Protective Effect of Aminoguanidine Against Cyclosporine Induced Nephrotoxicity and Hepatotoxicity in Rats: Histological and Biochemical Study

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Abstract

Cyclosporine A (CsA) is a potent and effective immunosuppressive agent used to prevent rejection in organ transplant surgery and autoimmune diseases. Its use is frequently accompanied by nephrotoxicity and hepatotoxicity. The present study was designed to investigate the possible protective effect of aminoguanidine on cyclosporine induced nephrotoxicity and hepatotoxicity in male albino rats. A total of sixty healthy adult male albino rats were used divided into four equal groups in this study. Group I rats served as control treated with distilled water orally only, group II was treated with CsA in a dose of 25mg/kg orally daily for 21 days, group III treated with CsA concurrently with aminoguanidine in doses of 25 and 20mg/kg orally daily for 21 days respectively and group IV was treated with olive oil orally (vehicle for CsA). Rats were sacrificed 24 hs after last dose, blood, kidney and liver samples were taken. Histopathological examination by light and electron microscopic, immunohistochemistry for active caspase-3 were done. Besides, biochemical measurement; serum nitric oxide level and kidney and liver function tests were done. CsA oral administration for 21 days significantly increased serum nitric oxide level impaired the renal and hepatic function tests and markedly distorted the renal and hepatic morphology in light and electron microscopic examination. Aminoguanidine administration improved serum nitric oxide level, kidney and liver function tests and preserved renal and hepatic morphological structures.

In Conclusion: Aminoguanidine has a protective effect against cyclosporine induced nephrotoxicity and hepatotoxicity.

Key Words: Aminoguanidine – Cyclosporine – Nephrotoxicity – Hepatotoxicity – Histological – Biochemical.

Introduction

Cyclosporine (CsA) is a potent immunosuppressive agent with inducible efficacy in the prevention of organ allograft rejection and autoimmune disease. However, the therapeutic treatment induces several side effects such as nephrotoxicity, cardiotoxicity, hypertension and hepatotoxicity [1,2]. Nephrotoxicity is a serious complication of the therapy. CsA nephrotoxicity is characterized by intense renal vasoconstriction that often progresses to chronic injury with irreversible structural renal damage [3]. Renal vasoconstriction is attributed to increase release of vasoconstrictive factors such as endothelin [4], thromboxane, angiotensin II and a decrease in vasodilating factors such as prostacyclin Perico et al., [5] and nitric oxide (NO) [6].

Renal dysfunction can occur at any time and ranges from an early reversible damage to a late progression to irreversible chronic renal failure. Acute nephrotoxicity may appear soon after transplantation or after weeks or months, with oliguria, acute decrease of glomerular filtration rate and renal plasma flow were also found. The acute toxicity is usually believed to be consequent to renal vasoconstriction [7]. Conversely, after prolonged CsA administration, chronic nephrotoxicity is characterized by a progressive and mostly irreversible impairment of renal function and it is supported by histological lesions ranging from striped fibrosis to ischemic collapse of the tuft, glomerular sclerosis and tubular atrophy; however, the increase in interstitial matrix preceding the interstitial fibrosis might be due to a direct toxic effect of CsA [8].

CsA-induced hepatotoxicity was characterized by morphological alterations in tissue architecture, changes in reduced glutathione (GSH) and malonaldehyde (MDA) levels and increase in stress protein expression. The imbalance between production of free oxygen radicals and antioxidant defense systems, due to CsA administration, is a mechanism responsible for oxidative stress [2].
Nitric oxide has been implicated as a mediator, messenger or regulator of cell function in physiologic situations as platelet function, vascular tone, memory, hepatocyte function and apoptosis [4].

NO is produced from L-arginine by the action of nitric oxide synthase (NOS) which exists in three forms (isoforms). Neuronal NOS (nNOS) which is markedly expressed in brain; inducible NOS (iNOS) is expressed in macrophages and endothelial NOS (eNOS) is expressed in endothelial cells [9]. All three NOS isoforms are present in the kidney. In renal cortex, nNOS exhibits a macula densa cell specific expression, iNOS has been observed in mesangial and proximal tubule cells of the afferent and efferent arterioles and glomerular capillaries [10].

