Renoprotective effect of rosuvastatin in DOCA–salt hypertensive rats

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Abstract

Background. Pleiotropic effects of statins represent potential mechanisms for the treatment of end organ damage in hypertension. This study has investigated the effects of rosuvastatin (10 mg/kg/day) on renal function impairment, glomerulosclerosis and tubulointerstitial fibrosis in deoxycorticosterone acetate (DOCA)–salt hypertensive (DSH) rat.

Methods. Rats were implanted with DOCA strips (200 mg/kg) on 1 week after unilateral nephrectomy. Rats received a controlled diet with or without rosuvastatin. Three weeks after DOCA implantation, systolic blood pressure (SBP) was measured by tail-cuff method. The glomerulosclerosis and tubulointerstitial fibrosis was determined by Masson's trichrome stain. The tumour necrosis factor (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), monocyte chemoattractant protein1 (MCP1), intercellular adhesion molecule-1 (ICAM-1) and endothelin-1 (ET-1) were determined by real-time polymerase chain reaction. The expression of ED-1, transforming growth factor-β1 (TGF-β1) and connective tissue growth factor (CTGF) was determined in the kidney by immunoblotting and immunohistochemistry.

Results. In DSH rats, SBP was increased, which was not affected by rosuvastatin treatment. Creatinine clearance was decreased while urinary albumin excretion ratio was increased in DSH rats compared with controls, which were attenuated by rosuvastatin treatment. Glomerulosclerosis and tubulointerstitial fibrosis in DSH rats were attenuated by rosuvastatin treatment. The messenger RNA expression of TNF-α, IL-1β, IFN-γ, MCP1, ICAM-1 and ET-1 was increased in DSH, which was attenuated by rosuvastatin treatment. The expression of ED-1, TGF-β and CTGF was increased in the kidney of DSH, which was counteracted by rosuvastatin treatment.

Conclusion. Rosuvastatin is effective in preventing progression of renal injury in DSH, the mechanism of which is associated with anti-inflammatory and anti-fibrotic effects.

Keywords: DOCA–salt; glomerulosclerosis; hypertension; rosuvastatin

Introduction

The incidence of chronic renal diseases is increasing worldwide, and there is a great need to identify therapies capable of arresting or reducing disease progression. Diffuse glomerulosclerosis and interstitial fibrosis contribute to the final common pathway for almost all forms of kidney disease [1]. The current treatment of chronic nephropathies is limited to angiotensin-converting enzyme inhibitors and angiotensin receptor blockers, but there is growing clinical and experimental evidence that statins could play a therapeutic role. Although it is difficult to distinguish the effects that are dependent on, or independent of, their cholesterol-lowering effects in clinical trials, there is emerging evidence that the renoprotection provided by statins is due to their pleiotropic and particularly anti-inflammatory properties. A double-blind, randomized trial investigating the effects of pravastatin treatment on proteinuria in normocholesterolemic patients concluded that it reduced proteinuria independently of its lipid-lowering effects by inhibiting the renal synthesis of endothelin-1, a potent proinflammatory agent [2], and a prospective, randomized trial involving patients with chronic kidney disease found that 20-week treatment with rosuvastatin (RUS) substantially reduced the levels of the inflammatory biomarker high-sensitivity C-reactive protein (hs-CRP) and increased the glomerular filtration rate (GFR) [3]. A number of in vivo studies have found that statins have therapeutic effects on a number of animal models of renal disease in the absence of any changes in lipid levels. Statins interfere with various pathological mechanisms but especially modulate inflammation and fibrotic processes. RUS is a new synthetic and chemically distinct member of the statin family [4]. The renoprotective effects of statins have been reported in animal models of kidney damage resulting from surgery or chemical challenges [5,6].

The deoxycorticosterone acetate (DOCA)–salt hypertensive (DSH) rat is a well-known established model of mineralocorticoid hypertension with renal dysfunction. Although DSH has low renin, the high levels of mineralocorticoid from the implanted DOCA strip mimics aldosterone overload, giving rise to a volume-dependent hypertension that is
also considered a model of human primary aldosteronism [7]. Although mineralocorticoid is traditionally known to promote sodium retention, recent evidence indicates that it causes oxidative stress [8] and stimulates inflammation and fibrosis by activating transcription factors such as nuclear factor KappaB (NF-κB) and activating protein (AP-1) [9]. Accordingly, glomerulosclerosis, tubular fibrosis and cardiac hypertrophy and fibrosis are commonly observed in DSH, along with activation of renal transforming growth factor (TGF)-β1 expression [10]. Thus, it is plausible that statin may have renoprotective effect in DSH, and a recent study demonstrated that fluvastatin treatment reduced glomerular inflammation in DSH [11].

