The Effects of Valsartan on Renal Klotho Expression and Oxidative Stress in Alleviation of Cyclosporine Nephrotoxicity

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Background. Nephrotoxicity side effect of the immunosuppressant drug, cyclosporine A (CsA), can be a major issue in transplantation medicine. Cyclosporine A–induced nephrotoxicity is multifactorial but oxidative stress has a critical role in this process. It has been demonstrated that Valsartan (Val) as an angiotensin receptor blocker has renoprotective effects, but the molecular mechanisms responsible for the renal protection, independent from its blood pressure lowering effect, have not yet been fully understood. The present study is aimed at evaluating the Val effect in alleviation of CsA nephrotoxicity by probable increase in renal Klotho expression and/or reducing oxidative stress. Methods. Thirty-two Sprague-Dawley rats were divided into 4 groups based on the administration of CsA and/or Val: group A (control, 1 mL/kg per day of olive oil as vehicle), group B (CsA, 30 mg/kg per day), group C (CsA + Val, 30 + 30 mg/kg per day), and group D (Val, 30 mg/kg per day). Real-time polymerase chain reaction and Western blotting were used to evaluate Renal Klotho expression. Serum Klotho level was measured by enzyme-linked immunosorbent assay. 8-Hydroxy-deoxy guanosine and malondialdehyde levels as markers of oxidative stress were measured by enzyme-linked immunosorbent assay and spectrophotometrically, respectively. Results. Cyclosporine A treatment reduced renal expression and serum levels of Klotho, improved malondialdehyde and 8-hydroxy-deoxy guanosine levels, and also deteriorated renal function. Valsartan prevented CsA-induced oxidative stress as well as Klotho downregulation and could alleviate CsA-induced renal histological changes and function. Conclusions. Administration of Val might lead to alleviation of CsA nephrotoxicity by probably diminishing CsA-induced renal Klotho downregulation and oxidative stress.

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According to previous studies,7–9 Valsartan (Val), an antihypertensive drug that belongs to the angiotensin receptor blockers (ARBs), has a renoprotective effect; so, it may lead to the alleviation of CsA nephrotoxicity. It blocks angiotensin II type 1 (AT1) receptor, which mediates blood pressure (BP) elevating effect of angiotensin.10 Valsartan can be used for the treatment of hypertension in patients with chronic kidney diseases (CKD), because hypertension is a prominent cause and also an outcome of kidney diseases.11 For the treatment of hypertension in the patients with diabetic nephropathy,
ARBs are usually considered as the first-line agents. Effects of different ARBs on BP reduction are close to each other, but some studies have demonstrated that Val is the most specific, most effective, and safest drug of all. Treatment with Val does not interfere with immunosuppressive therapy. All the molecular mechanisms responsible for the renal protection of Val, which is independent from its antihypertensive effect, have not yet been fully understood. Some studies have shown the association of ARBs in the elevation of Klotho (Kl) levels. Yoon et al in their study demonstrated that angiotensin II receptor blockade by Losartan upregulates the expression of Kl in an experimental model of chronic CsA nephropathy. Klotho is a recently known anti-aging gene that is expressed in multiple tissues, but predominantly in healthy kidneys. The Kl gene encodes a type I single-pass transmembrane protein. It also has a soluble secreted form (s-Kl) that can be derived by alternative splicing or cleavage from membrane Kl. Kl can then circulate throughout the body fluids.

The potential usefulness of Kl in clinical practice has at least 2 parts. First, Kl could serve as an early and sensitive biomarker of kidney diseases. Second, Kl supplementation may provide a novel therapy for the treatment of acute kidney injury by limiting damage and promoting recovery and also for the treatment of CKD by slowing progression as well as preventing and reversing complications. Mitani et al in their study demonstrated that renal Kl gene downregulation may have an aggravative role in the development of renal damage induced by angiotensin II and that induction of the Kl gene may have therapeutic possibilities in the treatment of angiotensin II-induced end organ damage.

