Early and delayed effects of AST-120 on chronic cyclosporine nephropathy

Su Hyun Kim1,*, Jung Yeon Ghee2,*, ShangGuo Piao2, Dong He Han2, Ji-Hyun Song2, Hye Eun Yoon3, Bum Soon Choi2,3, Jin Kim4, Fuyuhiko Nishijima5, Kazumi Obara5, Noriyuki Ishikawa5 and Chul Woo Yang2,3

1Department of Internal Medicine, Chung-Ang University, Seoul, Korea, 2Catholic Transplantation Research Center, The Catholic University of Korea, Seoul, Korea, 3Department of Internal Medicine, The Catholic University of Korea, Seoul, Korea, 4Department of Anatomy, Cell Death Disease Research Center, The Catholic University of Korea, Seoul, Korea and 5Biomedical Research Laboratories, Kureha Corporation, Tokyo, Japan

Correspondence and offprint requests to: Chul Woo Yang; E-mail: yangch@catholic.ac.kr
*Both authors contributed equally to this work.

Abstract

Background. Removal of uraemic toxins by AST-120 (Kremezin®) decreases the progression of chronic kidney disease by reducing oxidative stress. We performed this study to evaluate whether AST-120 has a similar effect on progression of cyclosporine (CsA)-induced renal injury.

Methods. Two separate studies were performed in adult Sprague–Dawley rats. First, AST-120 was administered with CsA (15 mg/kg) for 4 weeks (early treatment). Second, AST-120 was administered to the rats for 3 weeks after treatment with CsA for 3 weeks (delayed treatment). Uraemic toxin and oxidative stress were evaluated with plasma indoxyl sulphate (IS) levels and urinary 8-OHdG excretion. The effects of AST-120 on CsA-induced renal injury were evaluated in terms of renal function, interstitial fibrosis, inflammation, and apoptotic cell death.

Results. CsA treatment for 4 weeks showed 2-fold increase in plasma IS and urinary 8-OHdG levels compared with the VH group. Early treatment with AST-120 significantly decreased both parameters, and this was accompanied by improved renal function and decreased interstitial inflammation, fibrosis, and apoptotic cell death compared with those of rats that received CsA alone. Delayed treatment with AST-120 also decreased the plasma IS and urinary 8-OHdG levels, and reduced the progression of chronic CsA nephropathy. Furthermore, delayed AST-120 treatment decreased the epithelial–mesenchymal transition in chronic CsA nephropathy.

Conclusions. Removal of uraemic toxins with AST-120 treatment is effective in decreasing the progression of CsA-induced renal injury by reducing oxidative stress.

Keywords: AST-120; chronic cyclosporine nephropathy; indoxyl sulphate; oxidative stress

© The Author 2010. Published by Oxford University Press on behalf of ERA-EDTA. All rights reserved.
For Permissions, please e-mail: journals.permissions@oup.com
Introduction

Chronic cyclosporine (CsA)-induced nephrotoxicity is one of the important factors in chronic allograft dysfunction, characterized by progressive renal dysfunction, afferent arteriolopathy, inflammatory cell influx and striped tubulointerstitial fibrosis (TIF) [1,2]. Although the mechanism of CsA-induced renal injury is not completely understood, oxidative stress plays a pivotal role in the progression of CsA-induced nephrotoxicity [3,4].

AST-120 (Kremezin®, Kureha-Chemical Co., Tokyo, Japan), black spherical carbon adsorbent, removes uraemic toxins such as indoxyl sulphate (IS) in the gastrointestinal tract [5,6] and prevents the deterioration of renal function in experimental and clinical studies [7–10]. IS levels are increased significantly in patients with decreased renal function, and it is closely correlated with oxidative stress in several studies [11–14]. Moreover, oxidative stress plays an important role in the inflammation and tubulointerstitial fibrosis in both chronic kidney disease (CKD) and chronic allograft nephropathy [15–18].

The present study was undertaken to investigate whether the administration of an oral adsorbent AST-120 is effective in decreasing CsA-induced renal injury. To define our hypothesis, we evaluated the effect of AST-120 on removal of uraemic toxin and oxidative stress in an experimental model of chronic CsA nephropathy. The results of our study clearly demonstrate that removal of uraemic toxins by AST-120 treatment is effective in decreasing progression of chronic CsA nephropathy, and this protective effect of AST-120 is related to the decreased oxidative stress.

