Can Trimetazidine, Vinpocetine or Isosorbide Dinitrate Ameliorate Cyclosporine-Induced Nephrotoxicity in Rats?

Heba MI Abdallah1, Hisham A Salem2, Omar M Abdel Salam1, Salwa H Salama1, Sanaa A Kenawy2

1Department of Pharmacology, National Research Centre, Giza, Egypt
2Department of Pharmacology, Faculty of Pharmacy, Cairo University, Egypt

ABSTRACT

BACKGROUND: The present study was conducted to investigate the effect of concurrent administration of trimetazidine (a mitochondrial stabilizing anti-anginal agent), vinpocetine (a phosphodiesterase-1 inhibitor) and isosorbide dinitrate (a NO donor) on nephrotoxicity induced by cyclosporine A (CsA) treatment.

METHODS: Female albino rats were divided into eight groups. Group 1 rats were treated with corn oil and served as normal control. Group 2 received CsA (15mg/kg, s.c. for 4 weeks) and served as control. Groups 3 and 4 received CsA along with trimetazidine (5 & 10mg/kg, p.o). Groups 5 and 6 received CsA along with vinpocetine (5 & 10mg/kg, p.o). Groups 7 and 8 received CsA along with isosorbide dinitrate (3.6, and 7.2mg/kg p.o). Blood urea nitrogen (BUN), serum creatinine, serum uric acid and blood glucose were measured. Creatinine clearance (Ccr) and proteinuria were estimated. Reduced glutathione (GSH), lipid peroxides, nitric oxide (NO), and hydroxyproline contents were measured in kidney tissues.

RESULTS: Injection of CsA increased BUN, serum creatinine, and blood glucose levels as well as renal hydroxyproline content. It also decreased Ccr and renal NO content. Only isosorbide dinitrate (3.6mg/kg) could partially improve CsA-dependent changes in renal function as shown by decrease in elevated serum creatinine and renal hydroxyproline content as well as improvement of Ccr. However, administration of isosorbide dinitrate at a higher dose (7.2mg/kg) along with CsA deteriorated the renal function reflected by decreased Ccr and renal NO content associated with proteinuria, increased BUN and uric acid levels.

CONCLUSION: The current study demonstrates that isosorbide dinitrate at the dose of 3.6mg/kg could protect against CsA-induced nephrotoxicity, whereas both trimetazidine and vinpocetine are of unclear utility.

Key Words: Cyclosporine; Nephrotoxicity; Trimetazidine; Vinpocetine; Isosorbide Dinitrate

INTRODUCTION

Cyclosporine A (CsA) is a potent antifungal and immunosuppressive compound. It was approved in early 1980s for use as prophylactic antirejection therapy in patients receiving allogeneic transplants (kidney, liver, and heart). It has markedly improved survival of solid organ transplants as compared to conventional therapy consisting of prednisone and azathioprine (from 50% to 85%). In addition, CsA has also been used in the treatment of autoimmune diseases such as psoriasis, rheumatoid arthritis, atopic dermatitis, and nephrotic syndrome [1]. However, the clinical use of CsA is limited by chronic nephrotoxicity. Two types of CsA nephrotoxicity have been described: acute and chronic. Acute CsA treatment induces reversible reduction of glomerular filtration rate (GFR) and renal blood flow that is related to afferent arteriolar vasoconstriction. Imbalance in the release of vasoactive substances may account for this vasoconstriction. Either an increase in vasoconstricting factors such as endothelin, thromboxane, and angiotensin II, or a decrease in vasodilators such as prostacyclin,
and nitric oxide (NO) takes place. When given for a long term, CsA can lead to irreversible renal failure not only due to renal vasoconstriction, but also due to tubulo-interstitial fibrosis, tubular atrophy and glomerular sclerosis [2]. Trimetazidine is an anti-ischemic agent. Its effects have been experimentally assessed in various models including cell cultures, isolated tissues and perfused organs [3]. Trimetazidine can ameliorate oxidative stress. During acute and chronic ischemia, trimetazidine reduced the loss of intracellular potassium induced by oxygen free radicals and decreased the membrane content of peroxidized lipids. The metabolic effects of trimetazidine were accompanied by a significant decrease of endothelin-1 levels, suggesting a beneficial effect of trimetazidine on endothelial function. Furthermore, some studies have reported that trimetazidine can protect against experimentally-induced nephropathy [4-5]. Therefore, trimetazidine was expected to be a possible remedy for CsA-induced nephrotoxicity.

