming growth factor-β, sclerosis or segmental sclerosis, mesangial cell proliferation and mesangial matrix expansion, and decreased endothelial CD31 expression in glomeruli in this experimental model. In addition, in the disease control group of this study, PTC networks were decreased, which were also clinically recognized in relation to the histological findings of interstitial injury in IgA nephropathy as a marker of poor prognosis. Furthermore, renal dysfunction and elevated blood pressure were observed in the vehicle group. In a previous report, SKT was administered at doses 1.0 to 2.0 g/kg/d to stroke-prone spontaneously hypertensive rats. In the present study, rats develop moderate elevation of blood pressure. Then, we chose half of the dose in the previous study: 500 mg/kg/d, which is nearly 7.5 folds of the clinical dose. Blood pressure failed intermittently just after anti-rat thymocyte serum (ATS) injection. In Thy-1 nephritis, massive proteinuria is observed after ATS injection due to the renal damage of mesangiolysis. It is suspected that blood plasma is lost and hypovolemia is induced, and that, after this stage, fusion of glomerular foot process resists hypovolemia, leading to sodium retention and blood pressure elevation. Based on the background, the present experimental model is considered to be appropriate to elucidate the efficacy of SKT in CKD with renal dysfunction and hypertension.

To examine the involvement of hypertrophy and extracellular matrix (ECM) accumulation in the residual glomeruli after reduction of renal mass by unilateral nephrectomy, light microscopic observation of PAS and trichrome staining together with Western blotting for collagen IV were conducted. Previous studies revealed that the overload of glomerular filtration increases cellular reactive oxygen species (ROS), and that ROS up-regulate cell proliferation and ECM expression in mesangial cells. In this study, SKT suppressed both glomerular hypercellularity and ECM accumulation, together with recovery of tissue SOD-like activity, which is recognized as a ROS scavenger. Therefore, it is suspected that the antioxidative effect was one of the mechanisms of SKT treatment in the present study. Previously we have observed the recovery of tissue SOD-like activity by saireito treatment in experimental MsPGN without uninephrectomy, and it was suggested that flavonoids, including baicalin, partly suppressed experimental glomerular and peritoneal fibrosis by anti-oxidative effects. However, the Thy-1 model without uninephrectomy recovers one month after the induction of glomerulonephritis and is considered an acute mesangioproliferative model. In a preliminary experiment using irreversible Thy-1 nephritis, saireito suppressed mesangial cell proliferation, but not azotemia (data not shown). Clinically, saireito is used for early stage of renal diseases. On the other hand, SKT is used advanced stage of renal diseases with elevated blood pressure. Therefore, we tested SKT in the present study, using irreversible Thy-1 nephritis. In this study SKT recovered SOD-like activity, as in the previous report, however, baicalin was not included in SKT. As other candidates for anti-oxidative effects in spontaneously hypertensive rats, it was reported that alkaloids in Uncaria thorn, which is one of the herbal compositions of SKT, including hirsutine, hirsuteine, and geissoschizine methy1 ether, ameliorated glutamate-mediated oxidative stress, and protected against PC12 cell death. It is suspected that SKT suppressed consumption of tissue SOD-like activity through a quenching mechanism by some components in SKT. In the future study, as markers of direct oxidative damage, nitrotyrosine and malondialdehyde levels are needed evaluation.

Using a salt-induced model in Dahl strain rats, Hiwara et al. reported that SKT suppressed hypertension. In accordance with their report, SKT suppressed the elevation of blood pressure in this study using a CKD model. So far, the anti-hypertensive components in SKT have not been well determined. Rhynchophylline, an alkaloid constituent of Uncaria species, was supposed to induce an anti-hypertensive effect through mechanisms related to the modulation of calcium and potassium ion channels. Furthermore, the extract of Uncaria prevented learning deficits induced by the transient occlusion of bilateral carotid arteries. In this study, SKT exerted slight suppression of urinary protein as compared with the significant reduction of blood pressure and with the significant protective effect against histological injury. Because SKT is traditionally prescribed to the patients with hypertensive CKD, it is suspected that SKT may affect mainly hypertension and histological injury. Enhancement of serum NOx levels by SKT was also reported, and that nitric oxide synthase inhibitor in vivo, asymmetric dimethylarginine, progresses CKD through endothelial dysfunction. PTC injury is accepted as a final common pathway to end-stage renal failure. In these contexts, PTC injury was prevented in the SKT-treated group in this study.

In this study, single treatment of SKT was evaluated. Bai et al. reported that both SKT and ARB drug, losartan, suppressed blood pressure elevation in 5/6 nephrectomized rats, respectively. It is suspected that the combination of SKT with ARB or ACEi may present a synergistic effect, because mechanisms of SKT and ARB/ACE-I may be different. The experiment on the combination effects of SKT and ARB/ACE-1 is needed in the future study. In conclusion, the present findings suggest that SKT may be useful for the treatment of CKD during the progression to nephrosclerosis, through the mechanisms of anti-oxidative activity and maintenance of PTC networks. More studies are desired to clarify the precise
activity mechanisms of rhynchophylline and other components in the extract of Uncaria on endothelial protection and microcirculation.

REFERENCES