Animal disease models suggest that while high local concentrations of NO may play a key role in the initiation and progression of renal disease, low levels may also be essential for normal renal function and cell protection, possibly explaining the variable reports of both beneficial and detrimental responses of renal disease models following NO inhibition.

Because of the abundance of physiologic and pathophysiologic roles in which NO participates, the manipulation of NO offers therapeutic potential in a variety of disease states. Pharmacologic agents that inhibit NOS can decrease the generation of NO. Selective inhibitors of iNOS commonly used experimentally as aminoguanidine [11].

CsA induced nephrotoxicity through increase production of reactive oxygen species Rezzani et al. [12] and apoptosis of renal cells Kim et al. [13].

Caspase-3 is an important player in the final induction of apoptosis and a close relationship exists between Bcl-2 expression and caspase activity. The over expression of Bcl-2 inhibits the activation of Caspase-3 [14].

Aminoguanidine, structurally contains a guanidine moiety and a hydrazine group Jianmongkol et al., 2000. It acts by inhibition of iNOS. It has antioxidant properties and decrease lipid peroxides [15].

Aim of the work:

The present study was performed to investigate the possible role and mechanism of aminoguanidine in protection against cyclosporine induced nephrotoxicity, hepatotoxicity at histological and biochemical levels. Also the role of caspase-3 in the mechanism of CsA toxicity.

Material and Methods

1- Chemicals:

CsA (sandimmun) was purchased from Sigma Aldrich (USA) was dissolved in olive oil in a dose of 25mg/kg orally daily. The CsA dose has been chosen according to Josephine et al., [16]. Aminoguanidine was purchased from Sigma Aldrich (USA). Aminoguanidine was administered orally in a dose of 20mg/kg according to Abdel-Zaher et al., [17]. All other chemicals were of analytical grades.

2- Animals and treatments:

Sixty adult healthy Wister albino rats, with an average weight of 230-260gm were used in this study. They were kept under routine healthy laboratory conditions and were fed normal Purina chow and tab water ad libitium. They were divided into 4 equal groups.

Group I rats served as control administrated distilled water orally for 21 days. Group II was administrated orally CsA (25mg/kg oral for 21 days). Groups III treated with CsA along with aminoguanidine (25 and 20mg/kg orally for 21 days respectively. Group IV administrated orally olive oil (vehicle for CsA) for 21 days.

One day after the last injection all rats were sacrificed by decapitation. Blood samples were collected from orbital sinus of rats. Sera were obtained by centrifugation for measurements of nitric oxide, kidney and liver function tests. The kidneys and liver were obtained and prepared for histopathological examination.

3- Biochemical measurements:

I- Measurements of nitric oxide (NO) level:

NO was measured in the serum by assaying nitrate, one of the stable and non volatile end-products of NO oxidation. Serum nitrite concentration was measured using Griess reagents as described by Green and his coworker [18]. The absorbance measure at 550 nm using UV-Visible spectrophotometer and calculate the total serum NO level in samples from the standard curve and expressed as gml/ml.

II- Assessment of liver function tests:

Measurement of liver function tests by spectrophotometer using colorometric diagnostic kits (Diagnostics, Giza, Egypt).

• Serum alkaline phosphatase (ALP) expressed as IU/l was measured according to Belfield and Goldberg [19].
Glutamic oxalacetic transaminase (GOT or AST) was measured according to Reitman and Frankel [20].

Glutamic pyruvic transaminase (GPT or ALT) was measured according to Reitman and Frankel [20].

III- Assessment of renal function tests:

Measurement of renal function tests creatinine and uric acid using standard diagnostic kits by spectrophotometer using colorometric diagnostic kits (Biodiagnostic, Giza, Egypt).