The roles of this protein have not yet been fully known, and it has become obvious that Kl has antioxidative effects and may protect the kidneys from oxidative stress-inducing factors, such as CsA. The aim of this study is to evaluate Val effect in the alleviation of CsA nephrotoxicity. In addition, the probable alterations in renal Kl expression and oxidative stress are assessed to better understand the mechanisms involved in CsA nephrotoxicity and also Val renal protection.

MATERIALS AND METHODS

Animals

In this study, 32 male 12-week-old Sprague-Dawley rats weighing 220 to 280 g were purchased from Pasteur Institute of Iran (Tehran, Iran). According to Guide for Care and Use of Laboratory Animals [US Department of Health, Education, and Welfare, Publication number 78-23, National Institutes of Health, revised 1978] and local guidelines for compassionate use of animals in research, the animals were housed 2 per cage and provided free access to tap water and standard chow. The animals were kept in similar laboratory conditions (18-23°C room temperature and controlled humidity) with alternating 12-hour light and dark cycles.

Group Design (Drug Treatment)

After a 2-week acclimation period, the weight-matched rats were randomly allocated into 4 groups (8 rats per group): group A (control) received daily subcutaneous injection of vehicle (1 mL/kg of olive oil, Sigma Co.) for 6 weeks. Group B (CsA) received daily subcutaneous injection of CsA (NovaMatis Pharma) diluted in olive oil (15 mg/mL) at a dose of 30 mg/kg for 6 weeks. Group C (CsA + Val) received both CsA (30 mg/kg per day, subcutaneous injection) and Val (NovaMatis Pharma; 30 mg/kg per day, in drinking water) for 6 weeks. Group D (Val) received daily administrations of Val (30 mg/kg, in the drinking water) for 6 weeks. Based on previous studies, this dose of Val (30 mg/kg) does not alter BP in rats. The conditions of the administrations were based on previous studies. After the administration period, all the rats were weighed and anesthetized by a single dose (100 mg/kg) intraperitoneal injection of ketamine. Intravenous blood samples were collected and after clotting, the sera were separated by centrifugation at 3000 g for 15 minutes and stored at −80°C. Thereafter, the rats were killed with a single lethal dose (200 mg/kg) of ketamine, and the kidneys were excised and weighed separately. The right kidneys were collected and fixed in 10% formalin for histological assessments. The left kidneys were snap-frozen in liquid nitrogen and stored at −80°C until the analyses were performed.

Determination of Serum Parameters

To evaluate kidney function, the serum concentrations of calcium (Ca), phosphorus (P), urea, and creatinine (Cr) were determined colorimetrically using commercial reagents in an automated chemical analyzer (Roche Cobas Mira). In addition, deteriorated renal failure (DRF) index was calculated using the formula “(Cr + urea)/2” as a way to properly estimate the glomerular filtration rate. Serum level of Kl (s-Kl) was determined by a rat enzyme-linked immunosorbent assay kit (Hangzhou Eastbiopharm CO., LTD.; Hangzhou, China).

Determination of Oxidative Stress Markers

The rats’ renal tissues were collected from the freezer and tissue lysates were prepared using phosphate-buffered saline and butylated hydroxytoluene to prevent oxidative damage. Malondialdehyde (MDA) level as a lipid peroxidation marker was evaluated spectrophotometrically using thioarbituric acid reactive substances assay based on the method of Lapenna et al. 8- hydroxyl-deoxyguanosine (8-OHdG), another marker of oxidative stress, was measured with a rat enzyme-linked immunosorbent assay kit (Hangzhou Eastbiopharm CO., LTD.; Hangzhou, China).

Analysis of Renal Kl Expression

Quantitative Real-Time Polymerase Chain Reaction

Total RNA was extracted from the kidney tissues using RNeasy Mini kit (QIAGEN, Germany) based on the manufacturer’s protocol. Thereafter, 1 µg of the total RNA was used for complementary DNA (cDNA) synthesis with Oligo (dT) primers using the RevertAid first-strand cDNA synthesis kit (Thermo Fisher Scientific Inc.) based on the manufacturer’s instruction. Quantitative real-time polymerase chain reaction (PCR) analysis was conducted in duplicate using SYBR Premix Ex Taq II (Takara Bio Inc., Japan) on the Rotor-Gene 6000 Real-time PCR detection system (Corbett Research, Australia). Results were normalized to the gene expression of rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as the reference gene. Table 1 presents the used specific primer-sequences. Polymerase chain reaction program comprised a preincubation step at 94°C for 5 minutes,
followed by 40 cycles of denaturation (94°C, 15 seconds), annealing (64°C, 15 seconds), and extension (72°C, 15 seconds) steps with the final extension step at 72°C for 10 minutes. The 2−ΔΔCt formula was used to calculate the gene expression ratio of Kl to GAPDH with respect to the control group.

