Acute and chronic effects of dietary sodium restriction on renal tubulointerstitial fibrosis in cisplatin-treated rats

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ABSTRACT

Background. Renal interstitial fibrosis is a major complication of cisplatin (CP) treatment, and increased sodium intake may accelerate its progression by stimulating transforming growth factor (TGF)-β/Smad signaling. However, it is not clear whether a low-sodium diet has beneficial effects on the development of interstitial fibrosis because it activates the renin–angiotensin–aldosterone system. Here, we tested whether the TGF-β/Smad signaling pathway is stimulated in CP-treated rats, and whether the development of tubulointerstitial fibrosis in CP nephropathy can be checked by dietary sodium restriction.

Methods. Male Sprague Dawley rats were randomly divided into controls, CP treatment and CP treatment with low-sodium diet. The acute experiment lasted 7 days with a single intraperitoneal injection (6 mg/kg) of CP, and the chronic experiment involved weekly injections (2 mg/kg) for 7 weeks.

Results. In both sets of experiments, CP treatment produced pronounced tubulointerstitial injury, increased infiltration of ED1-positive cells and increased expression of monocyte chemoattractant protein-1 (MCP-1), α-smooth muscle actin (SMA), TGF-β1, phosphorylated Smad3, fibronectin and collagen III proteins. In the acute experiment, the increases in expression of osteopontin, MCP-1, α-SMA, TGF-β and collagen III were significantly reduced by dietary sodium restriction. In the chronic experiment, however, none of the measurements were improved by a low-sodium diet. Examination of CP-treated rat kidneys revealed de novo vimentin expression in tubular epithelial cells and invasion of α-SMA-positive tubular epithelial cells through the basement membrane into the interstitium.

Conclusions. The pro-fibrotic effect of TGF-β in CP nephropathy appears to be associated with the epithelial–mesenchymal transition and is ameliorated by dietary sodium restriction only during the acute phase.

INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum, CP) is an effective anticancer agent in head/neck, lung, cervical and bladder cancers [1]. However, the use of CP is limited by severe side effects in normal tissues including the kidney [2]. In vivo experiments have shown that CP-induced nephrotoxicity is characterized by acute focal damage to proximal tubules, dilatation of convoluted tubules and formation of luminal casts [3, 4]. The affected renal tubular epithelial cells undergo pathological alterations, such as necrosis, apoptosis and regeneration [5]. These may lead to extensive interstitial fibrosis and a persistent reduction in glomerular filtration rate (GFR) [3–5]. Thus, tubulointerstitial damage is the main component of CP nephropathy.

Interestingly, in many forms of renal disease, the deterioration of renal function is largely determined by the extent and severity of interstitial lesions [6]. Progressive interstitial fibrosis is considered the ultimate effect of different forms of renal injury and is currently accepted to be a more important predictor of functional impairment than glomerular injury [7]. Thus, animal models of CP nephropathy should be useful for testing clinical strategies in progressive renal disease.

Transforming growth factor-β (TGF-β) plays a pivotal role in the initiation and progression of interstitial fibrosis. Macrophages, tubular epithelial cells and myofibroblasts are all capable of synthesizing TGF-β at different stages in the development of renal fibrotic lesions [8]. In a number of experimental models, activation of TGF-β and its downstream Smad signaling pathway promotes fibrosis by...
autocrine and/or paracrine activation of matrix-producing myofibroblasts, promotion of the tubular epithelial–mesenchymal transition (EMT) and downregulation of many matrix-degrading proteases [9–12]. However, few studies have focused on the role of TGF-β/Smad signaling pathway in CP nephropathy and appropriate strategies for reducing CP-induced renal fibrosis.

