Renoprotective Effect of Vasopressin V2 Receptor Antagonist Tolvaptan in Dahl Rats With End-Stage Heart Failure

Mayuko Ishikawa, MD, Naohiko Kobayashi, MD, Fumihiro Sugiyama, MD, Sho Onoda, MD, and Toshihiko Ishimitsu, MD

SUMMARY

Tolvaptan is a highly selective and orally effective arginine vasopressin V₂ receptor antagonist, and is potentially useful for the treatment of heart failure (HF) patients. However, the renoprotective effect of long-term tolvaptan therapy and its underlying mechanisms remain unknown. We evaluated the effects of chronic treatment with tolvaptan on renal dysfunction, podocyte injury, inflammation, oxidative stress, Rho-kinase, epithelial-mesenchymal transition (EMT), and the extracellular signal-regulated protein kinase (ERK1/2) pathway in the renal cortex of Dahl salt-sensitive hypertensive (DS) rats with end-stage severe HF. DS and Dahl salt-resistant rats were fed a high-salt diet at 6 weeks of age. DS rats were treated with vehicle and tolvaptan (0.05% concentration in diet) from the age of 11 to 18 weeks. Vehicle-treated DS rats developed proteinuria, renal dysfunction, glomerulosclerosis, and interstitial fibrosis, which were ameliorated by tolvaptan without changing blood pressure. Decreased expression of nephrin and podocin and increased desmin-positive area in failing rats were restored by tolvaptan. Upregulation of NAD(P)H oxidase p22phox, p47phox, and gp91phox, EMT markers such as transforming growth factor-β1, vimentin, and fibronectin expression, and Rho-kinase and ERK1/2 phosphorylation in DS rats were significantly suppressed by tolvaptan. Tolvaptan administration resulted in significant inhibition of tumor necrosis factor-α and monocyte chemoattractant protein-1 expression, and nuclear factor-κB phosphorylation. We concluded that long-term tolvaptan therapy may improve renal dysfunction, glomerulosclerosis, podocyte injury, and inflammation associated with oxidative stress, as well as EMT, ERK, and the Rho-kinase pathway in the failing heart of DS rats. Thus, tolvaptan may be a therapeutic strategy for end-stage severe HF. (Int Heart J 2013; 54: 98-106)

Key words: Oxidative stress, Nephrosclerosis, Inflammation

Patients with chronic kidney disease (CKD) are at increased risk of both atherosclerotic and congestive heart failure (CHF), as well as stroke and peripheral arterial disease. Renal dysfunction can be due to both hemodynamic impairment and preexisting intrinsic renal disease, which can adversely affect cardiac function and contribute to the pathogenesis of cardiovascular disease. The increased risk of cardiovascular disease begins early in the natural history of CKD, so an understanding of that excess risk should begin with an assessment of the magnitude of any early changes in known causal risk factors. Several studies have shown renal function to be one of the strongest predictors of adverse outcomes in CHF patients, and renal dysfunction in particular is more highly prevalent in patients with CHF than previously reported and is an independent prognostic factor in diastolic and systolic dysfunction. These findings suggest that the kidney plays a critical role in CHF in terms of contributing directly both to myocardial performance and to alteration of myocardial structure.

The findings of recent studies have emerged as a central mechanism in the pathogenesis of tubulointerstitial fibrosis in epithelial-mesenchymal transition (EMT). EMT, a process by which fully differentiated epithelial cells undergo transition to a fibroblast phenotype, has emerged as an important pathway leading to generation of matrix-producing fibroblasts and myofibroblasts in diseased kidney. Many studies from different laboratories have illustrated that tubular epithelial cells in vitro undergo phenotypic conversion after incubation with fibrogenic transforming growth factor-β1 (TGF-β1); the transition is characterized by loss of epithelial proteins such as E-cadherin, and acquisition of new mesenchymal markers including vimentin, α-smooth muscle actin (α-SMA), interstitial matrix component type I collagen, and fibronectin. In different cellular systems, Smad proteins, Rho-kinase, extracellular signal-regulated protein kinase (ERK1/2), and p38 mitogen-activated protein kinase (p38MAPK) have all been shown to contribute to the induction of EMT by TGF-β. TGF-β is a central regulator of the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis and it is also one of the most potent growth factors to induce EMT in tubular cells.