Similarly, vinpocetine, a semisynthetic alkaloid derivative of an extract from the plant *Vinca minor* has been reported to possess cerebral blood-flow enhancing effect and was used in the treatment of cerebrovascular disorders and age-related memory impairment. Vinpocetine is also widely marketed as a supplement for vasodilatation possibly through the inhibition of Ca\(^{2+}\)-dependent phosphodiesterase-1 activity. Moreover, several studies have suggested that vinpocetine has good antioxidant properties [6-7]. Thereby, it may have a therapeutic benefit in diseases where vasoconstriction as well as oxidative stress plays a crucial role such as nephrotoxicity.

Isosorbide dinitrate is a long-acting organic nitrate used for the relief and prophylaxis of angina pectoris. It produces vasodilatation by forming nitric oxide NO (also known as endothelium-derived relaxing factor) in the vascular smooth muscle cells. It relaxes vascular smooth muscles and consequently produces dilatation of peripheral arteries and veins [8]. NO is also involved in the control of vascular growth. Since the availability of NO is decreased in a variety of conditions associated with endothelial dysfunction, it is possible that the isosorbide dinitrate, as an exogenous donor of NO, might have beneficial effects in these conditions. In addition, the role of NO in the pathogenesis of chronic CsA-induced nephrotoxicity has been studied. Exogenous supplementation with L-arginine effectively prevented CsA-induced renal dysfunction, arteriolopathy, and interstitial fibrosis in rats [9]. Hence, the present study was designed in order to investigate the effect of treatment with trimetazidine, vinpocetine or isosorbide dinitrate on CsA-induced renal damage in rats.

**MATERIALS AND METHODS**

**Animals:**
The study was performed on 64 female albino rats weighing 180–200 g obtained from National Research Center outbreed stock. Rats were housed in an air-conditioned atmosphere and kept on a standard diet and water ad libitum. Standard diet pellets (El-Nasr, Abu Zaabal, Egypt) contained not less than 20% protein, 5% fiber, 3.5% fat, 6.5% ash and a vitamin mixture. The experiments were conducted according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC).

**Drugs:**
Trimetazidine was obtained from Servier Egypt (Cairo, Egypt) and administered orally at two dose levels (5 and 10mg/kg). Vinpocetine was obtained from Gedeon-Richter (Budapest, Hungary) and administered orally in the same doses as trimetazidine. Isosorbide dinitrate was obtained from Eipico (Sharqia, Egypt) and was administered at two dose levels (3.6 and 7.2 mg/kg, respectively. Each dose of the investigated drug was given to different group of animals. The selected doses chosen for the drugs in the present study were based on human therapeutic doses after conversion to the corresponding rat dose using Paget’s table [10], except vinpocetine that was used according to previous experimental studies [11].

**Experimental design:**
Animals were classified into 8 groups, each consisting of eight rats. The first group received subcutaneous injection of corn daily for 4 weeks and served as normal group. The second group was injected subcutaneously with CsA (15mg/kg) after being dissolved in corn oil as a 1:10 dilution daily for 4 weeks. Groups from 3-8 received CsA similar to second group in addition to the drugs under investigation. Groups 3 and 4 were treated with trimetazidine at two dose levels (5 and 10mg/kg), respectively. Groups 5 and 6 were treated with...
vinpocetine at two dose levels (5 and 10mg/kg), respectively, while Groups 7 and 8 were treated with isosorbide dinitrate at doses of 3.6 and 7.2mg/kg, respectively. All drugs were administered orally, daily and concurrently with CsA for 4 weeks. At the end of the 4th week, urine samples were collected by placing the animals in the metabolic cages for 24 h with free access to tap water, without food, under the same temperature and light conditions. Urine samples were stored at -80°C for evaluation of creatinine clearance and protein level. Blood samples were also collected via the retro-orbital venous plexus of the rats and allowed to clot. Serum was separated by centrifugation at 1000 g for 10 min and used for the assessment of kidney function. Finally, rats were sacrificed and kidneys were removed, washed with ice-cold saline, and homogenized in saline solution.

Biochemical analysis:
Renal function tests were assessed by measuring BUN according to Tabacco et al [12]. Both serum creatinine level and creatinine clearance (C\text{cr}) were determined according to Fabiny and Erinhausen [13]. Proteinuria was estimated according to the method of Orsonneau et al [14]. Blood glucose level was determined according to the method provided by Teller et al [15]. Renal content of lipid peroxides was determined according to the method of Ohkawa et al [16]. Renal reduced glutathione content was assessed according to the method given by Ellman [17]. Renal nitric oxide (NO) was evaluated according to the method of Miranda et al [18]. As an index of renal fibrosis, hydroxyproline was estimated in the kidney using the method of Woessner et al [19].

