The Possible Protective Role of Candesartan on Cyclosporine Induced Nephrotoxicity in Rats

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ABSTRACT

Cyclosporine A (CsA), a fungal undecapeptide, is the most common immunosuppressive drug used in organ transplantation and autoimmune diseases. However, nephrotoxicity is the major adverse effect of CsA use. The molecular mechanisms of CsA nephrotoxicity are not well characterized, but more recent studies suggest an involvement of angiotensin II (ANG II) and reactive oxygen species in the development of cyclosporine nephrotoxicity. This study was thus designed to investigate the role of angiotensin II type I (AT1) receptor antagonist, candesartan, on CsA-induced nephrotoxicity. Three groups of male adult albino rats were employed in this study; group 1, served as control, group 2, rats were treated with CsA (20mg/kg/day subcutaneously) for 21 days, and group 3, received CsA (20mg/kg/day subcutaneously) along with candesartan (1mg/kg/day perorally) 24 hours before and 21 days concurrently. Renal blood flow (RBF) was estimated by flowometer. Estimation of plasma renin activity, serum creatinine, blood urea and tissue malondialdehyde content was done by using colorimetric methods. Renal tissue specimens were histopathologically examined by hematoxylin & eosin staining. CsA administration for 21 days resulted in a marked renal impairment and significantly decreases RBF, deranged the renal functions as well as renal morphology. All these factors were significantly improved by candesartan administration. These results clearly demonstrate the pivotal role of AT1 receptor antagonist (candesartan) in CsA- induced nephrotoxicity.

Keywords: Cyclosporine – Nephrotoxicity – Candesartan – Angiotensin - Renin.

INTRODUCTION

Due to its potent immunosuppressive effects, cyclosporine A (CsA) has improved allograft survival in organ transplantation and has been applied increasingly with considerable clinical benefit in the treatment of autoimmune diseases. Unlike most other immunosuppressive agents, CsA has no depressant effects on the bone marrow. However, the therapeutic benefits of CsA have been frequently limited by the occurrence of acute and chronic nephrotoxicity, which is the most serious common side effect. CsA nephrotoxicity therefore remains an important clinical challenge (1).

It has been suggested that activation of the renin-angiotensin system is involved in the pathogenesis of CsA-induced nephrotoxicity. Angiotensin receptor blocker (ARB) as irbisartan is effective in preventing CsA-induced vascular and renal adverse effects (2).
Candesartan cilexetil; an inactive ARB prodrug, which hydrolyzed to active form during absorption from GIT, due to its tight binding to angiotensin II type I (AT1) receptor, it has longer activity than other members of ARBs (3).

This study was thus designed to investigate the role of AT1 receptor antagonist, candesartan, on CsA-induced nephrotoxicity.

MATERIAL AND METHODS

I- Animals:

Twenty four male adult albino rats of locally breaded strain weighing between 150-250 g, at the beginning of the study, were used. They have been acclimatized for one week in groups (8/cage) in a fully ventilated room at ordinary room temperature in Pharmacology Department, Benha Faculty of Medicine. Rats were allowed to ad libitum access to water and balanced diet. All experimental protocols were approved by the Ethical Committee of Faculty of Medicine, Benha University during September 2012.

II- Experimental design (Study groups):

At the beginning of the experiment, rats were randomly divided into 3 groups; each of them consisted of 8 rats:

- **Group 1**: control normal rat.
- **Group 2**: (cyclosporine nephrotoxic group): rats of this group were received cyclosporine in a dose of 20 mg/kg daily subcutaneously (SC), for 21 days (4).
- **Group 3**: (candesartan treated group): rats of this group were given the same dose of cyclosporine for 21 days along with candesartan in a dose of 1mg/kg daily orally, 24 hours before and 21 days concurrently with cyclosporine treatment (4).
  - All drugs and chemicals were freshly prepared before each experiment.

III- All groups were subjected to the following investigations:

1. Renal blood flow by Doppler flow meter (5).
2. Estimation of plasma renin activity (6).
3. Estimation of blood urea (7).
4. Estimation of serum creatinine (8).
5. Estimation of tissue malondialdehyde (MDA) content.\(^{(9)}\).