Arginine vasopressin (AVP) is a nine-amino acid neuropeptide hormone synthesized in the nuclei of the hypothala-
NAD(P)H oxidase p22^phox^, p47^phox^, gp-91^phox^, nphrin, podocin, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), regulated upon activation, normal T cell expression and secreted (RANTES), TGF-β1, E-cadherin, vimentin, fibronectin, procollagen type I, AQP2, V1aR, V2R, and RhoA protein expressions were measured as described previously. Renal cortex was homogenized (25% wt/vol) in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1mmol/L EDTA, 1mmol/L DTT, 10 μg/mL leupeptin, and 2 μg/mL aprotinin at 0°C to 4°C with a polytron homogenizer. Protein concentrations were determined with bovine serum albumin as a standard protein. Equal amounts of protein were loaded in each lane of SDS-PAGE using 13% gels. The proteins in the gels were transferred electrothermally to PVDF sheets for one hour at 2 mA/cm². The sheets were immunoblotted with anti-NAD(P)H oxidase subunit, anti-nephrin, anti-podocin, anti-TNF-α, anti-MCP-1, anti-RANTES, anti-TGF-β1, anti-E-cadherin, anti-vimentin, anti-fibronectin, anti-procollagen type I, anti-AQP2, anti-V1aR, anti-V2R, and anti-RhoA antibodies (Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) in a buffer containing 10 mmol/L Tris-HCl, pH 7.5, 100 mmol/L NaCl, 0.1% Tween 20, and 5% skim milk. The proteins transferred to the sheets were detected using an ECL immunoblotting detection system (Amersham Life Science Inc).

**METHODS**

All experimental procedures and protocols used in this study were in accordance with the Dokkyo Medical University School of Medicine institutional guidelines for animal research and with the US National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Animal models and experimental designs:** Male inbred DS rats and Dahl salt-resistant (DR) rats (Eisai, Tokyo) were weaned and fed a diet containing 0.3% NaCl until the age of 6 weeks. Thereafter, they were fed a diet containing 8% NaCl until the age of 18 weeks. The systolic blood pressure was measured by the tail-cuff method at the start of the 8% NaCl diet and at 1-week intervals thereafter. Transthoracic echocardiography evaluating the left ventricular end-diastolic diameter (LVEDD), and percent fractional shortening (%FS) were performed at 18 weeks. At the age of 11 weeks, when left ventricular hypertrophy developed, DS rats were randomly divided into 2 groups: rats treated with vehicle (DSHF-V), and rats treated with tolvaptan (DSHF-T; added to the powdered food sheets). The V1a selective receptor antagonists inhibit recruitment of AQP2 water channels into the apical membranes of collecting duct epithelial cells, thereby reducing the ability of the collecting duct to resorb water. Tolvaptan is a potent, highly selective, and orally effective nonpeptide AVP V2R antagonist. A recent study indicated that tolvaptan improved glomerulosclerosis and suppressed the expression of endothelin-1 and type I collagen, and fibronectin mRNA in the kidney of Dahl salt-sensitive hypertensive (DS) rats with HF. However, no detailed report has been published on the renoprotective mechanisms of tolvaptan, such as podocyte injury, inflammation, oxidative stress including NAD(P)H oxidase and superoxide generation, EMT, Rho-kinase, and the MAPK pathway in the renal cortex of DS rats with HF. The purpose of the present study was to investigate the effects of long-term tolvaptan therapy on renal dysfunction, and renal damage such as glomerulosclerosis, podocyte injury, and inflammation, as well as oxidative stress, EMT, Rho-kinase, and ERK1/2 pathway in the renal cortex of DS rats with end-stage severe HF.