Materials and methods

Animals and drugs

Male Sprague–Dawley rats (Charles River Technology, Seoul, Korea), initially weighing 230–250 g, were housed in cages (Nalge Co., Rochester, NY, USA) in a temperature- and light-controlled environment. They were allowed a free access to a low-salt diet (0.01% sodium, Teklad Premier, WI, USA) and tap water. CsA (Novartis Pharma Ltd., Basel, Switzerland) was diluted in olive oil (Sigma-Aldrich, St. Louis, MO, USA). AST-120 was a generous gift from Kureha Corporation (Tokyo, Japan). Five percent and 10% AST-120 were made by mixing with the low-salt diet and were given to rats orally.

Experimental design

Preliminary study. The experimental protocol was approved by the Animal Care Committee of the Catholic University, in Seoul, Korea and all procedures performed in this study were followed up by ethical guidelines for animal studies. To evaluate the optimal effects of AST-120 on protective function of chronic CsA nephropathy, 5% and 10% AST-120 were used based on previous reports that these doses are effective in improving renal function [12]. AST-120 was given to rats orally, mixed with low-salt diet as 5% and 10%, respectively.

Basic protocol. After starting the treatment, the rats were pair-fed with low-salt diet; body weight was monitored daily. Systolic blood pressure was recorded at the end of study by the tail-cuff method with a plethysmography using a tail manometer–tachometer system (BP-2000, Visitech system, NC, USA). Prior to sacrifice, the rats were individually housed in
metabolic cages (Tecniplast, Gazzada, Italy) for 24-h urine collection. Afterwards, blood samples were obtained to evaluate renal function.

Measurement of renal function, blood CsA and IS levels. Serum and urine creatinine levels were measured by the enzymatic method using Daiichi reagent (Daiichi Pure Chemical Co. Ltd, Tokyo, Japan) on a Hitachi 7600 chemistry analyser (Hitachi Inc., Tokyo, Japan). Creatinine clearance was calculated using a standard formula (C = U/V.P). The whole blood CsA levels and serum IS levels were measured by a monoclonal radioimmuno-assay (Incastar Co., Stillwater, MN, USA) and HPLC, respectively.

Measurement of interstitial fibrosis
For interstitial fibrosis analysis, kidney paraffin sections were stained with Masson’s trichrome stain. A finding of TIF was defined as previously described [4]. Briefly, a minimum of 20 fields per section were assessed using a colour image analyser (TDI Scope EYETM version 3.0 for Windows, Olympus, Japan). Histopathologic analysis was performed in randomly selected cortical fields of sections by two pathologists blinded to the identity of the treatment groups.

Immunohistochemistry and in situ hybridization
For detection of ectodysplasin-A (ED1), osteopontin (OPN), 8-hydroxy-2-deoxyguanosine (8-OHdG), E-cadherin and vimentin in tissues, cryo-sections were stained as described previously [4]. Primary antibodies were used against ED1 (Serotec Inc., UK), OPN (Developmental Studies Hybridoma Bank, IA, USA), 8-OHdG (JaCa, Shizuoka, Japan), E-cadherin (BD Transduction Labs, CA) and vimentin (DAKO, Glostrup, Denmark). The number of ED1-positive cells was quantified in area (0.5 mm²) rat kidney using a computer programme (TDI Scope Eye). A minimum of 20 fields per section were assessed. Vimentin staining was scored with five grades as follows [19]: 0.5, very weak; 1, weak; 2, moderate; 3, strong; and 4, very strong. Grades were given by analysis of a complete kidney section in different fields at the magnification of ×40.

In situ hybridization for OPN was performed as described previously [4].

In situ TdT-mediated dUTP-biotin nick end-labelling assay. Assay cells undergoing apoptosis were identified by the ApopTag in situ apoptosis detection kit (Oncor, Gaithersburg, MD, USA). The number of TdT-mediated dUTP-biotin nick end-labelling (TUNEL)-positive cells was counted in 20 different fields in each section under ×200 magnification.

Western blot and northern blot analysis. Western blot analysis was performed as described previously [4]. Caspase-3, β-Ig-h3 and E-cadherin were detected by incubating for 1 h with a specific antibody (Chemicon International, Inc.,) Northern blot analysis was performed as previously described [20,21].