Salt has been linked to hypertension for many years [13]. In patients with chronic kidney disease (CKD), high sodium intake might be nephrotoxic as a result of hemodynamic mechanisms, e.g. by increasing blood pressure and by attenuating the antihypertensive and antiproteinuric effects of pharmacologic blockade of the renin–angiotensin system [14]. In addition, high sodium intake by itself, even without hypervolemia or arterial hypertension, is associated with renal fibrosis and EMT of the peritoneal membrane of the rat [14, 15]. Whereas restricting sodium intake reduces proteinuria, arrests further glomerular sclerosis and/or prevents decline in GFR [16–18], radical sodium restriction causes hypovolemia, with attendant adverse effects [19]. Dietary sodium restriction may upregulate the activity of the renin–angiotensin system, increase angiotensin II and upregulate NADPH oxidase, thus generating reactive oxygen species [20]. Thus, whether low sodium intake would have long-term beneficial effects in reducing renal damage is not clear. This study was undertaken to test the hypotheses that the TGF-β/Smad signaling pathway is activated and produces renal interstitial fibrosis in CP-treated rats and that the tubulointerstitial fibrogenesis in CP nephropathy can be reduced by dietary sodium restriction.

**MATERIALS AND METHODS**

**Animal experiments**

Pathogen-free male Sprague-Dawley rats (Orient Bio Inc., Seongnam, Korea) weighing 180–210 g (5 weeks old) were randomly divided into three groups: control, CP treated (CP-CS) and CP treated with a low sodium diet (CP-LS). Four rats were assigned to each group, and all were maintained on a daily food slurry, a mixture of base diet (18 g/200 g BW) and deionized water (9 mL/200 g BW). The base diet was a commercially available synthetic rat chow containing no added NaCl (DYET #113763 Sodium Deficient AIN-93G Purified Rodent Diet, Dyets Inc., Bethlehem, PA); it was given to the CP-LS rats. The controls and CP-CS rats were fed the base diet with added NaCl (0.5%). The control- and low sodium diet was initiated 3 days prior to CP administration and continued throughout the study period. Drinking water was freely accessible to all rats.

The acute experiment lasted 7 days with a single intraperitoneal injection (6 mg/kg) of CP (Choongwae Pharma Corp., Seoul, Korea), and the chronic experiment involved weekly doses (2 mg/kg) for 7 weeks. As the vehicle solution used for CP was normal saline (NS), controls were given the equivalent volume of intraperitoneal NS. This corresponds to a small amount of NaCl (21.6 mg and 7.2 mg per 200 g BW in the acute and chronic experiment, respectively), which will not contribute to volume expansion. The experimental protocols were approved by the institutional Animal Care and Use Committee of Hanyang University.

**Renal histopathology**

Renal tissue was fixed in 10% neutral-buffered formalin and embedded in paraffin. Four-micron paraffin sections were stained with hematoxylin and PAS. Tubulointerstitial injury was scored semiquantitatively on 10 cortical and 5 outer medullary fields of PAS-stained tissue, with a ×20 objective, as described previously [21]. Tubulointerstitial injury was defined as tubular dilation, tubular atrophy, sloughing of tubular epithelial cells, or thickening of the tubular basement membrane and was scored on a scale of 0–4, as follows: 0, no tubulointerstitial injury; 1, <25% of tubulointerstitial injured; 2, 25–50% of the tubulointerstitial injured; 3, 51–75% of the tubulointerstitial injured; 4, >75% of the tubulointerstitial injured.