• Serum urea was measured according to Coulombe and Favreau, [21].
• Serum creatinine was measured according to Larsen (1972) [22].
• Serum uric acid was measured according to Whitehead et al. [23].

4- Histopathological examination:

After scarification of animals, samples of kidneys and liver was isolated immediately washed with ice-cold saline were fixed in 10% formaline, dehydrated, cleared, embedded in paraffin and were sectioned at 5-7um. Deparaffinized sections were stained with haematoxylin and eosin (H.&E) stain for light microscope examination.

Immunolabeling labeling staining:

Kidney and liver tissue were fixed in phosphate buffered 10% formaldehyde, PH 7.2m embedded in paraffin wax and cut to at thickness of 5Mm. Deparaffinized tissue section were incubated with active anti-rabbit caspase-3 polyclonal antibodies at a concentration of 1:100. Immunolabelling of specimen was carried out by a modified avidin-biotion- peroxidase complex technique vectastain ABC kit (Universal Elite, Vector Laboratories) and the specimens were handled as described previously [24].

Electron microscope:

Other small samples of kidneys and liver were fixed in glutaldehyde and processed for electron microscope examination. Semithin sections were cut at 1/2-1 micron and stained with toluidine blue. Selected sectioned were contrasted and electron micrographs were taken with Jeol transmission electron microscope (TEM).

Statistical analysis:

The data were run on IBM personal computer and analyzed using SPSS computer program (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) Version 11. The data were presented in the form of mean ± standard error (SE). Student’s t-test was used. The difference was insignificant at $p>0.05$, significant at $p<0.05$ and highly significant at $p<0.01$.

Results

Histological results:

I- Kidney:

A- Light microscope results:

Group I: Control group: H&E stained sections; the renal cortex is characterized by the presence of the renal tubules together with the renal capsules. The renal capsule is formed of glomerular tuft of blood capillaries surrounded by the Bowman’s capsule. Each capsule consists of a parietal layer of simple squamous epithelium and a visceral layer. The two layers are separated by a narrow urinary space. The proximal convoluted tubules have dense acidophilic cytoplasm and round vesicular nuclei with apical brush border. The bound lies between the adjacent cells are indistinct. The distal tubules have pale cytoplasm and rounded vesicular nuclei. Their lumina are wider than those of the proximal tubules (Fig. 1). Semithin sections revealed large vesicular nuclei and eosin (H.&E) stain for light microscope examination.

Immunolabeling labeling staining:

Kidney and liver tissue were fixed in phosphate buffered 10% formaldehyde, PH 7.2m embedded in paraffin wax and cut to at thickness of 5Mm. Deparaffinized tissue section were incubated with active anti-rabbit caspase-3 polyclonal antibodies at a concentration of 1:100. Immunolabelling of specimen was carried out by a modified avidin-biotion-peroxidase complex technique vectastain ABC kit (Universal Elite, Vector Laboratories) and the specimens were handled as described previously [24].

Group II: H & E sections showed that the glomerular capillaries of some renal corpuscles of animals had dilatation and thin wall lining, most of the changes was seen in the P.C.T. some of them have less acidophilic cytoplasm and dark nuclei while others, their cytoplasm was pale or vacuolat-ed. The lumen of some P.C.T. have hyaline material. The proximal convoluted tubules have pale cytoplasm and rounded vesicular nuclei. Their lumina are wider than those of the proximal tubules (Fig. 1). Semithin sections revealed large vesicular nuclei and positively stained clumps mostly longitudinal and basically located. The apical cytoplasm stained lightly. The brush border stained faintly blue at the apical part of the cells (Fig. 4). Immunohistochemical stained sections showed negative immunoperoxidase reaction to anticaspase-3 antibodies (Fig. 7).

Electron microscope:

Other small samples of kidneys and liver were fixed in glutaldehyde and processed for electron microscope examination. Semithin sections were cut at 1/2-1 micron and stained with toluidine blue. Selected sectioned were contrasted and electron micrographs were taken with Jeol transmission electron microscope (TEM).