Measurement of urinary 8-OHdG and acrolein. Twenty-four-hour urinary concentrations of 8-OHdG and acrolein were determined using a competitive ELISA (Institute for the Control of Aging, Shizuoka, Japan).

Statistical analysis
The data are expressed as mean ± SEM. Multiple comparisons among groups were performed by one-way ANOVA with the post hoc Bonferroni test (SPSS software version 9.0, SPSS Inc., IL, USA). A two-tailed P-value of <0.05 was considered to be statistically significant.

Results

The dose–response of AST-120 in VH- and CsA-treated rats
In the VH group, administration of 5% or 10% AST-120 did not affect creatinine clearance. The creatinine clearance in the CsA group decreased compared with the VH group but increased by the concomitant administration of 5% or 10% AST-120 (Figure 2A). There was no significant difference of creatinine clearance between 5% and 10% AST-120 in the CsA group.

Similar results were observed in serum IS levels. During 4 weeks of treatment with CsA, the IS levels significantly increased in the CsA group compared with the VH group (0.047 ± 0.011 mg/dL vs. 0.03 ± 0.002 mg/dL; P = 0.01; Figure 2B). Treatment with 5% AST-120 significantly reduced the plasma IS levels relative to those of the VH or CsA groups (P < 0.05, respectively). However, treatment with 10% AST-120 showed no significant effect on plasma IS levels in VH-treated rats or CsA-treated rats.

To define why high-dose (10% AST-120) treatment failed to remove ureamic toxins, we evaluated the gross and microscopic findings of the intestines in the experimental groups. The gross examination of the intestines showed distended intestines filled with black AST-120 in rats with high dose, but other groups did not (Figure 2C). The microscopic examination of the intestines revealed no oedema in experimental groups (Figure 2D). This finding suggests that AST-120 is not toxic to the intestinal wall but is not tolerable for rats to digest. Based on the aforementioned findings, 5% AST-120 was selected in these experiments.

Effect of early treatment with AST-120 on CsA-induced renal dysfunction
To evaluate the renal function of rats, their serum creatinine and creatinine clearance at 4 weeks were measured. Table 1 shows the functional parameters of the experimental group. The CsA4+AST4 group showed significantly lower levels of serum creatinine and higher levels of creatinine clearance than those of the CsA4 group. No differences in systolic blood pressure, body weight change or CsA concentration were observed among the groups.

Effect of early treatment with AST-120 on CsA-induced interstitial inflammation and fibrosis
To evaluate the effects of AST-120 on the interstitial inflammation associated with chronic CsA nephropathy, ED1-positive cells were detected by immunohistochemistry, and the numbers of ED1-positive cells in the experimental groups were quantitatively analysed (Figure 3A and B). ED1-positive macrophages were rarely observed in the VH4 (1.2 ± 0.4 cells/0.5 mm²) or VH4+AST4 (1.5 ± 0.3 cells/0.5 mm²) groups. In contrast, the CsA4 group showed a significant increase in ED1-positive macrophages (182.6 ± 17 cells/0.5 mm²; P < 0.01). AST-120 treatment significantly reduced the infiltration of ED1-positive cells (59.9 ± 6.5 cells/0.5 mm²; P < 0.05). We also evaluated the effects of AST-120 on OPN, which is a well-known pro-inflammatory cytokine in animal models of CsA-induced renal injury. The administration of AST-120 reduced both OPN protein and mRNA expression compared with those of the CsA4 group (Figure 3C–E).
Fig. 2. The dose–response relationships of AST-120 in chronic cyclosporine nephropathy. (A) The renal function in the study groups (n = 6). The creatinine clearance in the CsA+5%AST group was significantly increased compared with that in the CsA+0%AST group, but there was no significant difference between CsA+5%AST and CsA+10%AST groups. *P < 0.001 vs. all VH groups. **P < 0.05 vs. the CsA+0%AST group. (B) The serum IS levels in study groups. Note the significantly lower level of IS in the CsA+5%AST group relative to that in the CsA+0%AST group. However, 10% AST treatment had no significant effects on IS levels in VH-treated rats or CsA-treated rats. *P < 0.05 vs. the VH+0%AST group. **P < 0.05 vs. the CsA group. (C) Gross findings of the intestines in study groups. Note that the bowel is dilated and filled with AST-120 in the VH+10%AST or CsA+10%AST groups. (D) Microscopic findings of duodenum in the study groups. There were no abnormalities such as oedema and cell infiltration of the duodenal walls in study groups.