6. Histopathological studies \(^{(4)}\).

At the end of the experiment, rats were anaesthetized with urethane (0.6 ml/100 gm of 25% freshly prepared solution), of about, the abdominal cavity was opened, blood samples were collected from abdominal aorta and processed for biochemical measurements. The kidneys were exposed, removed and divided into 2 parts. One part was placed in 10% formalin for histopathological examination. The second part was kept at 70°C and used for biochemical measurement of MAD.

IV- Statistical analysis:

All data were expressed as mean & standard deviation (mean ± SD); data were evaluated by the one way analysis of variance. Difference between groups were compared by Student's t-test with \( p < 0.05 \) selected as the level of statistical significance \(^{(10)}\).

RESULTS

Effect of candesartan on cyclosporine induced nephrotoxicity in rats:

a) Renal blood flow (RBF):

There was a significant (\( p < 0.05 \)) reduction of RBF from a mean of \( 9.4 ± 1.13 \) cm/s in control normal rats to a mean of \( 4.14±0.11 \) cm/s in rats received cyclosporine only, as shown in table (1) and Fig. (1, 6 & 7).

Meanwhile RBF was significantly (\( p < 0.05 \)) increased from a mean of \( 4.14±0.11 \)cm/s in cyclosporine treated rats to a mean of \( 8.02 ± 0.6 \)cm/s in candesartan treated group, as illustrated in table (1) and Fig. (1, 7 & 8).

b) Plasma renin activity:

The mean value of plasma renin activity in control normal rats was \( 1.50 ±0.06 \) ng/ml/h. In rats received cyclosporine only; this value was significantly (\( p < 0.05 \)) elevated to reach \( 4.37 ±0.08 \) ng/ml/h. The mean value of plasma renin activity in candesartan treated group was insignificantly (\( p > 0.05 \)) increased to \( 4.58 ± 0.09 \) ng/ml/h, in comparison to the corresponding value of cyclosporine treated rats, as shown in table (1) and Fig. (2).
c) **Blood urea level:**

The mean value of blood urea level in control normal rats was 20.4±2.1 mg/dl. In rats received cyclosporine only; this value was significantly (p < 0.05) elevated to reach 49.8 ± 4.2 mg/dl. The mean value of blood urea level in candesartan treated group was significantly (p < 0.05) decrease to 31.8±2.2 mg/dl, in comparison to the corresponding value of cyclosporine treated rats, as illustrated in table (1) and Fig. (3).

d) **Serum creatinine level:**

The mean value of serum creatinine level in control normal rats was 0.50±0.06 mg/dl. In rats received cyclosporine only; this value was significantly elevated (p < 0.05) to reach 1.38 ± 0.08 mg/dl. The mean value of serum creatinine level in candesartan treated group was significantly decreased (p < 0.05) to 0.58 ± 0.06 mg/dl in comparison to the corresponding value of cyclosporine treated rats, as shown in table (1) and Fig. (4).

e) **Tissue malondialdehyde (MDA) level:**

The mean value of MDA level in control normal rats was 7.50±0.06 µM/g. In rats received cyclosporine only; this value was significantly (p < 0.05) elevated to reach 16.38 ± 1.8 µM/g. The mean value of MDA level in candesartan treated group was significantly (p < 0.05) decreased to 10.58 ±1.06 µM/g, in comparison to the corresponding value of cyclosporine treated rats, as shown in table (1) and Fig. (5).

f) **Histopathological examination:**

Renal sections of control group showed normal glomeruli & tubules and absence of any calcification, as illustrated in Fig. (9).

In rats received cyclosporine only; it was noticed that cyclosporine induced tubular degeneration and vaculation, hyalinosis of basement membrane of glomeruli and renal tubules. Also, there was patchy dystrophic calcification at the cortico-medullary junction and in the dilated tubules indicating nephrocalcinosis, as illustrated in Fig. (10 & 11).

In the candesartan treated rats, the hyaline degeneration of tubular and glomerular basement membrane was markedly decreased, tubular atrophy and vaculation was much
less evident. No calcification was found in the renal interstitium or in the tubules, as illustrated in Fig. (12 & 13).