The present study investigated the morphological and cellular alterations occurring in the kidney of DSH and the potential pleiotropic effects of RUS on renal dysfunction and progressive kidney injury.

Materials and methods

Animals

The animal study was approved by the Ethics Committee of Chonnam National University Medical School. Male Sprague-Dawley rats weighing 180 to 200 g were used. DSH was induced by subcutaneous implantation of silicone rubber containing DOCA (200 mg/kg) on 1 week after unilateral nephrectomy. Rats received a controlled diet with or without RUS (Crestor®, 10 mg/kg/day) for 4 weeks after unilateral nephrectomy. The control group was also unilaterally nephrectomized but received silicon rubber implantation without DOCA implantation. Physiologic saline was supplied as a drinking water to all animals for 3 weeks after DOCA implantation. Systolic blood pressure (SBP) was measured by tail-cuff method (Power lab, ADI instrument, CA, USA). The rats were maintained individually in the metabolic cages for last 3 days to allow urine collections for the measurement of creatinine and urine microalbumin. They were killed for semiquantitative immunoblotting and immunohistochemical studies on 3 weeks after DOCA implantation. Under anesthesia with iso-flurane, blood samples were collected from the inferior vena cava and analyzed for creatinine. The left kidney was fixed by retrograde perfusion for immunohistochemical studies.

Another series of experiment was done for the assay of real-time polymerase chain reaction (PCR). The rats were decapitated under a conscious state, and their kidneys were taken and kept at −70°C until assayed for the messenger RNA (mRNA) expression by real time-PCR. In another set of experiments, rats received a control diet with or without RUS (Crestor®, 10 mg/kg/day) for 4 weeks after unilateral nephrectomy. The protein expression of ED-1, TGF-β1 and CTGF in the kidney was investigated to evaluate the effects of RUS alone in unilateral nephrectomy rats.

Pathologic examinations

The extent of glomerulosclerosis (GS) was graded from 0 to 4 by a semiquantitative score: 0, normal; 1, mesangial expansion/sclerosis involving <25% of the tuft; 2, moderate GS (25 to 50%); 3, severe GS (50 to 75%); and 4, diffuse GS involving >75% of the glomerular tuft. Glomerulosclerosis index (GSI) for each rat was calculated as a mean value of all scores obtained from quadruplicate measurements. Tubulointerstitial lesion indexes were determined by activating transcription factors such as nuclear factor κB (NF-κB) and activating protein (AP-1) [9]. Accordingly, glomerulosclerosis, tubular fibrosis and cardiac hypertrophy and fibrosis are commonly observed in DSH, along with activation of renal transforming growth factor (TGF)-β1 expression [10]. Thus, it is plausible that statin may have renoprotective effect in DSH, and a recent study demonstrated that fluvastatin treatment reduced glomerular inflammation in DSH [11].

The dissected whole kidneys were homogenized in ice-cold isolation solution containing 0.3 M sucrose, 25 mM imidazole, 1 mM EDTA, 8.5 μM leupeptin and 1 mM phenylmethylsulfonyl fluoride, with pH 7.2. The homogenates were centrifuged at 1,000 g for 15 min at 4°C to remove whole cells, nuclei and mitochondria. The total protein concentration was measured (Pierce BCA protein assay reagent kit, Pierce, Rockford, IL). All samples were adjusted with isolation solution to reach the same final protein concentrations and solubilized at 65°C for 15 min in SDS-containing sample buffer and then stored at −20°C. To confirm equal loading of protein, an initial gel was stained with Coomassie blue. SDS-PAGE was performed on 9 or 12% polyacrylamide gels. The proteins were transferred by gel electrophoresis (Bio-Rad Mini Protean II, Bio-Rad, Hercules, CA, USA) onto nitrocellulose membranes (Hybond ECL, RPN3032D, Amersham Pharmacia Biotech, Little Chalfont, UK). The blots were subsequently blocked with 5% milk in Phosphate Buffered Saline with Tween 20 (PBST) (80 mM Na2HPO4, 20 mM NaH2PO4, 100 mM NaCl, 0.1% Tween 20, pH 7.5) for 1 hour and incubated overnight at 4°C with 1:1000 dilutions of primary antibodies (TGF-β1, CTGF; Santa Cruz Biotechnology, Santa Cruz, CA, USA, ED-1, serotec, Denmark; β-actin, Sigma, St Louis, MO, USA), followed by incubation with 1:1500 dilutions of secondary anti-rabbit (P447, DAKO, Glostrup, Denmark) or anti-mouse (P447, DAKO, Glostrup, Denmark) horseradish peroxidase-conjugated antibodies. The labeling was visualized by an enhanced chemiluminescence system.