**Immunohistochemical staining and quantification**

Kidneys were preserved by *in vivo* perfusion with 1% phosphate-buffered saline (PBS) via the abdominal aorta to remove blood and then with periodate–lysine–paraformaldehyde (PLP; 0.01 M NaNO₂, 0.075 M lysine, 2% paraformaldehyde, in 0.0375 M Na₂HPO₄ buffer, pH 6.2) for 10 min to fix the kidneys. Each kidney was sliced into 5 mm sections, which were immersed in 2% PLP solution overnight at 4°C. Immunohistochemical staining for monocyte chemotactic protein-1 (MCP-1), ED1, collagen III, α-smooth muscle actin (α-SMA) and vimentin was performed on formalin-fixed, paraffin-embedded sections using a microwave antigen retrieval method [22]. Sections were deparaffinized with a graded series of ethanol and heated for 14 min in a microwave oven in 0.01 mM sodium citrate, pH 6.0, at 2450 MHz and 800 W. Endogenous peroxidase was inactivated by incubation in 1.4% H₂O₂ in methanol. After preincubation with 10% normal donkey serum (Jackson ImmunoResearch, West Grove, PA) for 1 h, sections were incubated overnight at 4°C with the respective primary antibodies. For ED1 immunostaining, they were pretreated with trypsin (Sigma) for 15 min at 37°C. Sections were washed with PBS and incubated for 2 h with a DAKO Envision kit (DAKO, Glostrup, Denmark) at room temperature. The sections were washed in 0.05 M Tris buffer, developed with a mixture 0.05% 3,3'-diaminobenzidine and 0.033% H₂O₂ in Tris buffer, counter-stained with hematoxylin and mounted with Canada balsam (Sigma, Saint Louis, MO).

To quantify immunohistochemical data, JPEG images of random areas of cortical and outer medullary fields in each kidney section were acquired with SPOT™ imaging software (Diagnostic Instruments, Inc., Sterling Heights, MI) and a SPOT™ flex FX1520 (2048 × 2048 pixels) CCD digital camera coupled to an Olympus BX 51 microscope with an Olympus U-CMAD3 and U-TV1X-2 adapters (Olympus, Optical company Ltd, Tokyo, Japan) and ×20 objective. Tubulointerstitial MCP-1, vimentin, collagen III and α-SMA immunostaining was assessed quantitatively using a computerized image analyzer (Media Cybernetics, Bethesda, MD).
A point-counting technique was used to quantitate the number of interstitial ED1-positive cells. Interstitial ED1-positive macrophages were counted in at least 20 consecutive high-power fields.

**Immunoblot analysis**

Manually dissected slices of whole kidney were homogenized in a buffer containing 250 mM sucrose, 10 mM triethanolamine, 1 µg/mL leupeptin and 0.1 mg/mL phenylmethylsulfonyl fluoride titrated to pH 7.6. Coomassie-stained 'loading gels' were made to assess the quality of the protein by the sharpness of the bands, and to adjust protein concentrations before immunoblotting [23]. Protein samples were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, using 15% gels for detection of TGF-β and α-SMA, 12% gels for fibronectin (FBN) and GAPDH and 10% gels for osteopontin (OPN), Smad3, phosphorylated Smad3 and Smad7. For immunoblotting, the proteins were transferred electrophoretically from unstained gels to nitrocellulose membranes (Bio-Rad, Hercules, CA). After being blocked with 5% skim milk in PBS-T (80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, 0.1% Tween-20, pH 7.5) for 1 h, membranes were probed overnight at 4°C with the respective primary antibodies. The secondary antibody was goat anti-rabbit IgG conjugated to horseradish peroxidase (Jackson ImmunoResearch, West Grove, PA). Sites of antibody-antigen reaction were viewed using an enhanced chemiluminescence substrate (GenDEPOT, Barker, TX) before exposure to X-ray film (Agfa-Gevaert, Mortsel, Belgium). The band densities on immunoblots were quantified by densitometry using a laser scanner and Quantity One software (Basic version 4.6.9, Bio-Rad).

**Primary antibodies**

We used the following commercially available antibodies: Rabbit polyclonal anti-human MCP-1 (Abcam, Cambridge, UK), mouse monoclonal anti-rat ED1 (Serotec, Oxford, UK), mouse monoclonal anti-rat OPN (MPIIIB10, the Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA), mouse monoclonal anti-α-SMA (Sigma), rabbit polyclonal anti-human FBN (Sigma), rabbit polyclonal anti-human TGF-β (Santa Cruz Biotechnology, Santa Cruz, CA), rabbit polyclonal antiphospho-Smad3 (Ser423/425) (Cell Signaling Technology, Beverly, MA), rabbit polyclonal anti-Smad3 (Cell Signaling Technology), goat polyclonal anti-human Smad7 (Santa Cruz Biotechnology), rabbit monoclonal anti-human GAPDH (Cell Signaling Technology); goat polyclonal anti-human collagen III (Sigma) and mouse monoclonal anti-human vimentin (Novocastra Laboratories Ltd, Newcastle, UK).