**Urine collection:** Twenty-four hour urine samples were collected from rats in metabolic cages at 7 weeks after tolvaptan therapy for measuring protein and creatinine levels. Urinary protein, creatinine in serum and urine, and serum blood urea nitrogen (BUN) were analyzed by standard methods. Creatinine clearance (Ccr) was calculated using standard formulas. **Renal morphology and glomerulosclerosis injury score (GIS):** After the right kidney sections were stained with hematoxylin and eosin (HE) and periodic acid-Schiff (PAS), glomerulosclerosis in renal cortex was scored as described previously. GIS was scored in 100 glomeruli in each section as G0 for a normal glomerulus; G1, mild sclerosis (25%); G2, moderate segmental sclerosis (25% to 50%); G3, severe segmental sclerosis (50% to 75%); and G4, global sclerosis.

**Renal interstitial fibrosis:** The right kidney was excised and immersed in neutralized formalin for histological examination. The area of fibrotic lesions in the interstitium (fibrosis area) in renal cortex was determined on sections stained by Masson’s trichrome method to stain collagen fibers (stained blue), using a computer-aided manipulator program as described previously.

**In situ detection of renal superoxide anion generation:** Superoxide anion generation in renal tissue of renal cortex was evaluated using the fluorescent dye dihydroethidium (DHE) as earlier reported.

**Western blot analysis:** NAD(P)H oxidase p22^phox^, p47^phox^, gp-91^phox^, nephrin, podocin, tumor necrosis factor-α (TNF-α), monocyte chemoattractant protein-1 (MCP-1), regulated upon activation, normal T cell expression and secreted (RANTES), TGF-β1, E-cadherin, vimentin, fibronectin, procollagen type I, AQP2, V1aR, V2R, and RhoA protein expressions were measured as described previously. Renal cortex was homogenized (25% wt/vol) in 10 mmol/L HEPES buffer, pH 7.4, containing 320 mmol/L sucrose, 1 mmol/L EDTA, 1 mmol/L DTT, 10μg/mL leupeptin, and 2 μg/mL aprotinin at 0°C to 4°C with a polytron homogenizer. Protein concentrations were determined with bovine serum albumin as a standard protein.

**Statistical analysis:** All values are expressed as the mean ± SEM. Mean values were compared among the 3 groups by
ANOVAs and the Bonferroni post hoc test for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

**RESULTS**

**Physiological profiles and urinary and serum parameters:** The physiological profiles and urinary and serum parameters of the 3 experimental groups are summarized in the Table. Body weight (BW) was significantly lower in DSHF-V rats than in DR-C rats. In contrast, DSHF-V rats had higher kidney weight/BW ratios compared with DR-C rats. Long-term tolvaptan therapy significantly increased the BW and significantly decreased the kidney weight/BW ratio in DSHF-T rats compared with DSHF-V rats. DSHF-V rats had markedly higher systolic blood pressure than DR-C rats. Long-term tolvaptan therapy did not affect the systolic blood pressure. Urinary volume and urinary protein excretion were significantly increased in DSHF-V rats compared with DR-C rats. Ccr was significantly decreased in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy significantly reduced urinary protein excretion and increased urinary volume and Ccr in DSHF-T rats compared with DSHF-V rats. Serum creatinine and BUN levels were also significantly increased in DSHF-V rats compared with DR-C rats. Chronic treatment with tolvaptan significantly decreased these increased creatinine and BUN levels in DSHF-T rats compared with DSHF-V rats.

**Effect of tolvaptan on cardiac function for LVEDD and %FS:** LVEDD was significantly higher in DSHF-V rats than in DR-C rats. Long-term tolvaptan therapy in DS rats significantly decreased LVEDD. In contrast, DSHF-V rats had lower %FS than did DR-C rats. Long-term tolvaptan therapy in DS rats significantly improved %FS (Table).