Immunohistochemistry

A perfusion needle was inserted into the abdominal aorta, and the vena cava was cut to establish an outlet. Blood was flushed from the kidney with cold phosphate-buffered saline (pH 7.4) for 15 s before switching to cold phosphate-buffered saline containing 1% paraformaldehyde for 1 min. The kidneys were immersed in 3% paraformaldehyde solution for 16 h, and then embedded in paraffin. 5 μm thick sections were cut and mounted on slides. Sections were immunostained using standard avidin-biotin-conjugated peroxidase technique (Universal ABC kit, DAKO, Denmark) on a microwave oven-cooked deparaffinized slides using 0.01 M citrate buffer (pH 6.0) for 15 min. Negative controls were run by omission of the primary antibody.

mRNA expression of inflammatory markers

The mRNA expression of TNF-α, IL-1β, INF-γ, ICAM-1, MCP1 and ET-1 was determined by real time-PCR. Complementary DNA (cDNA) was made by reverse transcribing 5 μg of total RNA using oligo (dT) priming and superscript reverse transcriptase II (Invitrogen, Carlsbad, CA, USA). CDNA was quantified using Smart Cycler II System (Cepheid, Sunnyvale, CA, USA), and SYBR Green was used for detection. PCR was done using Rotor-Gene™ 3000 Detector System (Corbett Research, New South Wales, Australia). Sequences of primers are listed in Table 1. The PCR was performed according to the following steps: (i) 95°C for 5 min; (ii) 95°C for 20 s; (iii) 58 to 62°C for 20 s (optimized for each primer pair); (iv) 72°C for 30 s; and (v) 85°C for 6 s to detect SYBR Green. Steps 2–5 were repeated for additional 45 cycles, while at the end of the last cycle temperature was increased from 60 to 95°C to produce a melt curve. Data from the reaction were collected and analyzed with the Corbett Research Software. The comparative critical threshold (Ct) values from quadruplicate measurements were used to calculate the gene expression, with normalization to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control [13]. Melt curve analysis was performed to enhance specificity of the amplification reaction.

Table 1. Primer sequences for real-time PCR

<table>
<thead>
<tr>
<th>Primers</th>
<th>Sense</th>
<th>Sequence</th>
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<tr>
<td>TNF-α</td>
<td>CTT CAG CTC CAC AGA GAA GAA CTG C</td>
<td>Senses: CAC GAT CAT GGG GAA CTG C</td>
</tr>
<tr>
<td>IL-1β</td>
<td>TCG CAT GGG GAA CTG C</td>
<td>Antisenses: CAC GAT CAT GGG GAA CTG C</td>
</tr>
<tr>
<td>INF-γ</td>
<td>AACCAGGCCCCGATGGAAACAACA</td>
<td>Senses: ACCCACTTCTGGACCTTCT</td>
</tr>
<tr>
<td>MCP1</td>
<td>AGCCCAAGGAGCAACA</td>
<td>Antisenses: GCCGACCTTCTGGACCTTCT</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>CCCGGGAGATGGACCAAAAGC</td>
<td>Senses: CTGGGGGCTGCGATGTAAGAGT</td>
</tr>
<tr>
<td>ET-1</td>
<td>ATGGGATTATTTTCCATGGT</td>
<td>Antisenses: AGGGATGTTGACCCAGATGA</td>
</tr>
<tr>
<td>GAPDH</td>
<td>ATCAAAATGCGGTGATCCTGTTGCTG</td>
<td>Antisenses: CAGGTTCTCCAGGGCGCATCAG</td>
</tr>
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to cold 3% paraformaldehyde in 0.1 M cacodylate buffer (pH 7.4) for 3 min. The kidney was removed and sectioned into 2–3-mm transverse sections and immersion fixed for additionally 1 hour, followed by 3 × 10 min washes with 0.1 M cacodylate buffer of pH 7.4. The tissue was dehydrated in graded ethanol and left overnight in xylene. After embedding in paraffin, 2-μm sections were made on a rotary microtome (Leica Microsystems A/S, Herlev, Denmark). Immunoperoxidase labeling was performed as previously described [14].