Statistical analysis:
Data are presented as the mean ± SEM. For statistical analysis of data, multiple comparisons were performed using one-way analysis of variance (ANOVA) followed by the LSD test for post hoc analysis. Statistical significance was accepted at a level of P < 0.05. Data were analyzed using SPSS (version 17; SPSS, Chicago, IL, USA).

RESULTS
Nephrotoxicity indices
Injection of CsA (15mg/kg, s.c.) daily for 4 weeks caused a significant change in the renal function tests. The nephrotoxicity indices (BUN and serum creatinine) significantly increased (35.8 ± 2.00 and 0.9 ± 0.05, respectively) as compared to the normal control group (16.0 ± 0.60 and 0.7± 0.03, respectively), while C\text{cr} significantly decreased (2.4 ± 0.21) as compared to the normal control group (4.7 ± 0.37). However, CsA injection did not induce any significant change in the level of urinary protein or serum uric acid as compared to the normal control group (Table 1).

Oral treatment of animals with trimetazidine (5 and 10mg/kg) daily and concomitantly with CsA for 4 weeks significantly decreased C\text{cr} as compared to the CsA-treated control group. However, a higher dose of trimetazidine (10mg/kg) increased serum uric acid and urine protein levels as compared to the CsA-treated control group (Table 1). Similar to trimetazidine, treatment of animals with vinpocetine (5 and 10mg/kg) significantly decreased C\text{cr} and increased urine protein level as compared to the CsA-treated control group (Table 1).

In contrast, treatment of animals with isosorbide dinitrate (3.6mg/kg, p.o.) daily for 4 weeks concomitantly with CsA significantly decreased the serum creatinine level and increased creatinine clearance as compared to the CsA-treated control group. This apparent beneficial effect of isosorbide dinitrate disappeared after oral treatment with a higher dose of isosorbide dinitrate (7.2mg/kg) which induced a significant increase in BUN level, urinary protein level and serum uric acid level as compared to the CsA-treated control group. In addition, it significantly decreased C\text{cr} as compared to the CsA-treated control group (Table 1).

Blood glucose level
Subcutaneous injection of CsA (15mg/kg) daily for 4 weeks showed a significant increase in blood glucose level (115.8± 4.80) as compared to the normal control group (94.19 ± 6.20) (Table 1). Oral treatment of animals with vinpocetine (10mg/kg) daily and concomitantly for 4 weeks significantly reduced the increased blood glucose level induced by CsA. On the other hand, oral treatment with isosorbide dinitrate (3.6 and 7.2mg/kg) significantly increased blood glucose level as compared to CsA-treated control group (Table 1).

Oxidative stress biomarkers
Subcutaneous injection of CsA for 4 weeks did not show any significant change in the renal lipid or GSH contents as compared to the normal control group. Administration of isosorbide dinitrate (7.2mg/kg) significantly increased the content of lipid peroxides as compared to the CsA-treated control group. Oral administration of
TMZ: trimetazidine, vinp: vinpocetine, ISDN: isosorbide dinitrate.

Figure 1: Effect of treatment with trimetazidine, vinpocetine, or isosorbide dinitrate on renal NO content in rats with chronic CsA intoxication.

Trimetazidine (5 and 10mg/kg), vinpocetine (5 and 10mg/kg) or isosorbide dinitrate (3.6 and 7.2mg/kg) was orally administered with daily subcutaneous injection of CsA in corn oil (15mg/kg) for 4 weeks. The normal group received corn oil (equivalent volume), while the control group received the same dose of CsA alone. Each value represents the mean of 8 rats ± S.E. Statistical analysis was carried out using ANOVA followed by LSD test as a post ANOVA test.