Table (1): Mean ± S.E. of RBF cm/s, plasma renin activity ng/ml/h., blood urea level, serum creatinine level in mg/dl and tissue MDA level µM/g, in control group, cyclosporine nephrotoxic group and candesartan treated group (No. of each= 8).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group 1 (control group)</th>
<th>Group 2 (cyclosporine nephrotoxic group)</th>
<th>Group 3 (candesartan treated group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBF cm/s</td>
<td>9.4±0.08</td>
<td>4.14±0.11*</td>
<td>8.02±0.6**</td>
</tr>
<tr>
<td>Plasma renin activity ng/ml/h.</td>
<td>1.5±0.06</td>
<td>4.37 ± 0.08*</td>
<td>4.58±0.09#</td>
</tr>
<tr>
<td>Blood urea level mg/dl</td>
<td>20.4±2.1</td>
<td>49.8±4.2*</td>
<td>31.8±2.2**</td>
</tr>
<tr>
<td>Serum creatinine mg/dl</td>
<td>0.5± 0.06</td>
<td>1.38 ±0.08*</td>
<td>0.58 ± 0.06**</td>
</tr>
<tr>
<td>Tissue MDA level µM/g.</td>
<td>7.5±0.6</td>
<td>16.38±1.2*</td>
<td>10.58±1.06**</td>
</tr>
</tbody>
</table>

*: Significant compared to control group, **: Significant compared to cyclosporine nephrotoxic group, #: Insignificant compared to cyclosporine nephrotoxic group, ± S.E.: standard deviation, RBF: renal blood flow, MDA: malondialdehyde.

Figure (1): A histogram showing the effect of candesartan treatment (group 3) on RBF cm/s, compared to cyclosporine nephrotoxic group (2) & control group (1), [*: significant compared to control group, **: significant compared to cyclosporine nephrotoxic group, RBF: renal blood flow].
Figure (2): A histogram showing the effect of candesartan treatment (group 3) on the plasma rennin activity ng/ml/h, compared to cyclosporine nephrotoxic group (2) & control group (1), [*: significant compared to control group, #: insignificant compared to cyclosporine nephrotoxic group].

Figure (3): A histogram showing the effect of candesartan treatment (group 3) on the blood urea level mg/dl, compared to cyclosporine nephrotoxic group (2) & control group (1), [*: significant compared to control group, **: significant compared to cyclosporine nephrotoxic group].

Figure (4): A histogram showing the effect of candesartan treatment (group 3) on the serum creatinine mg/dl, compared to cyclosporine nephrotoxic group (2) & control group.
(1), [*: significant compared to control group, **: significant compared to cyclosporine nephrotoxic group].

*Figure (5): A histogram showing the effect of candesartan treatment (group 3) on the tissue MDA level µM/g, compared to cyclosporine nephrotoxic group (2) & control group (1), [*: significant compared to control group, **: significant compared to cyclosporine nephrotoxic group, MDA: malondialdehyde].

*Figure (6): A trace showing renal blood flow (RBF) in control normal rats (group 1).

*Figure (7): A trace showing the renal blood flow (RBF) in cyclosporine nephrotoxic rats (group 2).
Figure (8): A trace showing renal blood flow (RBF) in candesartan treated rats (group 3).

Figure (9): A photomicrograph of cut section of rat’s kidney of control group (1) showing normal glomeruli and tubules (H & E x100)

Figure (10)

Figure (11)

Figures (10 & 11): Photomicrographs of cut sections of rat’s kidney of cyclosporine nephrotoxic group (2) showing cyclosporine induced changes in the form of tubular degeneration (a), vaculation and hyalinosis of the glomerular basement membrane (b) (H& E x40).
DISCUSSION

Cyclosporine A (CsA) is established as the immunosuppressant of choice in human organ transplantation, because it improves graft survival and is not associated with myelosuppression. Unfortunately, CsA can lead to a wide spectrum of nephrotoxic complications. Both functional and structural changes in the kidney of transplant patients and experimental animals have been reported (2).

The data of the present work revealed that daily administration of CsA 20mg/kg/day S.C. for 21 days produced significant reduction of renal blood flow, significant elevation of blood urea and serum creatinine levels. Histopathological examination of renal sections demonstrated that CsA produced tubular atrophy of the proximal convoluted tubules, interstitial fibrosis, hyalinosis of vascular basement membrane and nephrocalcinosis at the corticomedullary junction and calcified concretions in the tubules.