Statistical analyses
Results are expressed as mean ± SEM. Multiple comparisons among the groups were made by one-way ANOVA and post hoc Tukey honestly significant difference (HSD) test. Differences with values of \( P < 0.05 \) were considered significant.

Results
Blood pressure and renal function
Table 2 summarizes the changes of body weight, SBP, kidney and left ventricle weight in control, DSH and RUS-treated DOCA–salt rats (D + RUS). DSH rats showed 20% mortality rate, while all of the controls and D + RUS rats were survived during the experiments. SBP was markedly increased in DSH rats compared with control rats, which was not affected by RUS treatment. Kidney weights were increased in DSH rats compared with controls, which were counteracted by RUS treatment. Left ventricular weights were also increased in DSH rats, which were not affected by RUS treatment. Haematoxylin–eosin (H&E) stain revealed that left ventricular hypertrophy was prominent in DSH, which was not significantly affected by RUS treatment (Figure 1). These data support the notion that high blood pressure or haemodynamic factor is a more potent regulator in the pathogenesis of left ventricular (LV) hypertrophy rather than that of kidney hypertrophy, and other local factors may contribute as well.

Table 3 summarizes the changes of renal function, lipid profiles and hs-CRP. DSH rats decreased creatinine clearance and increased plasma creatinine levels. The fractional excretion of sodium increased significantly, suggesting an impaired tubular sodium reabsorption in DSH rats. In addition, urinary microalbumin excretion ratio (UAE) was markedly increased in DSH rats. RUS treatment lowered plasma creatinine levels, increased creatinine clearance and attenuated UAE. The level of total cholesterol, LDL and triglyceride did not show the difference among the three groups. These data suggest the pleiotropic effects of statin in DSH rats. However, hs-CRP was increased in DSH rats, which was attenuated by RUS treatment.
Effect of RUS on pathological changes in DSH rats

Figure 2 shows morphological change among three groups. In H&E stain, tubular cast, obstruction and dilatation were shown in DSH rats. The GSI attained values almost ten-fold as high as in control rats. Treatment with RUS was associated with a less pronounced increment of GSI. Interstitial expansion was also a prominent component of renal injury in DSH rat, which was attenuated by RUS treatment.

mRNA Expression of TNF-α, IL-1β, INF-γ, MCP1, ICAM-1 and ET-1

Figure 3 shows the mRNA expression of TNF-α, IL-1β, INF-γ, MCP1, ICAM-1 and ET-1 in the kidney. The abundance of TNF-α, IL-1β, IFN-γ, MCP1, ICAM-1 and ET-1 mRNA was significantly increased in the kidney of DSH rats compared with controls, which was counteracted by RUS treatment.

Immunohistochemistry staining and protein expression of ED-1, TGF-β1 and CTGF

ED-1-expressing macrophages accumulated into the cortical glomerulus and interstitium in the DSH rats compared with controls, which is reduced by RUS treatment (Figure 4). The expression of TGF-β and CTGF was also increased in DSH rats compared with controls, which was attenuated by RUS treatment (Figures 5 and 6).

Fig. 2. H&E stain and Masson’s trichrome (M-T) stain in cortex. Increased GS and interstitial fibrosis were shown in DOCA-salt rats (DSH), which were reversed by rosuvastatin treatment. *P < 0.05 compared with control. #P < 0.05 compared with DSH group.
Expression of ED-1, TGF-β1 and CTGF in unilaterally nephrectomized rats treated with RUS alone

In unilaterally nephrectomized rats, the protein expression of ED-1, TGF-β1 and CTGF was not changed by RUS treatment (Figure 7).

Discussion

This study demonstrated that RUS prevents progressive renal injury in DSH rats without affecting blood pressure. As expected, the DSH rats showed systemic arterial hypertension, proteinuria and impaired renal function. These functional changes were accompanied by severe GS and interstitial fibrosis, as well as expansion and intense macrophage infiltration of the interstitial area. The detection of inflammatory and profibrotic gene induction and macrophage infiltration in the kidneys supports the hypothesis that inflammatory processes may contribute to progressive renal injury and fibrosis.

