INTRODUCTION

Cyclosporin A (CsA) is still one of the most important immunosuppressive drugs used in solid organ and bone marrow transplantation. It has also become an important therapeutic regimen for patients with autoimmune diseases (1, 2). The therapeutic benefits of CsA are often limited by its main side-effect: nephrotoxicity. Mechanisms of chronic CsA-induced renal damage include: activation of renin-angiotensin-aldosterone system, upregulation of transforming growth factor beta (TGF-β), oxidative stress. This study was undertaken to investigate the protective effect of the peroxisome-proliferator-activated receptors gamma (PPAR-γ) agonists: rosiglitazone and 15-deoxy-D12,14-prostaglandin J2 (PGDJ2), against CsA-induced kidney injury in male Wistar rats. CsA was administered subcutaneously at a dose of 15 mg/kg/day for 28 days. Both PPAR-γ agonists were given for 28 days 0.5 hour before the administration of CsA. Rosiglitazone was administered orally at a dose of 8 mg/kg/day and PGDJ2 was given intraperitoneally at a dose of 30 µg/kg/day. CsA induced renal failure was evidenced by increased serum levels of urea, uric acid and creatinine. Serum concentrations of GSH and GSSG, lipid peroxidation products as well as NAD+/NADH, NADP+/NADPH and ADP/ATP ratios showed, that CsA induced oxidative stress and evoked an imbalanced red-ox state in the kidney. Light and electron microscope studies showed degenerative changes within renal tubules with damage to their mitochondria, interstitial fibrosis and arteriolopathy. Immunohistochemical expression of profibrotic TGF-β was assessed. The biochemical and morphological changes induced by CsA were limited by administration of both rosiglitazone and PGDJ2. Ultrastructural examination of renal tubular epithelial cells showed marked improvement within mitochondria. Our results indicate that both PPAR-γ agonists used in the experiment may play an important role in protecting against CsA-induced damage in the kidney.

Key words: cyclosporine A, nephrotoxicity, thiazolidinediones, peroxisome proliferator activated receptor gamma agonists, lipid peroxidation, transforming growth factor beta, glutathione

15-deoxy-Δ12,14-prostaglandin J2 (PGD₂J2); a product of prostaglandin D₂ metabolism, is known endogenous PPAR-γ ligand. This PPAR-γ ligand inhibits proinflammatory cytokines production in acute and chronic inflammation and prevents ROS generation induced by oxidative stress (37-40).

We performed this study to evaluate the protective affect of PPAR-γ ligands: endogeneous PGD₂J2 and exogeneous RGTZ on CsA-induced renal injury using experimental model of CsA nephropathy. Our data show that PPAR-γ ligands used in the experiment have protective effect against CsA - induced injury in the kidney.

**MATERIAL AND METHODS**

**Drugs and chemicals**

Cyclosporine A (Sandimmun, Novartis, Poland) was administered subeautaneously (SC) at a dose of 15 mg/kg/day diluted in olive oil. Dose of CsA was established on the basis of our previous study (41). The vehicle group received olive oil at a dose of 1 ml/kg/day SC. Rosiglitazone (Avandia, Glaxo SmithKline, Poland) was administered orally by gavage, at a dose of 8 mg/kg/day (22, 25). 15-deoxy-Δ12,14-prostaglandin J2 (Cayman Chemical, USA) was given intraperitoneally at a dose of 30 µg/kg/day (38-40).

**Experimental protocol**

The experimental protocols were conducted according to the guidelines of Institutional Animal ethics Committee (IAEC) of Medical University in Lublin. Adult male Wistar rats weighing 250–300 g were housed in a temperature controlled environment with an alternating cycle of 12 h light and dark. They were on low-sodium diet and had free access to water. The animals were divided into seven groups (C, G1-6) with 8 animals in each group.