Statistical analysis:

The data were run on IBM personal computer and analyzed using SPSS computer program (Statistical Package for Social Sciences, SPSS Inc., Chicago, IL, USA) Version 11. The data were presented in the form of mean ± standard error (SE). Student’s t-test was used. The difference was insignificant at $p>0.05$, significant at $p<0.05$ and highly significant at $p<0.01$. 
photo micrograph showing strong immulabelling of active-caspase-3 in P.C.T.

**Group III:** Combined treatment with amino guanidine and cyclosporine showed obvious improvement in the histological structure of the kidney compared to group II. The renal corpuscles appeared nearly similar to the control. The P.C.T. exhibits highly acidophilic cytoplasm and vesicular nuclei (Fig. 3). Semithin section showed that P.C.T. cells were more or less similar to (group I) except it stained deeply blue (Fig. 6). Immunohistochemically stained sections had negative immunoreaction against anti-caspase-3 antibodies like control group (Fig. 9).

**B- Electron microscope results:**

**Group I:** Ultrastructurally the cells of the proximal convoluted tubules show numerous microvilli. The cytoplasm contains numerous basal elongated mitochondria and basal infolding. Few cisternal profiles of rough endoplasmic reticulum and ribosomes were detected in the cytoplasm. The nucleus is rounded euchromatic large and centrally located (Fig. 10).

**Group II:** The proximal convoluted tubules were obviously affected. The nuclei are large with fine dispersed chromatin. The cytoplasm exhibits many vacuoles. Mitochondria were markedly affected. They were few with dense matrix and scattered in the cytoplasm. The tubular basement membrane displayed obvious demolishing of their basal infoldings. The apical brush border exhibited great loss of their microvilli (Fig. 11).

**Group III:** Combined treatment with cyclosporine and aminoguanidine. The cell of the proximal convoluted tubule exhibited numerous mitochondria scattered allover the cytoplasm and a well developed microvilli but still basal infolding are not well developed (Fig. 12).

**II- Liver:**

**A- Light microscope results (H & E stain):**

**Group I:** (Control group) the control liver show normal lobular architecture with central vein and radiating cords of hepatocytes, separated by blood sinusoids. Hepatocytes are polyhedral with acidophilic granular cytoplasm. The nucleus is rounded, vesicular with prominent nucleolus (Fig. 13).

**Group II:** Most of the changes have been seen in centrilobular area while area around portal triad looks normal. These histological changes were in the form of distortion in the hepatic organization, dilation of the blood sinusoids and thinner hepatocytes cords. The cytoplasm showed many vacuoles with very few cells look dark stained with dark nucleus (Fig. 14).

**Group III:** The hepatocytes appeared more or less similar to those of the control group (Fig. 15).

**Electron microscope results:**

**Group I:** The hepatocytes have many mitochondria, strands of rough endoplasmic reticulum free ribosomes, smooth endoplasmic reticulum and small electron lucent areas distributed through the cytoplasm. The nucleus is large rounded with prominent nucleolus (Fig. 16).

**Group II:** The hepatocytes showed clumping of cellular organelles which were mainly formed of rough endoplasmic reticulum and mitochondria. These cytoplasmic organelles were separated by large areas of rarified cytoplasm. The mitochondria looks normal and rough endoplasmic reticulum cisternae are prominent. Whorly appeared structures were seen in the rarified cytoplasm. Smooth endoplasmic reticulum and glycogen rosette are very few (Fig. 17-A, 17-B).

**Group III:** There is obvious improvement in the ultrastructure of the hepatocytes which appears nearly similar to the control (Fig. 18).

**I- Biochemical results:**

**Table (1):** Shows serum nitric oxide level in all treated rat groups:

There was no statistical difference between control and olive treated group. The mean of serum NO level was highly significant increase in CsA treated group (33 ±20) as compared to control group (23.7±1.3). There was a highly significant decrease in serum NO level to near control level in treatment with aminoguanidine when compared to CsA treated group (25.9±1.3) as shown in Table (1).