Western Blot Analysis

Rat kidney tissues were homogenized using ice-cold radioimmunoprecipitation assay buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate [SDS], 1 mM sodium orthovanadate, 1 mM NaF; hydrogen ion concentration [pH] 8) containing protease inhibitor cocktail (Roche Diagnostics, Germany). Fifty micrograms (50 μg) total protein (measured by Bradford method) was separated by 8% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and then electrotransferred onto polyvinylidine fluoride membranes (Merck Millipore, Germany). The membranes were blocked in 3% bovine serum albumin at room temperature for 2 hours. Thereafter, the blots were incubated in primary antibodies at 4°C overnight. The primary antibodies used as the target protein were goat polyclonal IgG against Kl (E-21, 1:500; Santa Cruz Biotechnology Inc., Germany) whereas goat polyclonal IgG against GAPDH (I-19, 1:500; Santa Cruz Biotechnology Inc.) was used as the internal control. After washing with Tris-buffered saline supplemented with 0.1% Tween 20 3 times, the membranes were incubated with horseradish peroxidase-labeled secondary antibody (donkey antigoat IgG-horseradish peroxidase, 1:10000; Santa Cruz Biotechnology Inc., Germany) for 1 hour at room temperature. After 5 times of washing with Tris-buffered saline supplemented with 0.1% Tween 20, BM chemiluminescence blotting substrate (Roche Diagnostics, Germany) was used to detect the immunoreactive proteins that were then recorded on X-ray films (FUJIFILM Corporation, Japan). Quantification was performed with relative density (Kl to GAPDH) using Image J (version 1.49 t) software.

Histological Assessment

Formalin-fixed kidney samples were subjected to dehydration in the ascending grades of ethanol and were kept in xylene overnight for complete dehydration. After embedding in paraffin, the tissue sections of 5 μ were cut and subsequently stained with hematoxylin-eosin. Thereafter, at least 10 fields of each slide were examined by a pathologist blinded to the grouping of the rats at 200× magnification under light microscope. For evaluation of tubulointerstitial damage (TID), each slide was scored semiquantitatively² from 0 to 3 according to the severity of pathological changes that included 0 (no changes), 1 (less than 25% of the field was changed), 2 (25-50% of the field was changed), and 3 (more than 50% of the field was changed). Score of each kidney sample was expressed as the mean value of all the achieved scores.

TABLE 1. The primary primer sequences of klotho and GAPDH genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Forward primer</th>
<th>Reverse primer</th>
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<tbody>
<tr>
<td>Klotho</td>
<td>5′GCGTGAATGAGGCTCTGAAAGC3′</td>
<td>5′GAGGCCGCTACTAAGCGGATACG3′</td>
</tr>
<tr>
<td>GAPDH</td>
<td>5′GAAGGCTCATGACACAGT3′</td>
<td>5′GGATGCAGGAGTAGTCTCT3′</td>
</tr>
</tbody>
</table>

Tubulointerstitial fibrosis (TIF) was estimated semiquantitatively¹⁵ from 0 to 3 by counting the percentage of injured areas per field using a color image analyser (TDI Scope Eye Version 3.5 for Windows, Olympus, Japan). Scores were given as follows: 0, normal interstitium; 0.5, less than 5% TIF; 1.0, 5-15% TIF; 1.5, 16-25% TIF; 2.0, 26-35% TIF; 2.5, 36-45% TIF and 3.0, more than 45% TIF.

Statistical Analysis

Data were presented as mean ± SD. Statistical comparisons of the groups were performed by 1-way analysis of variance and Bonferroni post hoc analysis. Pearson’s correlation coefficient was also calculated. Statistical significance was set at P value of less than 0.05. The analyses were carried out in SPSS 16.0 software.