- **C**: Control, NaCl 1 ml/kg/day
- **G1**: Vehicle, olive oil 1 ml/kg/day
- **G2**: CsA, 15 mg/kg/day
- **G3**: CsA 15 mg/kg/day with rosiglitazone 8 g/kg/day
- **G4**: CsA 15 mg/kg/day with PDGJ2 30 µg/kg/day
- **G5**: rosiglitazone 8 g/kg/day
- **G6**: PDGJ2 30 µg/kg/day

Animals were weighed daily. On 29th day of an experiment all animals were anesthetized with pentobarbital in a dose of 60 mg/kg (Morbital, Biowet, Poland). Blood sample and kidney specimens were obtained for biochemical, histological, ultrastructural and immunohistochemical analyses.

**Measurement of renal function**

Serum levels of urea, uric acid and creatinine were measured by using the diagnostic Kits of Cormay Diagnostic, according to standard procedures using commercially available kits (Cormay Diagnostic S.A., Poland). Blood sample and kidney specimens were obtained for biochemical, histological, ultrastructural and immunohistochemical analyses.

**Biochemical studies**

The kidney samples were homogenised in 20 mM phosphate buffer (pH 7.4); 0.5 g tissue in 2 ml. The homogenisation was made in cold-water bath (4°C) at 4000 rpm using homogeniser with teflon pestle (Glas-Col, USA) for 3 min. The homogenate was centrifuged for 20 min at 15,000 rpm) and the obtained supernatant was used for further biochemical studies. All spectrophotometric methods were performed using microtiter plate reader Power Wave xs (BioTek, USA).

1. Reduced glutathione (GSH), oxidized glutathione (GSSG), GSH/GSSG

Reduced (GSH) and oxidized (GSSG) glutathione determination was conducted using commercial kit BIOXYTECH GSH/GSSG-412 (OxisResearch, USA). The principle of the procedure is based on simultaneous determination of GSH and GSSG into two separate tubes. To determine GSSG, 1-methyl-2-vinylpyridinium trifluoromethane-sulfonate (M2VP) was used at a level that rapidly and completely scavenges GSH but does not interfere with the glutathione reductases, that in turn reverse GSSG to GSH in the next step of procedure. Subsequently the procedure is common for both parameters. GSH in separate tubes (created or native) was extracted and reacted with Ellman’s reagent (5,5'-dithiobis-2-nitrobenzoic acid) forming color product with the maximum of absorbance at 412 nm. The concentrations of GSH and GSSG were rewritten from the separate calibration curves. The GSH/GSSG ratio was calculated.

2. Lipid peroxidation products

The commercial kit BIOXYTECH LPO-586 (OxisResearch, USA) was used to measure the malondialdehyde (MDA) and 4-hydroxyalkenals (4HAE) as indicators of lipid peroxidation. The concept of the method is based on the reaction between MDA and 4HAE with N-methyl-2-phenyldiol at the temperature of 45°C for 60 minutes. In those reactions the stable chromophore with maximal absorbance 586 nm is yielded. Procedure was conducted according to manufacturer description . Concentration of MDA + 4HAE in tested samples was calculated from the formula of calibration curve $y = 0.0896x – 0.008$. Obtained results were expressed in nmol/g of kidney tissue.

3. NAD+/NADH and NADP+/NADPH ratios

The NAD(P)⁺ and NAD(P)H levels were measured using BioChain NAD⁺/NADH assay kits according to the manufacturer’s instructions (BioChain, Hayward, CA). The principles of assay kits are based on a glucose dehydrogenase cycling reaction, in which tetrazolium dye (MTT) is reduced by NAD(P)H in the presence of phenazine methosulfate (PMS). The intensity of the reduced product color, measured at 565 nm, is proportionate to the NAD⁺ concentration in the sample. The standards attached to the kits were used to preparing calibration curves needed to calculate NAD(P)⁺/NAD(P)H ratios.

4. ADP/ATP ratio

ADP/ATP ratio in tissue supernatant was calculated on the basis of fluorescence (luminescence) intensity measurement (BioVision kit, USA) using fluorescence microplate reader Victor3V (Perkin Elmer, Finland).