**Effect of tolvaptan on renal morphology, GIS, and interstitial fibrosis:** The morphological appearance of arterioles, glomeruli, and the interstitium were considered normal in the DR rats. Renal injury in the DS rats with HF consisted of segmental and global glomerular sclerosis and arteriolar sclerosis associated with inflammatory cell infiltration, interstitial fibrosis, atrophic and dilated tubules, and tubular casts. Moreover, these DSHF-V rats demonstrated marked medial and intimal thickening, with proliferation of vascular smooth muscle cells (VSMCs) in the interlobular arteries. Long-term tolvaptan therapy reduced these changes, especially in the glomeruli. Figure 1A to 1C shows typical light micrographs in 3 groups. DR-C rats showed few pathological findings. DS rats with HF demonstrated severe nephrosclerosis, and the damage was reduced in tolvaptan-treated DS rats. As a result, the total GIS was significantly greater in DSHF-V rats than in DR-C rats. Long-term tolvaptan therapy significantly ameliorated GIS (Figure 1D). In addition, the representative appearances of Masson’s trichrome-stained sections after chronic tolvaptan treatment are shown in Figure 1E to 1G. Interstitial fibrosis of the kidney was more prominent in DSHF-V rats than in DR-C rats. Long-term tolvaptan therapy ameliorated the interstitial fibrosis in DSHF-T rats compared with DSHF-V rats. The tubular structure was also preserved in DSHF-T rats compared with DSHF-V rats (Figure 1H).

**Effect of tolvaptan on podocyte injury:** To evaluate the podocyte injury in DS rats with end-stage HF, we tested the effect of tolvaptan on desmin-positive area, and nephrin and podocin expression in the renal cortex. Podocyte injury was assessed by immunohistochemical detection of desmin-positive area. Positive staining for desmin in the glomeruli was obviously increased in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy suppressed the appearance of desmin-positive area in DSHF-T rats compared with DSHF-V rats. Figure 2A to 2C. In addition, nephrin and podocin protein levels were significantly decreased in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy significantly increased the nephrin and podocin expression in DSHF-T rats (Figure 2).

**Effect of tolvaptan on NAD(P)H oxidase expression and superoxide generation:** To evaluate the oxidative stress in DS rats with end-stage HF, we tested the effect of tolvaptan on NAD(P)H oxidase 2A to 2C. In addition, tolvaptan significantly reduced superoxide anion generation in DSHF-T rats (Figure 3).

**Table. Physiological Profiles of the Three Experimental Groups**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>DR-C</th>
<th>DSHF-V</th>
<th>DSHF-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>8</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>BW, g</td>
<td>449 ± 6</td>
<td>361 ± 9*</td>
<td>386 ± 6**</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>138 ± 5</td>
<td>226 ± 11**</td>
<td>235 ± 13**</td>
</tr>
<tr>
<td>%FS</td>
<td>52.6 ± 0.7</td>
<td>24.8 ± 1.2*</td>
<td>44.3 ± 1.0**</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>6.5 ± 0.1</td>
<td>8.7 ± 0.3*</td>
<td>7.0 ± 0.2**</td>
</tr>
<tr>
<td>Kidney weight/BW, mg/g</td>
<td>3.53 ± 0.04</td>
<td>5.19 ± 0.19*</td>
<td>4.55 ± 0.06*</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>0.22 ± 0.01</td>
<td>0.47 ± 0.04*</td>
<td>0.35 ± 0.01*</td>
</tr>
<tr>
<td>Ccr, mL/minute</td>
<td>16.3 ± 0.9</td>
<td>33.6 ± 4.1*</td>
<td>18.3 ± 1.2*</td>
</tr>
<tr>
<td>Urine volume, mL/day</td>
<td>30.4 ± 6.8</td>
<td>53.6 ± 7.1*</td>
<td>83.3 ± 7.9**</td>
</tr>
<tr>
<td>UproV, mg/day</td>
<td>30.4 ± 6</td>
<td>307 ± 46**</td>
<td>124 ± 21**</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SEM. V, vehicle; T, tolvaptan. BW indicates body weight; SBP, systolic blood pressure; %FS, percent fractional shortening; LVEDD, left ventricular end-diastolic diameter; Ccr, creatinine clearance; and UproV, urinary protein excretion. *$P < 0.05$, **$P < 0.01$ versus DR-C; †$P < 0.05$, ††$P < 0.01$ versus DSHF-V.
Effect of tolvaptan on Rho-kinase pathway: To evaluate the Rho-kinase pathway in DS rats with end-stage HF, we tested the effect of tolvaptan on RhoA expression and phosphorylation of RhoA and MYPT-1. The levels of RhoA protein expression and phosphorylation of RhoA and MYPT-1, a target of Rho-kinase, were significantly greater in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy significantly decreased RhoA expression and RhoA and MYPT-1 phosphorylation in DS rats (Figure 4).