RESULTS

Basic Parameters

The data of body weight, left kidney weight, right kidney weight, Ca, P, urea, Cr, and DRF index are presented in Table 2. All the rats were weight matched before the intervention (P > 0.05), but after the intervention, the CsA-treated rats (group B) had significantly lower mean body weight than the other groups (P < 0.05). The mean levels of left kidney weight and right kidney weight in addition to serum Ca and P levels were not significantly different among the groups (P > 0.05), but the rats receiving CsA (group B) were detected to have significantly (P < 0.05) higher serum levels of urea and DRF-index than the control and Val groups. The mean level of Cr in CsA group was significantly (P < 0.05) higher than that in other study groups.

Renal Expression and Serum Levels of Kl

The results of real-time PCR and Western blotting showed that mRNA and protein expressions of renal Kl were significantly (P < 0.05) lower in CsA group than those in control and Val groups (Figures 1A and B). It is important to note that the changes in serum levels of Kl (s-Kl) also conformed to the results of expression alteration (Figure 1C).

Oxidative Stress Markers

As illustrated in Figure 2, the rats receiving CsA were detected to have significantly (P < 0.05) higher tissue 8-OHdG and MDA levels than the other groups.

Histological Findings

Cyclosporine A administration led to increased TID and TIF which were exhibited by histological alteration including hyperemia, inflammatory cell infiltration, interstitial fibrosis, tubular atrophy, and vacuolization. As illustrated in Figures 3B and D, the TID and TIF scores in CsA-treated rats were significantly (P < 0.05) higher than those in other groups. The histological changes were diminished in group C (CsA + Val) when compared with the CsA group. The tissue samples of Val-treated rats as well as controls did not show any remarkable renal histologic alterations.

Relationships Among Kl, Oxidative Stress, DRF, and TID Scores

As expected, the Kl mRNA and protein expression were correlated with each other and both were positively
correlated with the Kl serum level. As illustrated in Table 3, Kl protein expression was negatively correlated with MDA and 8-OHdG levels. These oxidative stress markers were positively correlated with each other. The TID, TIF, and DRF scores were positively correlated and Kl expression was inversely correlated with each of them. In addition, TID, TIF, and DRF scores were positively correlated with oxidative stress markers.

**TABLE 2.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group A (control)</th>
<th>Group B (CsA)</th>
<th>Group C (Val + CsA)</th>
<th>Group D (val)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW b.i., g</td>
<td>208.78 ± 12.58</td>
<td>207.14 ± 10.33</td>
<td>205.57 ± 6.00</td>
<td>210.11 ± 11.27</td>
</tr>
<tr>
<td>BW a.i., g</td>
<td>235.00 ± 17.03</td>
<td>190.57 ± 19.72a</td>
<td>223.71 ± 23.45b</td>
<td>252.32 ± 20.23c</td>
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<tr>
<td>LKW, mg</td>
<td>705.7 ± 119.8</td>
<td>614.0 ± 83.5</td>
<td>671.4 ± 46.4</td>
<td>738 ± 74.9</td>
</tr>
<tr>
<td>RKW, mg</td>
<td>733.6 ± 123.5</td>
<td>629.0 ± 88.6</td>
<td>666.4 ± 56.8</td>
<td>735.7 ± 89.5</td>
</tr>
<tr>
<td>Ca, mg/dL</td>
<td>10.97 ± 0.91</td>
<td>11.57 ± 1.55</td>
<td>12.91 ± 1.91</td>
<td>11.60 ± 1.83</td>
</tr>
<tr>
<td>P, mg/dL</td>
<td>9.22 ± 0.73</td>
<td>8.20 ± 1.92</td>
<td>9.23 ± 3.24</td>
<td>7.31 ± 1.62</td>
</tr>
<tr>
<td>Cr, mg/dL</td>
<td>1.04 ± 0.22</td>
<td>1.98 ± 0.71d</td>
<td>1.18 ± 0.65e</td>
<td>0.82 ± 0.37f</td>
</tr>
<tr>
<td>Urea, mg/dL</td>
<td>59.4 ± 14.5</td>
<td>100.86 ± 39.19g</td>
<td>78.0 ± 42.0</td>
<td>53.3 ± 11.6g</td>
</tr>
<tr>
<td>DRF index</td>
<td>30.24 ± 7.23</td>
<td>51.42 ± 19.78i</td>
<td>39.59 ± 21.14</td>
<td>27.07 ± 5.95f</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SD. **a** P < 0.001 vs control. 
**b** P = 0.006 vs CsA 
**c** P < 0.001 vs CsA 
**d** P = 0.005 vs control 
**e** P = 0.017 vs CsA 
**f** P = 0.001 vs CsA 
**g** P = 0.043 vs control 
**h** P = 0.015 vs CsA 
**i** P = 0.009 vs control 
**j** P = 0.013 vs CsA.