**Morphologic studies**

Kidney samples were fixed in 10% buffered formalin and embedded in paraffin. After dewaxing 4-µm sections were stained with hematoxylin and eosin (H + E), periodic acid-Schiff (PAS) or Masson’s trichrome. All slides were evaluated under light microscopy (Olympus BX45) by one pathologist to assess tubular
injury, arteriolopathy and interstitial fibrosis. A minimum of 20 cortical fields was examined in each biopsy (magnification ×100). Semiquantitative score was used to assess extension of microscopic changes (19, 20, 42). For tubular injury and arteriolopathy following score was used: 0: no changes, 1: up to 25% areas involved, 2: 26–50% areas involved, 3: 51–75 areas involved, 4: more than 75% areas involved. For interstitial fibrosis following score was used: 0: no fibrosis, 1: delicate fibrosis with mild interstitial widening between the tubules, 2: moderate fibrosis with moderate interstitial widening between the tubules, 3: severe fibrosis with severe interstitial widening between the tubules.

**Transforming growth factor-β immunohistochemistry**

TGF-β immunohistochemistry was assessed in the renal tissue. 4 µm thick slides were deparaffinized in xylene and rehydrated in graded water-ethanol solutions. Endogenous peroxidase activity was blocked for 20 minutes in 3% hydrogen peroxidase in methanol. Antigen unmasking was performed using DAKO Target Retrieval Solution high pH (DAKO, USA) for 10 min at 95°C. Slides were then incubated with monoclonal mice antibody against TGF β (Novocastra: NCL-TGF-β1, 1:20) for 60 minutes. DAKO Envision System-HRP (DAB, DAKO, USA) was used to visualize antigen/antibody complex. The slides were counterstained with hematoxylin followed by dehydration in graded ethanol and xylene.

**Electron microscopy**

Tissue sections for electron microscopic examination were fixed in 4% formaldehyde and 1% glutaraldehyde, and then processed to epoxy resin. Ultrathin samples were stained with uranyl acetate and lead citrate, and examined with Jeol-JEM 1011 electron microscope.

**Statistical analysis**

Results were presented as mean ± S.E.M. Data were analyzed by one way analysis of variance (ANOVA) followed by Duncan’s multiple range test and t-student test using a statistical software package (STATISTICA v.8.0 StatSoft, Poland). P-value <0.05 was considered statistically significant.

**RESULTS**

**Renal function**

CsA administration induced increase in serum level of creatinine, uric acid and urea when compared with control group (Table 1). Animals of groups G3 and G4 treated with CsA and PPAR-γ agonist significantly improved renal function, compared with animals treated with CsA alone (Table 1).

**Microscopic changes**

Kidney samples collected from CsA treated animals (G2) showed characteristic morphological changes of chronic CsA nephrotoxicity. We observed the presence of vacuolisation (Fig. 1A) and PAS positive inclusion bodies within tubular epithelial cells (Fig. 2A). Striped fibrosis was manifested by presence of narrow or broader areas extending from the subcapsular cortex towards the medulla (Fig. 3A). The samples of G2 group also presented focal infiltration of mononuclear cells within interstitium and arteriolar hyalinosis predominantly seen in afferent glomerular arterioles (Fig. 2A). All of these morphologic lesions were markedly reduced in G3 and G4 (PPAR gamma agonist + CsA) animals (Fig. 1B, 2B, 3B). Main microscopic features of the kidney in all 7 groups of the protocol are summarized in Table 2.