**Statistics**

Values are presented as means ± SE. Comparisons between groups were performed by the Mann–Whitney U-test using Statview software (Abacus Concepts, Berkeley, CA). P-values <0.05 were considered statistically significant.

**RESULTS**

**Acute CP nephropathy**

We induced an animal model of acute CP nephropathy by a single intraperitoneal injection (6 mg/kg) of CP, and the experiment lasted 7 days. Table 1 shows a comparison of the functional parameters obtained at the end of the experiment in three groups: controls, control sodium-fed CP-treated (CP-CS) rats and low-sodium-fed CP-treated (CP-LS) rats. CP
treatment increased urine output (3.5 ± 1.5 versus 9.9 ± 1.9 mL/day/100 g BW, \( P < 0.05 \)), but body weight was not affected. Plasma sodium concentration obtained at the end of the animal experiment was not significantly different between the groups. As expected, urinary sodium excretion was decreased by dietary sodium restriction in the CP-treated rats (5.0 ± 1.0 versus 1.3 ± 0.3 mEq/day/100 g BW, \( P < 0.05 \)). Although CP treatment induced a marked decrease in glomerular filtration rate as estimated by creatinine clearance, there was no significant difference in this respect between the CP-CS and CP-LS rats (Table 1).

The CP-treated rats suffered serious tubulointerstitial damage in the cortex and outer stripe of the outer medulla (OSOM), as estimated by a semiquantitative histologic analysis of periodic acid Schiff (PAS)-stained kidney sections. However, the severity of the tubulointerstitial injury was not significantly modified by the low-sodium diet (Figure 1). A similar pattern of changes in ED1-positive cell infiltration in the kidney is shown in Figure 2. In MCP-1 immunohistochemistry, however, the CP-LS rats showed a significantly reduced expression compared with the CP-CS rats in both cortex (0.8 ± 0.4 versus 2.5 ± 0.5\%area, \( P < 0.05 \)) and outer medulla (1.5 ± 0.7 versus 6.3 ± 1.7\%area, \( P < 0.05 \)) compared with controls (Figure 3).

Figures 4 and 5 illustrate the data obtained by immunoblot analysis of whole kidney homogenates. Compared with the controls, the CP-CS rats had increased osteopontin (OPN: 251 ± 34\%, \( P < 0.05 \)), \( \alpha \)-smooth muscle actin (\( \alpha \)-SMA: 599 ± 80\%, \( P < 0.05 \)), fibronectin (FBN: 172 ± 18\%, \( P < 0.05 \)), TGF-\( \beta \)1 (516 ± 79\%, \( P < 0.05 \)), phosphorylated Smad3 (261 ± 52\%, \( P < 0.05 \)) and Smad7 (551 ± 132\%, \( P < 0.05 \)). The increased phosphorylated Smad3 was not significantly reduced by the low-sodium diet. It is noteworthy that the increases in expression of OPN (164 ± 14\%, \( P < 0.05 \)), \( \alpha \)-SMA (284 ± 60\%, \( P < 0.05 \)) and TGF-\( \beta \)1 (226 ± 68\%, \( P < 0.05 \)) were significantly less marked in the CP-LS rats than in the CP-CS rats.

We also examined the expression of vimentin and collagen III by quantitative image analysis. In cortex, the vimentin-stained tubulointerstitial area was not significantly notable in CP-treated rat kidneys. In outer medulla, however, it was remarkably increased in the CP-CS rats (8.8 ± 2.6\%area, \( P < 0.05 \)) compared with controls and significantly relieved in the CP-LS rats (2.0 ± 0.9\%area, \( P < 0.05 \)) (Figure 6). Compared with the controls (cortex, 0.1 ± 0.1\% area; outer medulla, 1.8 ± 1.3\%area), the collagen III-stained

**FIGURE 1:** Histopathology of rat kidneys in the acute animal experiment. Representative periodic acid-Schiff (PAS)-stained tissue sections are shown from each group (A). The results of semiquantitative evaluation of tubulointerstitial injury are summarized in the bar graph (B). Although both cortex and outer medulla were affected by the cisplatin (CP) treatment, tubulointerstitial injury seemed more severe in the outer stripe of the outer medulla. CP-CS, control sodium-fed CP-treated rats; CP-LS, low-sodium-fed CP-treated rats. *\( P < 0.05 \), versus control.