*P < 0.05 versus control; #P < 0.05 versus CsA.

Data are shown as mean ± SD. a.i., after intervention; b.i., before intervention; BW, body weight; LKW, left kidney weight; RKW, right kidney weight.
FIGURE 2. 8-OHdG and MDA levels in kidney tissues affected by CsA and/or Val in the study groups. A, Levels of 8-OHdG in CsA group (1.96 ± 0.48 ng/mL) were significantly higher than those in the other study groups (control, 1.08 ± 0.45; CsA + Val, 1.23 ± 0.57; Val, 0.88 ± 0.13 ng/mL). B, MDA levels in CsA group (7.94 ± 1.97 nmol/L) were significantly higher than those in the other study groups (control, 4.55 ± 1.62; CsA + Val, 5.04 ± 1.37; Val, 4.27 ± 1.74; nmol/L). Data are expressed as mean ± SD. *P < 0.05 vs control; #P < 0.05 vs CsA.

FIGURE 3. Representative histologic findings. A, Hematoxylin-eosin (H&E) staining of the rats’ kidney-tissues in the 4 studied groups (magnification, ×200). Hyperemia (asterisks), inflammatory cell infiltration (arrows), tubular atrophy (arrowhead), and vacuolization (dashed arrow) were exhibited in the CsA-treated rats compared with those in the controls. Valsartan (Val) ameliorated these histological changes (CsA + Val group). There were no histologic alterations in Val and control groups. B, Graphs of TID scoring in the study groups. The TID score in CsA group (2.29 ± 0.70) was significantly (P < 0.001) higher than that in the controls (0.25 ± 0.25). The TID scores in CsA + Val group (0.93 ± 0.53) and Val-treated rats (0.23 ± 0.24) were significantly (P < 0.001) lower than in CsA group (2.29 ± 0.70). C, Photomicrographs of trichrome staining for each experimental group (magnification, ×200). CsA administration induced a typical interstitial fibrosis (blue collagen deposition, CsA group) that was ameliorated by Val treatment (CsA + Val group). D, Graphs of TIF scoring in the study groups. The TIF score in CsA group (1.43 ± 0.45) was significantly (P < 0.001) higher than that in the controls (0.0 ± 0.0). The TIF scores in CsA + Val group (0.43 ± 0.53) and Val-treated rats (0.0 ± 0.0) were significantly (P < 0.001) lower than in CsA group (1.43 ± 0.45). Data are shown as mean ± SD. *P < 0.001 vs controls; #P < 0.001 vs CsA.
DISCUSSION