### Table 1. Serum levels of creatinine, uric acid and urea in all experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine µmol/l</th>
<th>Urea mmol/l</th>
<th>Uric acid µmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (C)</td>
<td>73.23 ± 0.78a</td>
<td>8.32 ± 0.40</td>
<td>107.25 ± 2.06</td>
</tr>
<tr>
<td>Olive oil (G1)</td>
<td>79.53 ± 1.52</td>
<td>9.60 ± 0.50</td>
<td>108.50 ± 4.43</td>
</tr>
<tr>
<td>CsA (G2)</td>
<td>133.14 ± 2.22b</td>
<td>37.78 ± 1.80</td>
<td>182.31 ± 9.83b</td>
</tr>
<tr>
<td>CsA + Rosi (G3)</td>
<td>83.01 ± 2.0c</td>
<td>10.50 ± 0.34</td>
<td>121.18 ± 2.30c</td>
</tr>
<tr>
<td>CsA + PDGJ2 (G4)</td>
<td>81.08 ± 1.43c</td>
<td>13.18 ±0.60</td>
<td>122.29 ± 4.70c</td>
</tr>
<tr>
<td>Rosi (G5)</td>
<td>77.93 ± 1.04</td>
<td>9.73 ± 1.00</td>
<td>113.35 ± 2.17</td>
</tr>
<tr>
<td>PDGJ2 (G6)</td>
<td>81.08 ± 0.66</td>
<td>9.43 ± 0.42</td>
<td>110.01 ± 1.02</td>
</tr>
</tbody>
</table>

*a values are mean ± S.E.M., b versus control, c versus CsA, *P<0.0001.

### Table 2. Microscopic changes in all experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Tubular vacuolisation</th>
<th>Arteriolopathy</th>
<th>Interstitial fibrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (C)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Olive oil (G1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CsA (G2)</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>CsA + Rosi (G3)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>CsA + PDGJ2 (G4)</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Rosi (G5)</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PDGJ2 (G6)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Immunohistochemical expression of TGF-β was observed in renal interstitial cortical cells in animals treated with CsA (Fig. 1). The reaction was localized within areas of interstitial fibrosis. Animals treated with CsA and PPAR-γ agonists (group G3 and G4) showed focal TGF-β expression within single cells.
No TGF-β expression was seen in kidney samples in groups C, G1, G5 and G6.

**Ultrastructural changes**

Ultrastructural examination revealed dilatation of endoplasmic reticulum with formation of different sized vacuoles within renal tubular epithelial cells. This vacuolization corresponded to cytoplasmic vacuoles seen in light microscope. PAS positive inclusion bodies noticed in light microscope corresponded to mitochondrial changes observed in electron microscope. Marked swelling of mitochondria with presence of giant mitochondria in some tubular epithelial cells were present (Fig. 5A). In most of these organelles the disruption and loss of inner membrane and the cristae were seen. Formation of autolysosomes containing mitochondrial material was also observed. Some of nuclei were shrunken and performed karyorrhexis (Fig. 5A). Single necrotic cells were present. Morphological results of CsA-induced nephrotoxicity were presented as a separate study (43). PPAR-γ agonists markedly reduced ultrastructural changes associated with CsA treatment in animals of groups G3 and G4. Examination of tubular epithelial cells in these animals revealed some scattered vacuolization of the cytoplasm and mild swelling of single mitochondria (Fig. 5B).

Treatment of animals with CsA alone produced a significant increase in renal LPH and GSSG levels, and significant decrease in renal GSH level when compared with control (Table 3). Co-treatment of animals with CsA and one of PPAR-γ agonists: rosiglitazone (group G3) or PGDJ2 (group G4) significantly reversed changes in renal concentrations of LPO products, GSSG and GSH when compared with CsA group. Calculated GSH/GSSG ratio decreased by CsA treatment (14.11 ± 0.52 vs. 18.46 ± 0.20 in control group, P<0.0001) was significantly higher in both CsA + PPAR-γ agonist groups when compared with CsA group (Table 3).