Table 1. The effects of early treatment with AST-120 on basic parameters in experimental groups

<table>
<thead>
<tr>
<th></th>
<th>VH4</th>
<th>VH4+AST4</th>
<th>CsA4</th>
<th>CsA4+AST4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Body weight (g)</td>
<td>46 ± 2</td>
<td>43 ± 2</td>
<td>22 ± 4*</td>
<td>34 ± 3**</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>115 ± 13</td>
<td>113 ± 9</td>
<td>119 ± 12</td>
<td>114 ± 9</td>
</tr>
<tr>
<td>CsA C (ng/mL)</td>
<td>–</td>
<td>2145 ± 91</td>
<td>2145 ± 91</td>
<td>2276 ± 60</td>
</tr>
<tr>
<td>SCr (mg/dL)</td>
<td>0.54 ± 0.05</td>
<td>0.58 ± 0.07</td>
<td>0.86 ± 0.15*</td>
<td>0.71 ± 0.06**</td>
</tr>
<tr>
<td>ClCr (mL/min/100 g)</td>
<td>0.57 ± 0.08</td>
<td>0.54 ± 0.11</td>
<td>0.21 ± 0.05*</td>
<td>0.31 ± 0.06**</td>
</tr>
</tbody>
</table>

Δ Body weight, changes in body weight; SBP, systolic blood pressure; SCr, serum creatinine; ClCr, creatinine clearance; CsA C, cyclosporine concentration.

*P < 0.05 vs. VH4 group.

**P < 0.05 vs. CsA4 group.
Fig. 3. Effect of early treatment with AST-120 on ED1 and OPN expression in rats with chronic CsA nephropathy. (A) Representative photomicrographs of the immunohistochemical detection of ED1 protein (original magnification ×200). Strong signals for ED1 protein in the CsA4 group were reduced with AST-120 treatment. (B) Quantitative analysis of ED1-positive cells in the four groups. *P < 0.01 vs. the VH4 group; **P < 0.05 vs. the CsA4 group. Representative photomicrographs of the immunohistochemical detection of OPN protein (C), and in situ hybridization detection of OPN mRNA (D) in the rat kidney (original magnification ×200). Increased OPN expression with intense labelling of the tubules in the CsA4 group was reduced with AST-120 treatment. (E) Northern blot analysis for OPN mRNA. AST-120 treatment significantly reduced OPN mRNA expression, which was increased in the CsA-treated rat kidney. The data are relative optical densities, with the VH4 group designated as 100%. The data were normalized to 18S rRNA expression. *P < 0.05 vs. the VH4 group; **P < 0.05 vs. the CsA4 group.
Figure 4A shows the results of trichrome staining in the four groups. The CsA4 group showed typical striped interstitial fibrosis, inflammatory cell infiltration, and extracellular matrix deposition in the cortex, but lesions were significantly reduced by the administration of AST-120 (original magnification ×200). (B) Quantitative analysis of TIF. Note the significantly lower TIF score in the CsA4 + AST4 group relative to that in the CsA4 group. Representative photomicrographs of immunohistochemistry (C) and western blot analysis (D) for βig-h3 protein (original magnification ×200). CsA treatment upregulated the expression of βig-h3 protein, whereas this was significantly ameliorated with concomitant treatment with AST-120. *P < 0.01 vs. the VH4 or VH4 + AST4 group; **P < 0.01 vs. the CsA4 group.

Next, we evaluated whether AST-120 modulates apoptotic cell death, an important mechanism of cell death in CsA-induced renal injury. The number of TUNEL-positive cells increased significantly in the CsA4 group compared with that in the VH4 group (1.06 ± 0.37% vs. 0.02 ± 0.03%; P < 0.05; Figure 5A and B). However, the concomitant administration of AST-120 significantly reduced the number of apoptotic cells compared with that in the CsA4 group (0.23 ± 0.14%, P < 0.01). Western blot analysis showed that
expression of caspase-3 protein, an early mediator of apoptotic cell death, was significantly higher in the CsA4 group than in the VH4 group (160 ± 83% vs. 100 ± 5%; \( P < 0.01 \); Figure 5C). However, the addition of AST-120 significantly reduced the active caspase-3 protein compared with that in the CsA4 group (83 ± 24%; \( P < 0.01 \)).