* Significantly different from normal group, at p < 0.05.
@ Significantly different from CsA control group, at p < 0.05.
<table>
<thead>
<tr>
<th>Groups/Parameters</th>
<th>Blood urea nitrogen (mg/dl)</th>
<th>Serum Creatinine (mg/dl)</th>
<th>Creatinine clearance (ml/min)</th>
<th>Urinary Total protein (mg/L)</th>
<th>Uric acid (mg/dl)</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (corn oil)</td>
<td>16.0* ± 0.60</td>
<td>0.7* ± 0.03</td>
<td>4.7* ± 0.37</td>
<td>1.7 ± 0.07</td>
<td>0.64 ± 0.05</td>
<td>94.19* ± 6.20</td>
</tr>
<tr>
<td>CsA (15mg/kg)</td>
<td>35.8* ± 2.00</td>
<td>0.9* ± 0.05</td>
<td>2.4* ± 0.21</td>
<td>2.3 ± 0.04</td>
<td>0.69 ± 0.05</td>
<td>115.8* ± 4.80</td>
</tr>
<tr>
<td>CsA + TMZ (5mg/kg)</td>
<td>33.9* ± 1.90</td>
<td>1.0* ± 0.08</td>
<td>1.3**± 0.07</td>
<td>2.4 ± 0.22</td>
<td>0.77 ± 0.06</td>
<td>112.6* ± 3.30</td>
</tr>
<tr>
<td>CsA + TMZ (10mg/kg)</td>
<td>40.3* ± 1.90</td>
<td>0.9* ± 0.06</td>
<td>1.2**± 0.11</td>
<td>4.3**±0.36</td>
<td>0.88**±0.07</td>
<td>110.4 ± 7.60</td>
</tr>
<tr>
<td>CsA + Vinp (5mg/kg)</td>
<td>41.0* ± 2.90</td>
<td>0.7 ± 0.03</td>
<td>1.5**± 0.12</td>
<td>2.4 ± 0.11</td>
<td>0.63 ± 0.03</td>
<td>106.4 ± 7.40</td>
</tr>
<tr>
<td>CsA + Vinp (10mg/kg)</td>
<td>40.0* ± 2.10</td>
<td>0.9*± 0.05</td>
<td>1.1**± 0.06</td>
<td>4.3**±0.39</td>
<td>0.55 ± 0.04</td>
<td>88.8**± 6.10</td>
</tr>
<tr>
<td>CsA + ISDN(3.6mg/kg)</td>
<td>38.5* ± 1.70</td>
<td>0.7*±0.02</td>
<td>3.0**± 0.23</td>
<td>2.6* ± 0.25</td>
<td>0.64 ± 0.04</td>
<td>141.6**± 6.80</td>
</tr>
<tr>
<td>CsA + ISDN(7.2mg/kg)</td>
<td>56.0**±3.90</td>
<td>0.9* ± 0.03</td>
<td>1.1**±0.09</td>
<td>4.1**±0.18</td>
<td>0.92**±0.07</td>
<td>172.8**± 9.30</td>
</tr>
</tbody>
</table>

**Table 1:** Effect of trimetazidine, vinpocetine & isosorbide dinitrate on renal function tests and blood glucose in CsA intoxicated rats

Trimetazidine (5 and 10mg/kg), vinpocetine (5 and 10mg/kg) or isosorbide dinitrate (3.6 and 7.2mg/kg) was orally administered concomitant with daily subcutaneous injection of CsA in corn oil (15mg/kg) for 4 weeks. The normal group received corn oil (equivalent volume), while the control group received the same dose of CsA alone. Each value represents the mean of 8 rats ± S.E. Statistical analysis was carried out using ANOVA followed by LSD test as a post ANOVA test:

- *: Significantly different from normal group, at p < 0.05
- @: Significantly different from CsA control group, at p < 0.05

TMZ: trimetazidine, vinp: vinpocetine, ISDN: isosorbide dinitrate
vinpocetine (10mg/kg) and isosorbide dinitrate (3.6 and 7.2mg/kg) significantly increased the renal content of GSH as compared to the CsA-treated control group (Table 2).

**Renal NO content**
Injection of CsA (15mg/kg, s.c.) daily for 4 weeks produced a significant decrease in renal NO content (5.6* ± 0.22) as compared to the normal control value (6.9* ± 0.29). Administration of both trimetazidine at a dose of 10mg/kg and vinpocetine at doses of 5 and 10mg/kg further decreased NO content in kidney tissue as compared with CsA-treated control group. However, isosorbide dinitrate administration did not affect renal NO content as compared to CsA-treated control group (Figure 1).

**Renal hydroxyproline content**
CsA injection increased in the renal content of hydroxyproline, the sensitive marker of fibrosis (627 ± 49.70) as compared to the normal control group (189 ± 22.90). However, isosorbide dinitrate (3.6mg/kg) treatment significantly decreased the elevated hydroxyproline level induced by CsA (Table 2).

**DISCUSSION**
In the current study, the nephrotoxic effects of CsA were assessed by indices of kidney function. CsA (15mg/kg, s.c.) for 4 weeks significantly increased BUN and serum creatinine levels and decreased C_cr as compared to the normal control group. Our findings are similar to what have been previously reported by other authors [20-21]. One of the proposed mechanisms through which CsA affects renal hemodynamics is vasoconstriction of afferent arterioles and reduction in GFR. Long-term administration of CsA may also cause fibrosis, most likely independent of its vascular effect [22].