In the present study, it was initially hypothesized that Val treatment, due to its renoprotective effect, could alleviate the nephrotoxic side effect of CsA. Viberti et al \(^7\) and Katayama et al \(^8\) have shown that the renoprotective effect of Val is independent of BP changes. In our study, the dose of Val was 30 mg/kg per day; according to previous studies, this dose does not change BP in the rats.\(^{25,27}\) Because the BP lowering role has been eliminated, the other potential mechanisms for the renal protection of Val against CsA nephrotoxicity could be possible. Klotho may play a key role in this field. In our study, it was found that CsA treatment reduced renal Kl expression. The serum level of Kl as a derived form of membrane Kl positively correlated with each other, which is not unpredicted since the level of s-Kl as a derived form of membrane Kl was also reduced by CsA. In addition, we observed that the renal expression and serum level of Kl were positively correlated with each other, which is not unpredicted but still reflects the effect of CsA in the reduction of Kl gene expression. The exact mechanisms of CsA-induced oxidative stress are left to be fully elucidated. One considerable mechanism introduced by Yoon et al \(^31\) may be as follows: CsA could reduce the expression of manganese superoxide dismutase and hemeoxygenase-1 by changing the expression of forkhead box O (FoxO) transcription factors. It could eventually lead to improved sensitivity of cells to oxidative stress. As another possible mechanism, O’Connell et al \(^32\) revealed that thioredoxin interacting protein, an inhibitor of thioredoxin, which is an important ROS scavenger, was significantly increased after CsA treatment. This could then change cellular redox-oxidative balance to promote oxidative stress. In addition, inhibition of the mitochondrial electron transport chain and/or depletion of the cellular antioxidant system by CsA can also be determined.\(^{33}\) In our study, levels of 8-OHdG and MDA in kidney tissues were significantly higher in CsA-treated rats compared with the control. Moreover, Kl expression was negatively correlated with oxidative stress markers, which can be demonstrated that TID, TIF, and DRF scores had positive correlation with oxidative stress markers, which can be eventual lead to improved sensitivity of cells to oxidative stress.

According to the study of Mitobe et al,\(^30\) reduced Kl expression may be secondary to the oxidative stress-inducing effect of CsA. The exact mechanisms of CsA-induced oxidative stress are left to be fully elucidated. One considerable mechanism introduced by Yoon et al\(^{31}\) may be as follows: CsA could reduce the expression of manganese superoxide dismutase and hemeoxygenase-1 by changing the expression of forkhead box O (FoxO) transcription factors. It could eventually lead to improved sensitivity of cells to oxidative stress. As another possible mechanism, O’Connell et al\(^{32}\) revealed that thioredoxin interacting protein, an inhibitor of thioredoxin, which is an important ROS scavenger, was significantly increased after CsA treatment. This could then change cellular redox-oxidative balance to promote oxidative stress. In addition, inhibition of the mitochondrial electron transport chain and/or depletion of the cellular antioxidant system by CsA can also be determined.\(^{33}\) In our study, levels of 8-OHdG and MDA in kidney tissues were significantly higher in CsA-treated rats compared with the control. Moreover, Kl expression was negatively correlated with oxidative stress markers, which can be demonstrated that TID, TIF, and DRF scores had positive correlation with oxidative stress markers, which can be as tert butylhydroperoxide (TBP) scavengers.

In the present study, it was also demonstrated that the levels of MDA and 8-OHdG in CsA + Val group were significantly lower than those in CsA group, suggesting that Val treatment might improve resistance to CsA-induced oxidative stress. Because CsA-induced Kl-downregulation was diminished by Val and based on the negative correlation of Kl expression and oxidative stress, Val-induced oxidative stress resistance might be mediated by Kl. Klotho could lead to the activation of FoxO transcription factors by inhibiting the insulin/IGF-I signaling cascade. Active FoxO upregulates the expression of manganese superoxide dismutase and thereby facilitating the removal of ROS, which eventually leads to improved oxidative stress resistance. This mechanism was introduced by Yamamoto et al\(^{25}\). Also, before this study, it had been reported that Kl can also activate FoxO by inhibiting serum and glucocorticoid inducible kinase and/or activating c-Jun N-terminal kinase (JNK)\(^{37}\) or β-catenin.\(^{38}\)

Effects of CsA and, subsequently, Val treatments on kidney tissue were also evaluated histologically and functionally. The TID and TIF scores for the renal histological assessment in CsA group were significantly higher than those of the other groups. In addition, DRF score for renal functional evaluation was significantly higher in CsA group than in control and Val groups, but not in CsA + Val group, indicating that 30 mg/kg per day Val administration for 6 weeks might not be enough for the enhancement of renal function. The results also demonstrated that TID, TIF, and DRF scores had positive correlation with oxidative stress markers, which can be