**FIGURE 2:** Immunohistochemical analysis of ED1 in rat kidneys in the acute animal experiment. Representative tissue sections are shown from each group (A). The results of ED1-positive cells in interstitium are summarized in the bar graph (B). Whereas ED1-positive cells were seldom detected in the controls, overt infiltration of ED1-positive cells was observed in the CP-treated rats, especially in the outer medulla. CP-CS, control sodium-fed CP-treated rats; CP-LS, low-sodium-fed CP-treated rats. *\( P < 0.05 \), versus control.
tubulointerstitial area increased in the CP-CS rats (cortex, 5.2 ± 1.0% area, P < 0.05; outer medulla, 12.8 ± 4.5% area, P < 0.05), and again the effect was less pronounced in both cortex (0.3 ± 0.1% area, P < 0.05) and outer medulla (2.5 ± 0.5% area, P < 0.05) in the CP-LS rats (Figure 7).

**Chronic CP nephropathy**

An animal model of chronic CP nephropathy was induced by weekly doses (2 mg/kg) of CP for 7 weeks. Table 2 shows a comparison of the functional parameters obtained at the end of the experiment in the controls, CP-CS rats and CP-LS rats. Although body weight was not affected by CP treatment, two CP-treated rats (one on a control sodium diet and the other on a low-sodium diet) did not survive the experiment. The CP-LS rats had a tendency to lower plasma sodium concentration although the differences did not reach statistical significance. As expected, urinary sodium excretion was again decreased by dietary sodium restriction in the CP-treated rats (4.1 ± 0.8 mEq/day/100 g BW, P < 0.05). Although the decrease in glomerular filtration rate estimated by creatinine clearance was not significantly different between the CP-CS and CP-LS rats, proteinuria was only evident in the CP-LS rats (Table 2).

Figure 8 reveals that the CP-treated rats suffered severe tubulointerstitial injury irrespective of dietary sodium level, and there was a similar pattern of changes in ED1-positive cell infiltration in the kidney (data not shown). Figure 9 illustrates the results of an immunoblot analysis of whole kidney homogenates. The CP-CS rats had higher expression of FBN (887 ± 210%, P < 0.05) and phosphorylated Smad3 (637 ± 356%, P < 0.05) proteins than the controls, and these effects were not significantly reduced by dietary sodium restriction. Instead, TGF-β1 protein expression increased (395 ± 95%, P < 0.05) compared with the controls.

Figure 10 shows the results of immunohistochemical staining of kidneys for collagen III. Quantitative image analysis revealed that the collagen III-stained tubulointerstitial area was significantly increased in the CP-treated rats, and in contrast with the acute animal experiment, it was not reduced by dietary sodium restriction. Very similar results for α-SMA immunohistochemistry are shown in Figure 11. At high magnification from CP-CS rat kidney, however, α-SMA-positive...
tubular epithelial cells could be seen to invade the interstitium through the basement membrane (Figure 12A). Vimentin immunohistochemistry also revealed de novo vimentin expression in the tubular epithelial cells (Figure 12B).

**DISCUSSION**

In the present study, we demonstrated that the TGF-β/Smad signaling pathway was activated, and its related EMT was involved in the process of CP-induced renal interstitial fibrosis. In addition, we showed that the increased expression of OPN, MCP-1, TGF-β1, α-SMA and collagen III in CP-treated rat kidneys during the acute phase was ameliorated by dietary sodium restriction.