Our finding that CsA did not cause proteinuria is also consistent with other studies [23]. However, in contrast to previously reported studies, we did not find an increase in serum uric acid levels with CsA administration (Table 1). This disparity may be due to species difference, the CsA dose, or the route of administration [24-25].

In the present study, screening for the potential nephroprotective effects of trimetazidine, vinpocetine, or isosorbide dinitrate revealed that these drugs cause further reduction in renal function as compared with CsA treatment. Only administration of isosorbide dinitrate (3.6mg/kg) could counteract the elevation in serum creatinine level produced by CsA injection and improved C_cr. One possible explanation for these finding is that mechanisms other than vasoconstriction may have a major role in CsA-induced renal toxicity. We also cannot rule out the possibility of a nephrotoxic interaction between these drugs.

NO may play a role in CsA-induced nephrotoxicity. Exogenous supplementation with L-arginine effectively prevents CsA-induced renal dysfunction in rats, indicating a potential role of NO in CsA-induced renal dysfunction [9]. We have confirmed the findings from previous studies on experimental animals and cultured renal cells that subcutaneous injections of CsA for 4 weeks decrease the renal NO as compared to the normal group (Figure 1) [26-27]. Administration of both trimetazidine (10mg/kg) and vinpocetine (5 and 10mg/kg) significantly decreased NO content in kidney tissue as compared with CsA-treated control group (Figure 1). This was consistent with the deterioration in renal function elicited by both drugs (Table 1). Surprisingly, both doses of isosorbide dinitrate administration, a known NP donor, did not increase renal NO levels as compared to CsA-treated group (Figure 1). One explanation for this finding is that NO might have been exhausted in neutralizing reactive oxygen species produced by combined administration of isosorbide dinitrate and CsA. Some studies in parallel showed that nitrate therapy may enhance the deleterious effects, probably through the generation of reactive oxygen species and promotion of vasoconstrictor effect of CsA [28-29].

It has been postulated that oxidative stress plays an important role in CsA-induced nephrotoxicity through increased ROS production and CsA has been shown to increase lipoperoxidation in the rat kidney and deplete hepatic and renal pool of glutathione [30]. However, we show here that CsA injection for 4 weeks did not induce any significant change in the renal content of lipid peroxides or glutathione as compared to the normal group (Table 2). Some studies [31] have suggested that the increase in oxidative stress level takes place early during CsA-mediated nephrotoxicity (at 6 days) and by 4 weeks, the oxidative stress returns to normal state. The antioxidant potential of vinpocetine (10mg/kg) and isosorbide dinitrate (3.6 and 7.2mg/kg) is confirmed by our finding that these two drugs significantly increased the renal content of GSH as compared to the CsA-treated group [7]. On the other hand, we have also found that isosorbide dinitrate (7.2mg/kg) administration significantly increased the level of lipid peroxides as
Table 2: Effect of trimetazidine, vinpocetine or isosorbide dinitrate on renal lipid peroxide, and GSH and hydroxyproline contents in rats with chronic CsA intoxication.

Trimetazidine (5 and 10mg/kg), vinpocetine (5 and 10mg/kg) or isosorbide dinitrate (3.6 and 7.2mg/kg) was orally administered concomitant with daily subcutaneous injection of CsA in corn oil (15mg/kg) for 4 weeks. The normal group received corn oil (equivalent volume), while the control group received the same dose of CsA alone. Each value represents the mean of 8 rats ± S.E. Statistical analysis was carried out using ANOVA followed by LSD test as a post ANOVA test:

*: Significantly different from normal group, at p < 0.05.
@: Significantly different from CsA control group, at p < 0.05.

Similar observations have been reported previously for the induction of fibrosis with CsA injection [32]. Only isosorbide dinitrate treatment (3.6mg/kg) significantly decreased the elevated hydroxyproline content induced by CsA. It was demonstrated that NO-generating compounds,
such as isosorbide dinitrate, showed a marked reduction of fibrosis in cardiac tissues, and this is consistent with the present results [33].

CONCLUSION
In conclusion, we show that isosorbide dinitrate at the dose of 3.6mg/kg may be protective against CsA-induced nephrotoxicity. However, both trimetazidine and vinpocetine are of unclear utility. The present study was constructed and intended to study renal outcomes after 4 weeks. Hence, further studies are recommended to test the potential nephroprotective effect of the aforementioned drugs when administered concomitantly with CsA for shorter and longer periods.

REFERENCES