Effect of tolvaptan on EMT, ERK1/2, AQP2, and AVP receptors: To evaluate EMT, the MAPK pathway, and AQP2 and AVP receptors in DS rats with end-stage HF, we tested the effects of tolvaptan on EMT markers such as α-SMA, TGF-β1, E-cadherin, vimentin, fibronectin, procollagen type I expression, and ERK1/2 phosphorylation, and AQP2, V$_2$R and V$_1$R expression. The induction of interstitial myofibroblasts was assessed by immunohistochemical detection of α-SMA. Positive staining for α-SMA was seen in VSMCs, but not in the inter-
Interstitial space in DR-C rats. Intense immunostaining of α-SMA was observed in the peritubular interstitium in addition to the VSMCs of the arterioles in DSHF-V rats. Long-term tolvaptan therapy reduced the appearance of α-SMA-positive myofibroblasts in the interstitium in DSHF-T rats compared with DSCF-V rats (Figure 5A to 5C). Expression of TGF-β1, vimentin, fibronectin, and procollagen type I protein levels were significantly higher in DSHF-V rats than in DR-C rats. Long-term tolvaptan therapy significantly decreased the expression of these EMT markers in DSHF-T rats. E-cadherin protein expression was significantly lower in DSHF-V rats than in DR-C rats. Chronic treatment with tolvaptan significantly increased the E-cadherin expression in DSHF-T rats. In addition, ERK1/2 phosphorylation was significantly upregulated in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy significantly suppressed the ERK1/2 phosphorylation in DSHF-T rats. Furthermore, the expression levels of AQP2, V2R, and V1αR protein were significantly higher in DSHF-V rats than in DR-C rats. Chronic treatment with tolvaptan significantly reduced these expressions in DSHF-T rats. In addition, NF-κB phosphorylation was significantly upregulated in DSHF-V rats compared with DR-C rats. Long-term tolvaptan therapy significantly suppressed NF-κB phosphorylation in DSHF-T rats (Figure 6).

Discussion

In this study, we administered the AVP V$_2$R antagonist tolvaptan to DS rats with end-stage HF and observed an inhibition of glomerulosclerosis, interstitial fibrosis, podocyte injury, and inflammation. In addition, we demonstrated that oxidative stress such as NAD(P)H oxidase and superoxide generation, and Rho-kinase, EMT, and ERK pathways were upregulated at end-stage HF and that these signaling pathways were restored by long-term tolvaptan therapy. These findings suggest that long-term tolvaptan therapy may improve renal dysfunction, glomerulosclerosis, and interstitial fibrosis associated with oxi-
EFFECT OF TOLVAPTAN IN CHF