These results are in agreement with (11, 12); they reported that, CsA reduced glomerular filtration rate and renal blood flow by causing vasoconstriction of the glomerular afferent arterioles.
(13) Reported that cyclosporine decreases renal blood flow by constricting the afferent arteriole proximal to the glomerulus. Also, (1) reported that CsA produced renal vasoconstriction and systemic hypertension. Meanwhile (14) reported that CsA causes a dose related decrease in renal functions in experimental animals. It produced a significant increase in serum creatinine and blood urea and a significant decrease in creatinine clearance.

(1) Reported that in chronic CsA-induced nephrotoxicity, the main histological findings in the kidney are the vascular lesions in the endothelium and smooth muscle cells, severe atrophy, vacuolization and thickening of the basal membrane can be also found.

Moreover, (15) observed that chronic CsA administration leads to loss of proximal tubular epithelial cell integrity and tubular atrophy, a variable interstitial injury with secondary fibrosis.

(16) Reported that croticomedullary calcification was seen with CsA. Calcified laminated concretions in tubules and microcalcification of single tubular cells were also seen with CsA.

(17) Reported that chronic cyclosporine nephrotoxicity is characterized by interstitial fibrosis and afferent arteriolar hyalinosis.

In the present work, it was observed that daily oral administration of candesartan 24 hour before and 21 days along with CsA significantly increased renal blood flow, prevented CsA induced elevation in blood urea and serum creatinine. Histopahtological examination of renal sections of candesartan treated group revealed marked improvement; as no calcification was detected at all, tubular degeneration and hyalinosis of the blood vessels were much less evident.

These results are in agreement with (4) who reported that candesartan inhibited the increase in blood urea and serum creatinine induced by CsA.

(18) Concluded that candesartan cilexetil can effectively control hypertension and proteinuria without deterioration in renal allograft function. These data suggest that
treatment with candesartan cilexetil may be useful for maintaining long-term renal allograft function.

(19) Reported that upregulation of vascular endothelial growth factor seem to be related to upregulation of angiotensin II (ANG II) in cyclosporine nephrotoxicity.

When renal blood flow is substantially reduced, angiotensin II contributes to the maintenance of glomerular filtration rate by constricting the efferent glomerular arterioles. In such condition, dilation of the efferent arterioles by inhibitors of the renin-angiotensin system may potentiate the reduction in glomerular filtration rate. Captopril and saralasin have previously been shown to increase glomerular filtration rate and renal blood flow in CsA treated normotensive rats (20).

Enalapril was also able to prevent the CsA-induced decline in glomerular filtration rate in diabetic patients (21).

The exact mechanism of CsA-induced nephrotoxicity is not known, but there are several lines of evidence suggesting an involvement of the renin-angiotensin system (4). This is also supported by our study through the finding that plasma renin activity was higher in the CsA-treated rats than in the control group. CsA has been reported to stimulate renin production and release in isolated juxtaglomerular cells (22). An increase in plasma renin activity has been demonstrated in CsA-treated rats either on sodium depletion (3), on standard chow (23), or during high intake of sodium (24, 25).

CsA has also been reported to elevate plasma angiotensin II levels (26). Furthermore, long-term treatment with CsA upregulates AT1 receptors in vascular and renal tissue (27) and increases vasoconstrictive effect of angiotensin II (28, 29).

Chronic administration of angiotensin II in rats produces renal injury similar to that observed in CsA nephropathy (30, 31). Both angiotensin II and CsA cause an overexpression of transforming growth factor (TGF-β1), a cytokine which has been implicated in the pathophysiology of many fibrotic diseases of the kidney and other organs (31, 32). In addition, tubulointerstitial expression of osteopontin, an adhesion molecule associated with renal fibrosis, is increased by both angiotensin II (30) and CsA (32). Thus, activation of the renin-angiotensin system by CsA may be at least partly responsible for the interstitial fibrosis.
fibrosis through stimulation of TGF-β1 and osteopontin expression. This is also supported
by the finding that the AT1 receptor antagonist losartan reduced the CsA-induced interstitial fibrosis concomitantly with decreased renal TGF-β1 and beta1 inducible gene-
h3 (betaig-h3) expression (15).