Effect of early treatment with AST-120 on CsA-induced oxidative stress

To gain an insight into the mechanisms underlying the protective effects of AST-120 in chronic CsA nephropathy, oxidative stress was evaluated using urinary 8-OHdG and acrolein which are reliable markers of cellular oxidative stress [22,23]. Intense 8-OHdG immunoreactivity was observed in the nuclei of the injured tubules in the kidney cortices of the CsA4 group (Figure 6A). However, the concomitant administration of AST-120 significantly reduced the distribution and intensity of 8-OHdG immunoreactivity. Urinary 8-OHdG excretion was significantly higher in the CsA4 group than in the VH4 group (163.1 ± 84.0 vs. 85.5 ± 33.6 ng/day; \( P < 0.05 \); Figure 6B). The concomitant administration of AST-120 significantly reduced the urinary 8-OHdG excretion levels (37.4 ± 22.1 ng/day; \( P < 0.05 \)) compared with that of the CsA4 group. Urinary acrolein excretion in the CsA4+AST4 group showed a similar pattern (Figure 6C).

Effect of delayed treatment with AST-120 on renal function in chronic CsA nephropathy

To determine the effects of the delayed administration of AST-120 on CsA nephropathy, the rats were randomly categorized into four groups, and each group was treated for
6 weeks as previously described (experimental protocol II). The whole-blood CsA levels did not differ between the CsA6 and CsA6+AST3 groups (Table 2). The serum creatinine levels were significantly increased in the CsA6 group compared with those of the VH6 and VH6+AST3 groups (P < 0.01, for both). However, delayed treatment with AST-

Table 2. The effects of delayed treatment with AST-120 on basic parameters in experimental groups

<table>
<thead>
<tr>
<th></th>
<th>VH6</th>
<th>VH6+AST3</th>
<th>CsA6</th>
<th>CsA6+AST3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Δ Body weight (g)</td>
<td>51 ± 6</td>
<td>52 ± 5</td>
<td>24 ± 2*</td>
<td>31 ± 3**</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>103 ± 5</td>
<td>118 ± 2</td>
<td>97 ± 3</td>
<td>107 ± 3</td>
</tr>
<tr>
<td>CsA C (ng/mL)</td>
<td>–</td>
<td>–</td>
<td>2835 ± 43</td>
<td>2809 ± 44</td>
</tr>
<tr>
<td>SCr (mg/dL)</td>
<td>0.57 ± 0.03</td>
<td>0.58 ± 0.03</td>
<td>0.96 ± 0.03*</td>
<td>0.80 ± 0.04**</td>
</tr>
<tr>
<td>ClCr (mL/min/100 g)</td>
<td>0.63 ± 0.08</td>
<td>0.52 ± 0.04</td>
<td>0.25 ± 0.01*</td>
<td>0.36 ± 0.03**</td>
</tr>
</tbody>
</table>

Δ Body weight, changes in body weight; SBP, systolic blood pressure; SCr, serum creatinine; ClCr, creatinine clearance; CsA C, cyclosporine concentration.

*P < 0.05 vs. VH4 group.

**P < 0.05 vs. CsA4 group.

Fig. 6. Effect of early treatment with AST-120 on oxidative damage in rats with chronic CsA nephropathy. (A) Immunohistochemical analysis of 8-OHdG. CsA treatment for 4 weeks increased the immunoreactivity for 8-OHdG in the injured tubule cortex. However, AST-120 treatment reduced this immunoreactivity for 8-OHdG (original magnification ×200). The urinary concentrations of 8-OHdG (B) and acrolein (C) Urinary 8-OHdG and acrolein levels were increased in the CsA4 group, but AST-120 treatment significantly reduced these measures. *P < 0.05 vs. the VH4 or VH4+AST4 group; **P < 0.05 vs. the CsA4 group.
120 significantly reduced the serum creatinine levels compared with those of the CsA6 group (P < 0.05). Similar results were observed for creatinine clearance.