Podocytes appear to be a major functional component of the glomerular filtration size and charge barrier, and serve as the final filtration barrier to prevent the leak of plasma proteins. Recent studies indicate that glomerular podocyte injury is a cardinal feature of diabetic nephropathy, and is closely involved in the progression of proteinuria, glomerular sclerosis, and tubulointerstitial injury. In the present study, we demonstrated that nephrin and podocin protein expression were decreased and desmin staining was increased in DS rats with end-stage HF and that these expressions were restored by the V₂R antagonist tolvaptan. Nishiyama, et al reported that podocyte injury with decreased nephrin and podocin expression is observed in some experimental models. With regard to the V₂-mediated action of vasopressin, the use of a highly selective, orally active V₂R antagonist, SR-121463, in diabetic rats established the critical role played by the antidiuretic effects of vasopressin in urinary albumin excretion associated with diabetes. Recently, Okada, et al showed that tolvaptan is protective against podocyte damage and proteinuria, and that tolvaptan exerts a renoprotective effect by affecting podocyte morphology and function in puromycin aminonucleoside nephrosis. Therefore, these findings suggest that the renoprotective mechanisms of tolvaptan may at least in part be explained by restoration of proteinuria and renal function through inhibiting podocyte injury in DS rats with end-stage HF.

NAD(P)H oxidase has been shown to be one of the most active stress, and Rho-kinase, EMT, and ERK pathways in failing heart of DS rats. Thus, the AVP V₂R pathway may play a critical role in DS rats with end-stage HF, and tolvaptan may be a potential therapeutic strategy in end-stage severe HF.

Figure 5. Effects of long-term tolvaptan therapy on α-SMA-positive area, and TGF-β1, E-cadherin, vimentin, fibronectin, procollagen type I, AQP2, V₂R, and V₁αR expression, and ERK1/2 phosphorylation. A refers to DR-C; B, DSHF-V; and C, DSHF-T. Values are means ± SEM. n = 5 per group. * P < 0.05 versus DR-C; † P < 0.05 versus DSHF-V. Bar indicates 100 μm.
powerful pro-oxidants in both the vasculature and in the kidney, and exists not only in the migrating macrophages but also in the renal vessels, glomerular podocytes, mesangial cells, and distal tubules, and it produces superoxide anion.\(^{19}\) We have previously demonstrated that NAD(P)H oxidase subunit expression is enhanced in the kidney of DS rats.\(^{20}\) In the present study, the expression of NAD(P)H oxidase subunits such as p22\(^{\text{phox}}\), p47\(^{\text{phox}}\), and gp91\(^{\text{phox}}\) and superoxide generation were increased in DS rats with HF, and long-term tolvaptan therapy inhibited the NAD(P)H oxidase subunits and superoxide generation. In a previous study, Armstead, \textit{et al}\(^{21}\) showed that vasopressin, in concentrations present in cerebrospinal fluid after fluid percussion brain injury, increased superoxide generation in a PKC-dependent manner and contributes to such production after fluid percussion brain injury. Moreover, Li, \textit{et al}\(^{22}\) demonstrated that vasopressin increases vascular superoxide levels by stimulating endothelin-1 formation in mineralocorticoid hypertension. Based on these considerations, it is possible to speculate that the amelioration of renal dysfunction and damage by tolvaptan in DS rats may in part be due to the inhibition of NAD(P)H oxidase and superoxide generation, and that tolvaptan may have an important role in activation of the antioxidant system in DS rats with end-stage severe HF.