Lipid peroxidation (LPO) products, reduced glutathione (GSH), oxidized glutathione (GSSG), and GSH/GSSG ratio

NADP+/NADPH, NAD+/NADH and ADP/ATP ratios

CsA induced significant increase in NADP+/NADPH ratio and decrease in NAD+/NADH ratio when compared with control animals (Table 4). Mean ADP/ATP ratio in CsA treated animals was significantly higher compared with control (0.19 vs. 0.02; P<0.0001). Administration of RGTZ and PGDJ2 to CsA treated animals significantly reversed the values of these parameters when compared to CsA group (Table 4).
as striped interstitial fibrosis. Demonstrated severe renal tubular and arteriolar damage as well histopathological and ultrastructural findings, which levels. Kidney dysfunction was further confirmed by significant increase in serum urea, uric acid and creatinine CsA resulted in functional disturbances manifested by a values are mean ± S.E.M., b versus control, c versus CsA, *P<0.0001.

Table 3. Renal levels of LPO products, GSH, GSSG and GSH/GSSG ratio in all experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA + 4HAE nmol/g</th>
<th>GSH nmol/g</th>
<th>GSSG nmol/g</th>
<th>GSH/GSSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (C)</td>
<td>144.27 ± 9.02*</td>
<td>98.90 ± 0.71</td>
<td>5.36 ± 0.05</td>
<td>18.46 ± 0.20</td>
</tr>
<tr>
<td>Olive oil (G1)</td>
<td>142.75 ± 10.14</td>
<td>97.67 ± 1.01</td>
<td>5.39 ± 0.04</td>
<td>18.13 ± 0.17</td>
</tr>
<tr>
<td>CsA (G2)</td>
<td>569.97 ± 27.37**</td>
<td>85.49 ± 3.46**</td>
<td>6.05 ± 0.05***</td>
<td>14.11 ± 0.52****</td>
</tr>
<tr>
<td>CsA + Rosi (G3)</td>
<td>182.97 ± 10.92**</td>
<td>96.87 ± 0.97**</td>
<td>5.66 ± 0.04****</td>
<td>17.04 ± 0.19***</td>
</tr>
<tr>
<td>CsA + PDGJ2 (G4)</td>
<td>129.73 ± 12.43**</td>
<td>96.64 ± 0.83****</td>
<td>5.64 ± 0.04****</td>
<td>17.14 ± 0.20****</td>
</tr>
<tr>
<td>Rosi (G5)</td>
<td>125.10 ± 17.04</td>
<td>97.48 ± 1.08</td>
<td>5.37 ± 0.05</td>
<td>18.15 ± 0.29</td>
</tr>
<tr>
<td>PDGJ2 (G6)</td>
<td>119.73 ± 12.02</td>
<td>96.73 ± 0.92</td>
<td>5.63 ± 0.1</td>
<td>17.25 ± 0.48</td>
</tr>
</tbody>
</table>

* values are mean ± S.E.M., † versus control, ‡ versus CsA, ††P<0.0007, †‡P=0.002, †§P=0.0002, †¶P<0.0001.

Table 4. Renal NADP+/NADPH, NAD+/NADH and ADP/ATP in all experimental groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>NADP+/NADPH</th>
<th>NAD+/NADH</th>
<th>ADP/ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl (C)</td>
<td>1.84 ± 0.08*</td>
<td>3.51 ± 0.23</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>Olive oil (G1)</td>
<td>1.74 ± 0.12</td>
<td>3.18 ± 0.42</td>
<td>0.03 ± 0.004</td>
</tr>
<tr>
<td>CsA (G2)</td>
<td>7.61 ± 0.85**</td>
<td>1.46 ± 0.09**</td>
<td>0.19 ± 0.01*</td>
</tr>
<tr>
<td>CsA + Rosi (G3)</td>
<td>1.80 ± 0.15**</td>
<td>3.50 ± 0.19**</td>
<td>0.06 ± 0.01**</td>
</tr>
<tr>
<td>CsA + PDGJ2 (G4)</td>
<td>1.82 ± 0.15**</td>
<td>3.31 ± 0.18**</td>
<td>0.05 ± 0.004*</td>
</tr>
<tr>
<td>Rosi (G5)</td>
<td>1.73 ± 0.13</td>
<td>3.27 ± 0.21</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>PDGJ2 (G6)</td>
<td>1.66 ± 0.14</td>
<td>3.25 ± 0.18</td>
<td>0.03 ± 0.01</td>
</tr>
</tbody>
</table>

* values are mean ± S.E.M., † versus control, ‡ versus CsA, ††P<0.0001.