Statins have been reported to reduce blood pressure in both a randomized, double-blind crossover trial in humans [15] and hypertensive rodent models [16], including DSH [17]. In contrast, we found that RUS did not affect blood pressure in DSH rats, which is consistent with the results.
of a recent study [11]. This difference may be related to model and species differences or the relative hydrophilicity of RUS, which limits its uptake through the plasma membrane. Independent of any anti-hypertensive effects, however, RUS attenuated renal injury, GS, and tubulointerstitial fibrosis associated with DSH.

Inflammatory cell infiltration is present in various types of progressive renal disease in both humans and experimental animal models. The number of inflammatory cells within the kidney closely correlates with the severity of glomerular and tubulointerstitial lesions and loss of renal function. Inflammatory cells and activated intrinsic kidney cells can produce various cytokines, which can promote the progression of glomerular sclerosis and interstitial fibrosis. Therefore, reducing inflammation may be a novel therapy strategy for treating progressive kidney disease.

**Fig. 5.** (A) Semiquantitative immunoblotting of TGF-β1 in the whole kidney. Densitometric analysis revealed that the protein expression of TGF-β1 was increased in DOCA–salt rats, which was counteracted by rosuvastatin treatment. *P < 0.05 compared with control. †P < 0.05 compared with DSH group. (B) Immunoperoxidase microscopy of TGF-β1 in cortex. An increased immunolabeling was shown in DOCA–salt rats, which was reversed by rosuvastatin treatment. Magnification: ×400.

**Fig. 6.** (A) Semiquantitative immunoblotting of CTGF in the whole kidney. Densitometric analysis revealed that the protein expression of CTGF was increased in DOCA–salt rats, which was counteracted by rosuvastatin treatment. *P < 0.05 compared with control. †P < 0.05 compared with DSH group. (B) Immunoperoxidase microscopy of CTGF in cortex. An increased immunolabeling was shown in DOCA–salt rats, which was reversed by rosuvastatin treatment. Magnification: ×400.
Indeed, blocking inflammation using the TNF-α inhibitor and etanercept suppresses renal injury in DSH rats [19]. Moreover, RUS abrogates the renal expression of some inflammatory mediators (e.g. MCP1 and TGF-β1) in stroke-prone spontaneously hypertensive rats [20]. The inhibitory effects of RUS on TNF-α, IL-1β, IFN-γ, MCP1 and ICAM-1 may be partially explained by the decreased infiltration of monocyte/macrophages. Therefore, it is possible that RUS attenuates inflammation by reducing the overexpression of inflammatory cytokines and chemokines in the DSH rat kidney. This mechanism may lead to the prevention of renal disease progression.

ET-1 induces vasoconstriction and has been demonstrated to play a role in renal and cardiac target organ damage [21]. Recent studies have demonstrated that ET-1 mRNA expression is increased in the kidney and is associated with increased blood pressure and decreased GFR. These findings suggest that enhanced vascular and renal production of ET-1 plays a role in the development of hypertension and renal dysfunction. In the present study, upregulation of ET-1 mRNA expression was attenuated by RUS treatment in DSH rats. This finding is consistent with a previous study, which demonstrated that statins reduce the synthesis of ET-1 at a transcriptional level [22]. Statins inhibit the isoprenoid intermediates in the cholesterol pathway [23], which are essential for the function of signal transduction molecules in the Rho family [24]. Inhibition of Rho signaling by statins can activate peroxisome proliferator-activated receptors [25], which in turn suppress ET-1 secretion [26]. Thus, it is plausible that RUS suppresses ET-1 synthesis through the inhibition of Rho family signaling in the kidney. Other secondary mechanisms, however, may also contribute to the suppression of ET-1 by statins. Most inflammatory diseases show elevated levels of ET-1, possibly due to an alteration in vascular structure and function with the activation/accumulation of inflammatory cells. In the kidney, microvascular endothelial cells [27], mesangial cells [28,29] and renal tubular epithelial cells [30] synthesize and release ET-1. Furthermore, ET-1 synthesis and release is increased in mesangial cells and vascular endothelial cells upon exposure to inflammatory cytokines such as TNF-α and CRP [31,32]. In the present study, increased ET-1 expression was associated with the upregulation of inflammatory cytokines such as TNF-α, IL-1β and γ-IFN in DSH rats, and these effects were counteracted by RUS. These findings suggest that RUS decreases ET-1 synthesis by the inhibition of tissue inflammation in DSH.