Consistent with previous studies [24, 25], our models of CP nephropathy showed marked tubulointerstitial injury. OSOM involvement was severe in the acute CP nephropathy, and both cortex and outer medulla were strongly affected in the chronic CP nephropathy. Likewise, tubulointerstitial infiltration with ED1-positive cells was prominent in the OSOM in the acute CP nephropathy, and it extended to both OSOM and cortex in the chronic state. In response to tissue injury macrophages seem to act as early triggers in the genesis of interstitial fibrosis by producing profibrotic factors [5].

MCP-1 is an important chemokine that drives the interstitial inflammatory response in models of kidney disease [26], and we showed that CP nephropathy accompanied an increase in MCP-1 protein expression. Roncal et al. reported that MCP-1 mRNA and protein were increased in the hyperuricemic rats with CP-induced renal injury [27]. α-SMA and
FIGURE 7: Immunohistochemistry for collagen III in rat kidneys from the acute animal experiment. Representative tissue sections are shown from each group (A). The results of quantitative evaluation of interstitial immunostaining are summarized in the bar graph (B). Collagen III expression significantly increased in the CP-CS rats, and this effect was significantly reduced by dietary sodium restriction. CP-CS, control sodium-fed CP-treated rats; CP-LS, low-sodium-fed CP-treated rats. *P < 0.05, versus control; †P < 0.05, versus CP-CS.

FIGURE 8: Histopathology of rat kidneys from the chronic animal experiment. Representative periodic acid-Schiff (PAS)-stained tissue sections are shown from each group (A). The results of semiquantitative evaluation of tubulointerstitial injury are summarized in the bar graph (B). Both cortex and outer medulla were severely damaged by CP treatment. CP-CS, control sodium-fed CP-treated rats; CP-LS, low-sodium-fed CP-treated rats. *P < 0.05, versus control.

Table 2. Functional parameters in the chronic animal experiment

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 4)</th>
<th>CP-CS (n = 3)</th>
<th>CP-LS (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (BW, g)</td>
<td>267 ± 3</td>
<td>272 ± 4</td>
<td>262 ± 7</td>
</tr>
<tr>
<td>Plasma Na⁺ (mEq/L)</td>
<td>149 ± 1</td>
<td>140 ± 6</td>
<td>141 ± 1</td>
</tr>
<tr>
<td>Urine volume (mL/day/100 g BW)</td>
<td>6.5 ± 1.4</td>
<td>10.4 ± 2.6</td>
<td>9.0 ± 1.7</td>
</tr>
<tr>
<td>Urine Na⁺ (mEq/day/100 g BW)</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.8</td>
<td>0.5 ± 0.1***</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>7.8 ± 0.8</td>
<td>91.8 ± 50.4*</td>
<td>58.4 ± 8.5*</td>
</tr>
<tr>
<td>Cr (mg/dL)</td>
<td>0.58 ± 0.05</td>
<td>2.23 ± 1.10*</td>
<td>1.20 ± 0.15*</td>
</tr>
<tr>
<td>Cr clearance (μL/min/100 g BW)</td>
<td>385 ± 30</td>
<td>127 ± 61*</td>
<td>162 ± 26*</td>
</tr>
<tr>
<td>Proteinuria (mg/day/100 g BW)</td>
<td>2.6 ± 0.2</td>
<td>2.6 ± 0.3</td>
<td>5.2 ± 1.2***</td>
</tr>
</tbody>
</table>

Values are mean ± SE. *P < 0.05, versus control; **P < 0.05, versus CP-CS.
collagen III expression also increased in the interstitial area adjacent to damaged tubules and OPN expression was elevated in the acute CP nephropathy. OPN appears to be critical for macrophage recruitment and the production of certain cytokines during cell-mediated immunity [28, 29] and may contribute to the regeneration of tubular epithelial cells during the acute to late recovery phases of cortical tubular damage induced by CP [30].