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Kl</th>
<th>8-OHdG</th>
<th>MDA</th>
<th>TID score</th>
<th>TIF score</th>
<th>DRF score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kl (r, P)</td>
<td>—</td>
<td>−0.623, &lt;0.001</td>
<td>−0.624, &lt;0.001</td>
<td>−0.584, &lt;0.001</td>
<td>−0.585, &lt;0.001</td>
<td>−0.488, 0.005</td>
</tr>
<tr>
<td>8-OHdG (r, P)</td>
<td>−0.623, &lt;0.001</td>
<td>—</td>
<td>0.858, &lt;0.001</td>
<td>0.676, &lt;0.001</td>
<td>0.734, &lt;0.001</td>
<td>0.712, &lt;0.001</td>
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<tr>
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<td>0.858, &lt;0.001</td>
<td>—</td>
<td>0.631, &lt;0.001</td>
<td>0.658, &lt;0.001</td>
<td>0.660, &lt;0.001</td>
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<tr>
<td>TID (r, P)</td>
<td>−0.584, &lt;0.001</td>
<td>0.676, &lt;0.001</td>
<td>0.631, &lt;0.001</td>
<td>—</td>
<td>0.869, &lt;0.001</td>
<td>0.559, 0.001</td>
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<td>TIF (r, P)</td>
<td>—</td>
<td>0.712, &lt;0.001</td>
<td>0.660, &lt;0.001</td>
<td>0.559, 0.001</td>
<td>0.709, &lt;0.001</td>
<td>—</td>
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</table>

r, correlation coefficient.
documented with other studies\textsuperscript{32,39–41} to confirm the role of CsA-induced oxidative stress in the kidney damage; nevertheless, in the study of Galletti et al,\textsuperscript{4} there was no direct link between CsA-mediated oxidative stress and adverse renal effects. On the other hand, based on the negative correlations of KI with TID, TIF, and DRF scores; Val alleviation of CsA-induced renal histologic and functional changes might be mediated by enhancing KI and, subsequently, reducing oxidative stress levels. The entire molecular mechanisms responsible for Val renal protection, independent from its antihypertensive effect, have not yet been fully understood, and it may perform its renal protective effect through several known and unknown mechanisms.\textsuperscript{34} According to the results of the present study, KI may play an important role in this regard and upregulating of KI gene by Val and subsequent reduction in oxidative stress (due to antioxidative effect of KI) can be considered as 1 of the mechanisms involved in Val renal protection. This mechanism may be the most important in kidney tissue as the predominant source of the KI.\textsuperscript{18,19} Antioxidative effect of KI is due to its soluble form that circulates throughout the body fluid.\textsuperscript{21,24,25} So, KI may also have a role to play in other beneficial properties of Val such as on the cardiovascular system\textsuperscript{42–44} but this needs further studies.

In addition to KI, other factors such as heat shock proteins (HSPs) may also play important roles in the renal protection induced by AT1 receptor blockade. HSPs have beneficial roles in protein processing and protection against cell injury. Chander et al\textsuperscript{45} in their study proposed that HSPs might have roles in ameliorating CsA nephrotoxicity by Irbesartan as another member of ARBs.

The present study was performed in a clean environment (healthy, nontransplanted rats) that can be appropriate for evaluating molecular interactions.\textsuperscript{20} Nevertheless, it is well expected that kidney disease, transplantation, alloimmunity, and additional immunosuppression might influence the outcome of the study. So, further experimental (transplanted animal model) and clinical (transplant patients) studies are required in this regard to eventually provide clinical application.

Based on previous studies, 30 mg/kg of Val that was used in our study does not alter BP in rats.\textsuperscript{36,23} Therefore, BP measurement was not done in the present study. However, it could be a limitation of the study. Evaluation of serum inflammatory markers was not performed, which can be another limitation of the study.

CONCLUSIONS

It was demonstrated in the present study that CsA might lead to renal KI downregulation by improving oxidative stress. Valsartan treatment might diminish CsA-induced oxidative stress as well as KI downregulation and might eventually lead to the alleviation of CsA nephrotoxicity and improvement of renal function. This may be considered as a mechanism for CsA-induced nephrotoxicity and/or Val renal protection but further studies are needed in this regard.

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REFERENCES