**Table (2):** Shows serum ALP, GOT and GPT levels in all treated rat groups:

There was no statistical difference between control and olive treated group. The mean of serum levels of ALP, GOT and GPT were highly significantly increased in CsA treated group (215 ±8.52), (166.3±6.41) and (151.1±6.7) respectively as compared to control group (154.7±5.39), (71.2±4.3) and (59.2±3.37) respectively. There was a highly significant decrease in the elevated serum level of ALP, GOT and GPT in treatment with aminoguanidine (181.6±5.28), (132.2±4.4) and (96.6±4.3) respectively when compared with CsA treated group as shown in Table (2).
Table (3): Shows serum urea, creatinine and uric acid levels in all treated rat groups:

There was no statistical difference between control and olive treated group. The mean of serum levels of serum urea, creatinine and uric acid levels were highly significantly increased in CsA treated group (62.5±2.4), (0.622±0.02) and (8.17±0.34) respectively as compared to control group (23.8±1.25), (0.328±0.01) and (4.96±0.24) respectively. There was a highly significant decrease in the elevated serum level of urea, creatinine and uric acid in treatment with aminoguanidine (48.1±2.2), (0.478±0.01) and (6.59±0.29) respectively when compared with CsA treated group as shown in Table (3).

Table (1): Shows serum nitric oxide levels in all rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum NO (µmol/L)</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>23.7±1.3</td>
<td></td>
</tr>
<tr>
<td>Group II: Cyclosporine</td>
<td>33.5±20</td>
<td></td>
</tr>
<tr>
<td>Group III: Cyclosporine + aminoguanidine</td>
<td>25.9±1.3 **</td>
<td></td>
</tr>
<tr>
<td>Group IV: Olive oil</td>
<td>23.30±1.2</td>
<td></td>
</tr>
</tbody>
</table>

* Significant group II versus group I.
** Highly significant group III versus group II.

Table (2): Shows serum ALP, GOT and GPT levels in all rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALP (IU/l)</th>
<th>Mean ± SE</th>
<th>GOT (IU/l)</th>
<th>Mean ± SE</th>
<th>GPT (IU/l)</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>154.7±5.39</td>
<td></td>
<td>71.2±4.3</td>
<td></td>
<td>59.2±3.37</td>
<td></td>
</tr>
<tr>
<td>Group II: Cyclosporine</td>
<td>215±8.52*</td>
<td></td>
<td>166.3±6.41 *</td>
<td></td>
<td>151.1±6.7*</td>
<td></td>
</tr>
<tr>
<td>Group III: Cyclosporine + aminoguanidine</td>
<td>181.6±5.28**</td>
<td></td>
<td>132.2±4.4**</td>
<td></td>
<td>96.6±4.3**</td>
<td></td>
</tr>
<tr>
<td>Group IV: Olive oil</td>
<td>152.4±5.96</td>
<td></td>
<td>68.95±4.59</td>
<td></td>
<td>58.2±2.79</td>
<td></td>
</tr>
</tbody>
</table>

* Highly significant group III versus group I.
** Highly significant group III versus group II.

Table (3): Shows serum urea, creatinine and uric acid levels in all rat groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea (mg/dl)</th>
<th>Mean ± SE</th>
<th>Creatinine (mg/dl)</th>
<th>Mean ± SE</th>
<th>Uric Acid (mg/dl)</th>
<th>Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I: Control</td>
<td>23.8±1.25</td>
<td></td>
<td>0.328±0.01</td>
<td></td>
<td>4.96±0.24</td>
<td></td>
</tr>
<tr>
<td>Group II: Cyclosporine</td>
<td>62.5±2.4*</td>
<td></td>
<td>0.622±0.02*</td>
<td></td>
<td>8.17±0.34*</td>
<td></td>
</tr>
<tr>
<td>Group III: Cyclosporine + aminoguanidine</td>
<td>48.1±2.2**</td>
<td></td>
<td>0.478±0.01**</td>
<td></td>
<td>6.59±0.29**</td>
<td></td>
</tr>
<tr>
<td>Group IV: Olive oil</td>
<td>24.11±1.4</td>
<td></td>
<td>0.3316±0.015</td>
<td></td>
<td>4.86±0.25</td>
<td></td>
</tr>
</tbody>
</table>