We showed that expression of α-SMA and vimentin, reliable markers of EMT, increased in both the acute and chronic diseases. In addition, histologic evidence of EMT was found by immunohistochemistry of α-SMA and vimentin (Figure 12). When a diverse array of inflammatory cells including macrophages is recruited, EMT can be triggered through the release of profibrotic growth factors, such as TGF-β, platelet-derived growth factor, epidermal growth factor and fibroblast growth factor-2 [31]. In particular, matrix metalloproteinases degrade the tubular basement membrane and induce and promote EMT [25, 31]. Thus, in chronic CP nephropathy, continuing EMT may eventually lead to extensive interstitial fibrosis.

According to previous studies, Smad3 is the most important effector of the autoinduction of TGF-β1 and is associated with apoptosis, matrix overproduction and EMT [9–12, 31–33]. We also found above that activation of TGF-β1/Smad3 signaling pathway was involved in the CP nephropathy. Expression of TGF-β1 protein, phosphorylated Smad3 and Smad7 protein increased in response to both acute and chronic CP administration. Taken together, our findings suggest that appropriate modulation of monocyte/macrophage infiltration and proper control of activation of Smad3 signaling and initiation of EMT might make it possible to
prevent CP-induced interstitial inflammation and the development of renal fibrosis [34, 35]. Dietary sodium restriction may also be considered to achieve this end.

We found that dietary sodium restriction may temporarily ameliorate the development of renal fibrosis in CP nephropathy. The increased expression of OPN, MCP-1, α-SMA, TGF-β and collagen III were suppressed by dietary sodium restriction in the acute experiment. We believe that these results are related to the direct or nonhemodynamic effect of sodium on renal fibrosis. There has been experimental evidence to suggest direct effects of high salt intake on kidney tissue injury [36–40]. In particular, high salt intake causes increased TGF-β expression in the kidney without changes in blood pressure [41], and salt-induced organ damage may involve Smad signaling in endothelial cells [42]. Previous studies have also reported that dietary sodium restriction can reduce proteinuria and prevent further glomerular injury in animals and humans [16–18].

However, the beneficial effect of a low-sodium diet did not persist in the chronic experiment. Although creatinine clearance in the CP-treated rats was not significantly affected by dietary sodium restriction in either the acute or chronic experiment, proteinuria and TGF-β1 expression appeared to actually increase in the chronic CP-LS rats. Furthermore, plasma sodium concentration tended to decrease in the chronic CP-LS rats. When subjects are repeatedly exposed to CP treatment, dietary sodium restriction would no longer be of help. Thus, long-term dietary sodium deprivation in chronic tubulointerstitial disease may be detrimental rather than beneficial. According to Hamming et al., an extremely low-sodium diet overrides the beneficial effects of angiotensin-converting enzyme inhibition on proteinuria and has an adverse effect on tubulointerstitial inflammation and interstitial fibrosis in adriamycin nephrosis [43].

In summary, we have demonstrated a role of EMT and activation of TGF-β/Smad signaling in CP-induced tubulointerstitial inflammation and fibrosis in rat kidneys. We also showed that these processes could be temporarily relieved by dietary sodium restriction. Further studies are required to
establish the long-term effects of dietary sodium deprivation on chronic CP nephropathy.

CONCLUSION

This paper demonstrates two novel findings; (i) cisplatin-induced renal interstitial fibrosis may be relieved by dietary sodium restriction, but the effect was short-lived; (ii) epithelial–mesenchymal transition occurs during the process of cisplatin-induced tubulointerstitial fibrosis. Now that we have very limited measures to control renal tubulointerstitial fibrosis, the role of dietary sodium restriction could be explored in cisplatin-induced nephrotoxicity. This was conceivable because high sodium intake was known to contribute to renal progression. No previous studies were reported regarding dietary sodium restriction or epithelial–mesenchymal transition in cisplatin nephropathy.

AUTHOR CONTRIBUTIONS

Gheun-Ho Kim designed research; Joon-Sung Park, Chor Ho Jo and Sua Kim performed experiments; Joon-Sung Park and Gheun-Ho Kim wrote the paper.

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CONFLICT OF INTEREST STATEMENT

None declared.

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