Multifunctional cytokines such as TGF-β1 and CTGF are elevated in diabetic nephropathy and are likely to be prime mediators in the progression of renal disease [33]. Some mechanisms by which TGF-β1 induces fibrogenesis include renal cell proliferation, stimulation of extracellular matrix proteins and inhibition of matrix degradation [34,35]. In the present study, we found that RUS downregulated protein expression of these growth factors in the DOCA–salt kidney, thus indicating that RUS may prevent progressive renal fibrosis by reducing the upregulation of these growth factors. This may be a second possible mechanism for the observed renoprotective effects.

Although increased expression levels of ET-1, TGF-β1 and CTGF were observed in the DSH rats, it is not clear whether these inflammatory markers have a causative role in the progression of kidney injury. It is possible that inflammation may occur, but not be the cause of injury. Inhibition of TGF-β1 using a neutralizing antibody or gene silencing reduces the severity of GS and proteinuria, thus suggesting a critical role for TGF-β1 in hypertensive renal injury [36,37]. In the present study, RUS inhibited protein expression of TGF-β1 and CTGF, which was associated with marked decreases in proteinuria. Thus, it is plausible that the reduction of TGF-β1 by RUS treatment may play a role in the reduction of UAE. Furthermore, reduced pro-
teinuria may decrease the trigger for tubulointerstitial inflammation [38,39]. The molecular signals/mechanisms underlying inflammation and fibrosis may activate one another; therefore, the causative role of inflammation and fibrosis in the progression of kidney injury in DSH remains to be further elucidated.

Unilateral nephrectomy results in a number of changes in the remnant kidneys, such as hypertrophy and cell proliferation [40]. Thus, it is assumed that RUS treatment alone induces different baseline changes in uninephrectomized contralateral kidneys. The present study, however, demonstrated that RUS alone did not affect the abundance of ED-1, TGF-β1, and ET-1 overexpression and the downregulation of TGF-β1 and CTGF in the DOCA–salt rat kidney.

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Conflict of interest statement. None declared.

References

Renal phenotype of the cystinosis mouse model is dependent upon genetic background

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Abstract
Background. Cystinosis is caused by mutations in CTNS that encodes cystinosin, the lysosomal cystine transporter. The most severe and frequent form is characterized by a proximal tubulopathy that appears around 6 to 12 months of age. In the absence of treatment, end-stage renal disease is reached by 10 years. Ctns⁺/− mice of a mixed 129Sv × C57BL/6 genetic background show elevated renal cystine levels; however, proximal tubulopathy or end-stage renal disease is not observed.

Methods. As renal phenotype can be influenced by genetic background, we generated congenic C57BL/6 and FVB/N Ctns−/− mice and assayed renal lesions and function by histological and biochemical studies.

Results. C57BL/6 Ctns−/− mice showed significantly higher renal cystine levels than the FVB/N strain. Moreover, C57BL/6 mice presented with pronounced histological lesions of the proximal tubules as well as a tubulopathy and progressively developed chronic renal failure. In contrast, renal dysfunction was not observed in the FVB/N strain.

Conclusions. Thus, the C57BL/6 strain represents the first Ctns−/− mouse model to show clear renal defects. In addition to highlighting the influence of genetic background on cystinosin function in the kidney and, specifically, in the proximal tubules.

Keywords: chronic renal failure; cystinosis; genetic background; mouse model; proximal tubule dysfunction

Introduction

Cystinosis is an inherited disorder caused by mutations in the CTNS gene, which encodes cystinosin, the lysosomal cystine transporter [1]. Defective or absent cystinosin prevents cystine efflux thus resulting in lysosomal cystine storage [2]. At high concentrations, free cystine becomes insoluble and eventually forms crystals in certain tissues [3]. Individuals affected with the severe infantile form (MIM 219800) of the disease develop a proximal tubulopathy (de Toni-Debré-Fanconi syndrome) by 6 to 12 months of age. In the absence of treatment, end-stage renal disease (ESRD) can occur by 10 years. Also within the first year of life, cystine crystals begin to appear in the cornea, which lead progressively to severe photophobia. Continuous widespread cystine storage results in a multisystemic disorder involving the kidneys, bones, and skeleton; liver; pancreas; thymus; and the central nervous system.