* Highly significant group III versus group I.
** Highly significant group III versus group II.
Fig. (4): A photograph of semithin section of proximal convoluted tubules of rats of (group I) showing large round vesicular nucleus (n) and the cytoplasm contains basophilic structures (mitochondria) m and pale bluish brush border b (toludine blue x 1000).

Fig. (5): A photograph of semithin section of proximal convoluted tubules of rats of (group II) showing the nuclei are large with nucleoli but no fine chromatin (n). The cytoplasm pale, vaculated and contains small round blue granules scattered all over it (C). Rupture of the cell membranes can be seen. Dark cells with irregular shape and pyknotic nucleus can be seen lining the tubule (p). Disruption of the brush border is obvious (b).

Fig. (6): A photograph of semithin section of the proximal convoluted tubules of rats of (group III) where they look more or less normal. The basophilic structure in the cytoplasm look more deeper in colour (M).

Fig. (7): A photograph of control renal cortex of rats of (group I) showing immunostained with anti active caspase-3 antibodies showing negative immunolabelling reaction (x 200).

Fig. (8): A photograph of renal cortex of rats of (group II) showing proximal convoluted tubules have positive immunoperoxidase labeling. Some of these tubules have weak positive reaction (w) while others showing strong positive reaction (s) (x 200).

Fig. (8-A): Amagnified part of the former micrograph showing strong +ve reaction in some P.C.T. cells (S) Distal tubules looks normal (d).

Fig. (9): A photograph of renal cortex of rats of (group III) showing appearance which is more or less like the control group.
Fig. (10): An electron micrograph of proximal convoluted tubules of rats of (group I) showing long microvillus border (MV), many elongated mitochondria, basal spherical nucleus with euchromatin and prominent nucleolus (N). Note: Normal basal infolding (F) (x 5000).

Fig. (13): A photo micrograph of a section in the liver of rats of control adult male albino rat of (group I) showing central vein (c.v) and hepatocytes (h) with large round nuclei with prominent nucleoli (l) separated by blood sinusoids (s) (H & E. x 400).

Fig. (11): An electron micrograph of proximal convoluted tubules of rats of (group II) showing loss of microvilli (MV), polymorphic mitochondria with electron dense matrix: Note vacule (V) inside the degenerated mitochondria. Electron dense like lysosomal (L) bodies especially in the apical cytoplasm. Note rarefaction of some areas of the cytoplasm (R) (x 5000).

Fig. (14): A photo micrograph of a section of in the liver of rats of (group II) showing hepatocytes are thin and contain vacuolated cytoplasm (l). The blood sinusoids separating the hepatocytes are wide (s) (H & E. x 400).

Fig. (12): An electron micrograph of proximal convoluted tubules of rats of (group III) showing well developed microvilli (MV), many mitochondria scattered all over the cytoplasm and basal infolding are not well developed (x 5000).

Fig. (15): A photo micrograph of a section of in the liver of rats of (group III) showing hepatocytes appeared more or less similar to control liver (H & E. x 400). Note: Some blood congestion can be seen in blood sinusoids (l).
Fig. (16): An electron micrograph of control liver cells of rats of (group I) showing euchromatic nucleus (N). The cytoplasm have numerous mitochondria (M), cisternae of rER and fine glycogen granules (g) showing (x 4000).

Fig. (17-A): An electron micrograph of liver cells of rats of (group II) showing a clumping of cell organelles, whorly appeared structures (T) (mostly; mitochondria and prominent (rER) in a rarified cytoplasm (r) (x 4000).