The activation of RhoA and its target Rho-kinase is an important signaling pathway, and the Rho-kinase pathway may play a pivotal role in the pathogenesis of glomerulosclerosis and tubulointerstitial fibrosis.\(^{10}\) Bauer, \textit{et al}\(^{23}\) reported that the activation of Rho-kinase is common to AVP action in the kidney. Shimogai, \textit{et al}\(^{24}\) reported that AVP-induced vasoconstriction was mediated by activation of the Rho-kinase pathway in rat aortic smooth muscle. With regard to using a Rho-kinase inhibitor, Song, \textit{et al}\(^{25}\) indicated that treatment with the Rho-kinase inhibitor, fasudil, attenuated the renal vasoconstrictor responses to AVP, implicating the Rho-kinase pathway is one of the renal vascular smooth muscle signaling mechanisms activated by this agonist in the kidney of spontaneously hypertensive rats. Interestingly, Satoh, \textit{et al}\(^{26}\) demonstrated that they developed a new model of vasopressin-induced chronic myocardial damage with progression of myocardial fibrosis in rats, and that fasudil significantly prevented the development of fibrosis induced by vasopressin in this model. In this study, we showed that RhoA protein expression and levels of phosphorylation of RhoA and MYPT-1 were upregulated in DS rats, and that these protein and phosphorylation levels were inhibited by tolvaptan. Therefore, these findings suggest that the Rho-kinase pathway may be partly responsible for the renal pathogenesis of DS rats, and that long-term tolvaptan therapy could be an important therapeutic strategy of end-stage HF through the inhibition of the Rho-kinase pathway.

In the present study, we showed that EMT marker expression and ERK1/2 phosphorylation were upregulated in DS rats with HF, and that these increased indices were restored by long-term treatment with tolvaptan. In DS rats with HF, Nishikimi, \textit{et al}\(^{27}\) showed that TGF-\(\beta\) and collagen I/III mRNA expression increased in the renal cortex of this model, and that the TGF-\(\beta\)-collagen cascade may play a central role in the development of glomerulosclerosis and tubulointerstitial fibrosis. Wang, \textit{et al}\(^{28}\) supported the importance of cAMP in the pathogenesis of polycystic kidney disease (PKD), demonstrated the effectiveness in the PCK rat of an AVP V\(_2\)R antagonist to be used in autosomal-dominant PKD (ADPKD) clinical trials (OPC-41061), and suggested that OPC-41061 inhibits Ras/MAPK signaling in polycystic kidneys. Reif, \textit{et al}\(^{29}\) demonstrated that AVP increased intracellular cAMP, increased the level of P-ERK, and accelerated the proliferation of ADPKD cyst epithelial cells, and that tolvaptan inhibited AVP-induced cAMP production, decreased P-ERK/ERK, and suppressed ADPKD cell proliferation. These results suggest that long-term tolvaptan therapy inhibits the activity of EMT and the ERK signaling pathway, which may, at least in part, contribute to the pathogenesis of glomerulosclerosis.

In the present study, we showed the effect of tolvaptan on
cardiorenal protection in DS rats with end-stage severe HF. CHF causes marked alterations in renal hemodynamics and when CHF is treated the hemodynamics return to normal. Because tolvaptan restores hemodynamics in HF, it is possible that the renoprotective effect of tolvaptan is secondary to ameliorating hemodynamics and the role of an AVP V2R antagonistic effect in renoprotection may be questionable. We have previously demonstrated that other diuretics and a selective aldosterone blocker eplerenone, ameliorated cardiac dysfunction and renal damage in the same model of DS rats with severe HF. 12-20 Decreased %FS and increased LVEDD by echocardiography in failing rats were significantly ameliorated by eplerenone using the dose without changing blood pressure. Renal damage evaluated by the increase in serum creatinine levels, proteinuria, and glomerulosclerosis in DS rats was improved by eplerenone. Moreover, the expressions of NAD(P)H oxidase p22phox, p47phox, and TGF-β1 and fibronectin levels in the renal cortex of failing DS rats were decreased by eplerenone. These findings suggest that eplerenone and tolvaptan may have similar cardiorenal protection effects in DS rats with end-stage severe HF, and that tolvaptan may play a pivotal role in renoprotection in CHF.

In conclusion, we administered the AVP V2R antagonist tolvaptan to DS rats with end-stage severe HF and observed an inhibition of glomerulosclerosis, interstitial fibrosis, podocyte injury, and inflammation including NF-κB inhibition of glomerulosclerosis, interstitial fibrosis, podocyte injury in puromycin aminonucleoside nephrotic rats. Clin Exp Nephrol 1999; 10: 647-63. (Review)

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