(19) Reported that the increased vascular endothelial growth factor (VEGF) expression in chronic CsA nephrotoxicity seems to be related to upregulation of ANG II. In addition, VEGF probably exerted its effect via the kinase insert domain/fetal liver kinase1 (KDR/FLK-1) receptor; the actions of VEGF in this model remain speculative, but may be related to its effect on macrophage infiltration or matrix deposition.

The role of reactive oxygen species formation in the CsA-induced impairment of renal function has been suggested by (33). (16) Observed that oxygen free radicals are one of different mediators for CsA-induced nephrotoxicity. There was increased production of the lipid peroxidation product malondialdhyde (MDA) with CsA indicating the oxidative stress associated with CsA nephrotoxicity. (34) Reported that melatonin, through its antioxidant properties, significantly antagonized CsA-induced renal impairment.

(35) Showed that curcumin attenuates cyclosporine induced renal dysfunction and oxidative stress in rat kidneys through its antioxidant properties.

(2) Observed that ANG II induces oxidative stress in vitro and in vivo, and demonstrated the pivotal role of AT1 receptor blocker (irbisartan) in amelioration of cyclosporine nephrotoxicity through its antioxidant properties.

Thus, it is suggested that candesartan produces nephroprotective effect in the rat model of CsA-induced nephrotoxicity. This nephroprotection of candesartan is based on a combination of angiotensin receptor antagonism, improvement of endothelial function and its potent antioxidant properties.

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الملخص العربي

الدور الوقائي المحتمل لعقار الكانديسارتان على التسمم الكلوي المستحدث بعقار السيكلوسبورين في الجرزان المشتركون في البحث

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السيكلوسبورين، ذلك المستخلص الفطري، هو الدواء الأكثر شيوعاً لتثبيت المناعة، والمستخدم كعلاج ما بعد زراعة الأعضاء، وفي علاج أمراض المناعة الذاتية. ومع ذلك فإن التسمم الكلوي له تأثير سلبي كبير على استخدام السيكلوسبورين. وعلى الرغم من أن الآليات الجزيئية للتسمم الكلوي للسيكلوسبورين ليست واضحة تماماً، لكن تشير الدراسات الحديثة إلى إشراك الأنجيوتنسين وأنواع الأكسجين التفاعلي في حدوث التسمم الكلوي للسيكلوسبورين. وقد صممت هذه الدراسة لتعرف على دور أحد مغلقات مستقبلات الأنجيوتنسين (الكانتداسرتان) على التسمم الكلوي الذي يسببه السيكلوسبورين. تم استخدام ثلاث مجموعات من الجرزان البيضاء الذكور في هذه الدراسة: المجموعة (1) بمثابة مجموعة المراقبة العادية، المجموعة (2) تم حقنها بالسيكلوسبورين (20 مجم/كمج / يوماً تحت الجلد) لمدة 21 يوماً، المجموعة (3) تم تلقيت الكانتداسرتان (1 مجم/كمج/يومياً بالفم) 24 ساعة قبل حقن السيكلوسبورين، و لمدة 21 يوماً بالتزامن مع حقن السيكلوسبورين. وقد تم قياس تدفق الدم الكلوي باستخدام مقياس الجريان و كذلك قياس مستوى الرينين، الكرياتينين والبولياورا في الدم، و قياس محتوى الأنسجة الكلوية من مادة المالونالدهيد باستعمال الطرق اللونية. وقد تم فحص عينات الأنسجة الكلوية باستخدام صبغتي الهيماتوكسيل و الأيزوسين. وقد أظهرت نتائج الدراسة ما يلي: حقن الجرزان بالسيكلوسبورين لمدة 21 يوماً أسفر عن حدوث قصور كلوي ملحوظ حيث قل تدفق الدم الكلوي، و أختل وظائف الكلي و أختل تركيب النسيج الكلوي. وقد تحسنت كل هذه العوامل بشكل كبير وملحوظ مع استخدام الكانتداسرتان. وقد خلصت الدراسة إلى أن الدور المحوري الواقي لمغلق مستقبلات الأنجيوتنسين (الكانتداسرتان) على التسمم الكلوي المستحدث بالسيكلوسبورين.