Fig. (17-B): Amagnified part of the former electron micrograph.

Discussion

Cyclosporine (CsA) is a potent immunosuppressive agent with inducible efficacy in the prevention of organ allograft rejection and autoimmune disease [25].

CsA induced nephrotoxicity and hepatotoxicity has been reported previously by Rezzani and Josephine et al. [1,16].

Several mechanisms have been involved in CsA nephrotoxicity which include; increase production of reactive oxygen species Rezzani et al. [12] and increased kidney cell apoptosis have been proposed as an important cause of chronic cyclosporine nephrotoxicity [13].

In the present study the mean of serum NO level was highly significant increased in CsA treated group. This was in agreement with Amudha et al. [26] who mentioned that CsA induced increased NO level and oxidative stress accompanied by renal toxicity. Also coincide with Erik et al., (1997) who mentioned that cyclosporine side effects are due to increase NO production through enhanced receptor mediated NO release increase. In addition Amore A. et al. [8] reported that cyclosporine enhanced gene transcription of NOS-III and increase NO activity in the rat kidney cells in vitro.

In the present study the serum NO level was improved after aminoguanidine treatment which attributed to decrease NO production.

In the present study the mean of serum levels of serum ALP, GOT (or (AST) and GPT (or ALT) as a liver function tests were highly significantly increased in CSA treated group. This was in agreement with Hagar (2004) [27] who mentioned that
subcutaneous injection of CsA a dose of 20mg/kg subcutaneously daily for 21 days to rats induced hepatotoxicity manifested biochemically by reduced serum proteins levels, increased serum gamma glutamyl transferase (GGT), ALT and AST. CsA hepatotoxicity was attributed to reactive oxygen species due to oxidative stress.

In the present study treatment with aminoguanidine corrected the hepatotoxicity induced by cyclosporine. This coincide with the results of Raza et al. [15] who reported that aminoguanidine administrated in a dose of 100mg/kg intraperitoneally for 7 days after the immunosuppressive azthioprine has protective effect against hepatotoxicity and it improved the AST and ALT levels.

In the current study the mean of serum levels of urea, creatinine and uric acid as a kidney function tests were highly significantly increased in CSA treated group. This was in agreement with Chander and Chopra [28] who mentioned that CsA given to rats in a dose of 20mg/kg subcutaneously daily for 21 days induced nephrotoxicity attributed to renal oxidative stress which manifested by renal histopathological and biochemical changes manifested by increased serum urea and creatinine.

In the current study administration of CSA for 21 days induced significant damage in the structure of the adult rat kidney. The damage was most prominent in the proximal convoluted tubules and most of the work done was constricted on the P.C.T. damage. Histologically, the cells of the P.C.T. showed sever damage or injury. These cells showed clear cytoplasm, dense nuclei while other showed pale acidophilic cytoplasm with vacuoles especially in the base of the cells and the brush border has been interrupted or even lost. Some scattered few dark acidophilic cells with dark nuclei have been shown. This was in agreement with (Rezzani 2004) [1] who reported that prolonged CsA treatment associated with interstitial fibrosis, tubular atrophy, arteriolar hyalinosis and sometimes focal glomerular scarring.

It has been postulated that nephrotoxicity is dose dependent and has been associated with significant morphological changes, in which CsA toxicity was manifested by a variety of lesions affecting tubules vessels and the renal interstitium [29].

In the current study the CsA treated group, the blood vessels of kidney looks fine by light microscope. By E.M. examination of CsA treated group showed vaculated cytoplasm, mitochondria were less and there was loss of their arrangement, some were dark, others had vacuoles in the center. There were many lysosomal like dense bodies especially at the apical border. The basal infoldings were lost. Also the brush border showed great loss. These findings coincide with Razzani et al. [2] who mentioned that in CsA group the mitochondria were very big and swollen. They attributed CsA nephrotoxicity to oxidative stress. This is in agreement with our finding that P.C.T. damage was in the form of necrosis and/or apoptotic changes.