Effect of delayed treatment with AST-120 on interstitial inflammation and fibrosis in chronic CsA nephropathy

As shown in Figure 7, immunohistochemical analysis of ED1 and OPN also demonstrated the delayed protective effects of AST-120 on chronic CsA nephropathy. AST-120 treatment significantly reduced the number of ED1-positive cells compared with the CsA6 group (246.4 ± 6.4 vs. 510.8 ± 61.4 cells/0.5 mm²; P < 0.01). The OPN mRNA expression in the CsA6+AST3 group also showed a significant reduction to approximately the baseline levels of the VH6 group.

The rats that underwent delayed treatment with AST-120 showed less interstitial fibrosis and relatively well-preserved renal architectures compared with those of CsA-treated rats after 6 weeks (Figure 8A). Treatment with AST-120 showed a significant reduction in interstitial fibrosis compared with those in the CsA6 group (26.6 ± 4.4% vs. 39.4 ± 1.9%; P < 0.01; Figure 8B). At a molecular basis, upregulated βig-h3 protein in the CsA6 group was markedly decreased with delayed administration of AST-120 (Figure 8C).

Effect of delayed treatment with AST-120 on epithelial–mesenchymal transition in chronic CsA nephropathy

The effects of AST-120 on the epithelial–mesenchymal transition of tubular cells were investigated by staining for vimentin, a mesenchymal marker, and E-cadherin, an epithelial cell marker. Vimentin expression was strongly upregulated, and E-cadherin expression was reduced in the tubular cells of the CsA6 group compared with those of the VH6 group. AST-120 substantially inhibited the ex-
pression of vimentin and prevented the loss of E-cadherin in tubular cells (Figure 9).

**Effect of delayed treatment with AST-120 on apoptotic cell death in chronic CsA nephropathy**

As shown in Figure 10A, the delayed administration of AST-120 significantly reduced the number of TUNEL-positive cells compared with the CsA6 group (0.31 ± 0.23% vs. 1.11 ± 0.21%; P < 0.01; Figure 10B). To confirm the presence of apoptosis, caspase-3 protein was detected with western blot analysis. The delayed treatment with AST-120 partially inhibited the caspase-3 protein activation induced by CsA (672 ± 202% vs. 1510 ± 190%; P < 0.01; Figure 10C).

**Effect of delayed treatment with AST-120 on oxidative stress and uraemic toxin in chronic CsA nephropathy**

Figure 11 shows the effect of delayed treatment with AST-120 on oxidative stress in chronic CsA nephropathy. The urinary 8-OHdG excretion was significantly higher in the CsA6 group than in the VH6 group (163.1 ± 84.0 vs. 85.5 ± 33.6 ng/day; P < 0.05), but the delayed administration of AST-120 significantly reduced the urinary 8-OHdG excretion levels (37.4 ± 22.1 nmol/day; P < 0.05) than the CsA6 group. Six weeks of treatment with CsA significantly elevated serum and urine IS levels, and this increase was decreased by the delayed administration of AST-120 (Figure 12).
Discussion

Many studies showed that AST-120 has a beneficial effect on attenuating the progression of CKD [24–26]. The present study was undertaken to investigate whether an oral adsorbent AST-120 has protective effect on progression of chronic CsA nephropathy. The results of this study reveal that removal of uraemic toxins with AST-120 is effective in decreasing progression of chronic CsA nephropathy. This finding provides a rationale for use of AST-120 in patients with impaired renal function due to long-term CsA treatment.

It is well known that uraemic toxins play an important role in the progression of CKD by inducing oxidative stress [27]. IS, one of the important uraemic toxins, induces reactive oxygen species production and decreases the levels of glutathione, one of antioxidant systems of the cell, in human umbilical vein endothelial cells [28]. Furthermore, removal of uraemic toxins with AST-120 treatment is effective in reducing oxidative stress in experimental and clinical studies [29–31]. Based on the aforementioned findings, we evaluated whether removal of uraemic toxin is effective in decreasing oxidative stress in CsA-induced renal injury. The results of our study showed that the plasma levels of IS and urinary concentrations of 8-OHdG and acrolein in CsA-treated rats were twice the levels of VH-treated rats, and AST-120 treatment reduced the plasma IS levels to baseline and significantly reduced urinary 8-OHdG and acrolein concentrations compared with the VH group. This finding suggests that a substantial proportion of the oxidative stress is associated with uraemic toxins in chronic CsA nephropathy, and it can be reduced via concomitant treatment with AST-120.

In general, AST-120 is recommended for CKD patients with renal dysfunction [7,29,32]. In this study, delayed treatment with AST-120 in established chronic CsA nephro-
pathy improved renal function, reduced interstitial inflammation, fibrosis and its associated cytokines (OPN and \( \beta \text{-ig-h3} \)), and decreased the apoptotic cell death and caspase-3 activity compared with the CsA group. Furthermore, AST-120 treatment decreased the epithelial–mesenchymal transition in chronic CsA nephropathy, demonstrated by decreased expression of vimentin and increased expression of E-cadherin in tubular cells. These findings confirm that AST-120 treatment is effective in attenuating progression of chronic CsA nephropathy. Recently, several studies have suggested that AST-120 may delay the progression of not only advanced CKD but also early-stage overt diabetic nephropathy and CKD [33,34]. Therefore, we further evaluated the effect of early treatment with AST-120 on CsA-induced renal injury and found that early treatment also improves renal function and histopathology, as observed in delayed treatment. This finding suggests that early treatment with AST-120 before overt renal dysfunction is also effective in decreasing CsA-induced renal injury and may provide a rationale for use of AST-120 in the early stages of CKD.

It is well known that CKD progresses to end-stage renal failure by common pathways [35–37], and one of the common pathways involves the accumulation of uraemic toxins [38,39]. Therefore, AST-120 is recommended in CKD patients with diverse aetiologies, such as chronic glomerulonephritis or diabetic nephropathy [40,41]. Like CKD in native kidneys, transplanted kidneys progress to chronic allograft dysfunction due to immunologic or non-immunologic causes [42]. Therefore, it is expected that AST-120 treatment is useful to prevent the progression of chronic allograft dysfunction. In current practice, it is recommended to reduce or withdraw calcineurin inhibitor and add non-nephrotoxic immunosuppressant for reducing chronic allograft dysfunction, but this may increase the risk of acute rejection in some pa-
In this situation, the use of AST-120 in renal transplant recipients may provide two benefits. First, AST-120 reduces the progression of chronic allograft dysfunction caused by CsA-induced chronic nephropathy. Second, the reduction of uraemic toxins may itself inhibit the progression of chronic allograft dysfunction, regardless of the underlying cause.

In contrast to a previous study that showed dose-dependent effects of AST-120 [7], our study showed no dose-dependent effects. High doses of AST-120 (10 mg/kg) failed to decrease uraemic toxin and improve renal function. The reason for no dose-dependent effects seems to be related to the experimental model of chronic CsA nephropathy. Compared with the VH group, the CsA group with high-dose AST-120 showed adverse effects such as loss of appetite and weight loss, and the intestines were distended with AST-120. This finding suggests that rats are not tolerable to high-dose AST-120 in a setting of CsA-induced renal injury. We also suspected a direct effect of AST-120 on the intestinal wall, but microscopic examination revealed no intestinal oedema in rats with high-dose AST treatment in both VH and CsA groups. Therefore, we excluded the toxic effect of AST-120 on the intestinal wall as a cause of no response to high-dose AST-120.

CsA is usually given to patients via the oral route. Therefore, AST-120 may affect absorption of orally administered CsA in clinical practice. However, this concern may be excluded by a recent report that states orally administered AST-120 had no influence on the absorption and the enterohepatic circulation of orally administered CsA in olive oil [45]. Another concern is that we evaluated only one toxin (plasma IS). Recent reports show that AST-120 effectively reduces other uraemic toxins, such as carboxymethyllysine, which is an advanced glycation end product [46,47]. Thus, there is a possibility that other uraemic toxins are also involved in the protective effect of AST-120 on chronic CsA nephropathy.

In conclusion, AST-120 is an effective drug for the reduction of uraemic toxins. This effect may be applicable to renal transplant recipients with chronic allograft dysfunction. This study provides new insights into the usefulness of AST-120 in chronic allograft dysfunction caused by CsA-induced renal injury.

Acknowledgements. The authors wish to acknowledge the financial support of Kureha Corporation. This work was also supported by the Korea Science and Engineering Foundation (R13-2002-005-03001-0) through the Medical Research Center for Cell Death Disease Research Center at the Catholic University of Korea.

Conflict of interest statement. None declared.

References