Anna et al. (2007) mentioned that administration of drugs which induce angiogenesis in CsA treated rats improve nephrotoxicity and they postulated that the function and role of these drugs were in improving over all tissue oxygenation and anti-apoptotic role.

In the present study aminoguanidine protect against CsA nephrotoxicity. This coincides with Busauschina et al. [3] who attributed its protective effect due to its antioxidant action.

The affection of mitochondria reflects great disturbance in the mitochondrial oxidative phosphorylation that affects kidney tubular function [30,31]. This is in agreement with our findings.

In the current study light microscopic examination of aminoguanidine treated rats kidney showed obvious improvement of the P.C.T. structure. They look similar to the control group except in E.M. examination where these cells showed a lot of mitochondria not well arranged which reflect the highly acidophilic cytoplasm in the H.E. sections. Also, the basal infoldings not well develop. In contrast the brush border was highly reserved. The lysosome like dense bodies was also less in number.

Immunolabelling with anti-active caspase-3 antibodies revealed increase in the immunolabeling in the P.C.T. in the renal cortex while in the D.C.T. or the collecting lobules in the Medullary rays showed –ve immunolabelling capase-3 are present in the cell cytoplasm as pro-structure when they are activated they shave in a long chain which leads to cell damage. This was in agreement [32,33].

The current study demonstrated that active caspase-3 involved in tubulopathy of CSA treated kidney rats and aminoguanidine ameliorate these change and also decreased the active caspase-3 labelling.

These results is in agreement with Li et al. [14] who documented that colchicine administration
decreases the apoptotic cell death in chronic CsA-induced nephrotoxicity. Moreover, a marine tubular epithelial cell line exposed to CsA underwent apoptosis with evidence of caspase-3 activation, the administration of caspase inhibitors prevented the cell from undergoing cyclosporine induced apoptosis [34].

In the present study, the hepatic morphological changes in the CsA treated group were greatly less marked than morphological changes in the kidney section. These changes are more in the centrilobular cells while cells around the portal triad looks fine. The hepatocytes in the centrilobular area look thinner and wide blood sinusoids. This was in agreement with (Rezzani, 2004) [1].

Other studies showed that by E.M. the hepatocytes are swollen, the nucleus looks fine, the cytoplasmic organelles (mitochondria and rER) concentrated in some areas leaving a rarified cytoplasm. The rER looked very prominent in the CSA treated rats. The smooth endoplasmic reticulum can't be detected easily active caspase-3.

Immunolabelling had no effect in hepatic damage with aminoguanidine treatment. The hepatocytes look fine and more or less like normal cells. In the past it had been postulated liver showed also oxidative stress but this is strictly related with biochemical parameters that are responsible for liver toxicity [27]. They showed a significant increase in serum alanine amino transferase, aspartate aminotransferase and gamma glutamyl transferase levels and decrease in serum total proteins.

Nevertheless, the new finding of (Razzani, 2004) [1] showed that CsA induces changes in stress protein expression. Its fundamental role inside mitochondria has been demonstrated in different mammalian cell line damage.

In the present study improvement in the morphological changes in the liver induced by CsA was in agreement with Raza et al. [15] who reported that aminoguanidine improved the hepatic morphology induced by the immunosuppressive drug azathioprine.

In the present study improvement in the morphological changes in the kidney induced by CsA was in agreement with Mansour et al. [35] who reported that aminoguanidine has a protective effect on the kidney morphology induced by the cisplatin.

Conclusion and recommendations:

Nephrotoxicity and hepatotoxicity of rats treated with cyclosporins may be due to oxidative stress associated with increased NO level. Aminoguanidine treatment improves the biochemical and morphological changes in the liver and kidney especially in renal tubules induced by CsA. Active caspase-3 had a role in the apoptosis as another mechanism of CsA nephrotoxicity. Histologically the smooth endoplasm reticulum was affected in CsA hepatotoxicity which had a role in drugs detoxification.

References