DISCUSSION

In this study we report that PPAR-γ agonists: rosiglitazone and PDGJ2 reduce the severity of chronic experimental CsA nephrotoxicity. The animal model used in the study mimics the microscopic and functional changes observed in human lesions of chronic CsA nephrotoxicity.

The present investigation revealed that administration of CsA resulted in functional disturbances manifested by a significant increase in serum urea, uric acid and creatinine levels. Kidney dysfunction was further confirmed by histopathological and ultrastructural findings, which demonstrated severe renal tubular and arteriolar damage as well as stripped interstitial fibrosis.

The use of PPAR-γ agonists significantly improved renal function and morphology of the kidney in animals treated with CsA. Microscopy showed that CsA induced interstitial fibrosis was markedly limited in rats treated with rosiglitazone and PDGJ2. We also observed reduced expression of TGF-β, known profibrotic factor in the kidney. CsA enhances TGF-β1 expression in the kidney (5, 11), which is associated with progressive interstitial fibrosis (5, 11) and glomerulosclerosis (44). PPAR gamma agonists reduce TGF-β expression, glomerulosclerosis and interstitial fibrosis in experimental diabetic nephropathy (45, 46) by reduction of mesangial matrix expansion and production of extracellular matrix (47). Chung et al. (29) and Pereira et al. (48) have documented that PPAR gamma agonists reduce TGF-β expression and interstitial fibrosis in the course of experimental CsA nephrotoxicity. Our findings are in agreement with above mentioned studies and show, that both exogenous (rosiglitazone) and endogenous (PDGJ2) PPAR-γ agonist markedly reduce TGF-β expression and interstitial fibrosis in the kidney. CsA induced arteriolopathy is a hallmark of vascular injury in the kidney. Chung et al. (29), on the basis of their experimental study noticed that rosiglitazone treatment protected animals against CsA induced arteriolopathy. Such observations were a part of our findings in both PPAR-γ agonists + CsA groups. We may speculate that reduced arteriolopathy was one of the factors that slowed down the progression of interstitial fibrosis associated with CsA nephrotoxicity.

The role of oxidative stress in chronic CsA treatment was examined in several studies. CsA increases oxidative stress and ROS production, inhibits mitochondrial glucose metabolism (the Krebs cycle and oxidative phosphorylation) and ATP production (14, 18-20, 49-54). It is postulated that CsA is an inhibitor of mitochondrial transport system. CsA-induced ROS generation in the kidney is activated by NADPH oxidase, xanthine oxidase, cytochrome P450 CsA metabolism, or decreased intracellular antioxidant systems (61). ROS level in the kidney is dose-related (52, 56). Increase of ROS results in lipid peroxidation and increase of its products, such as MDA. CsA treatment reduces also GSH, an important antioxidant (6, 13, 16, 19, 57). Reduced glutathione (GSH) converts lipid peroxides to non-toxic products, thus maintaining the integrity of the mitochondria and cell membranes. GSH, in glutathione redox cycle, is converted by glutathione peroxidase to GSSG. Regeneration of GSH by glutathione reductase uses NADPH. In present study we observed significant increase in MDA and decrease in GSH levels in animals treated with CsA. Our results are in agreement with other studies (6, 13, 14, 16, 19, 57). We may speculate that marked decrease in GSH was produced not only by engagement in ROS reduction but also could be the result of impaired regeneration by glutathione reductase.

Microscopic examination of renal tubular epithelial cells revealed, in CsA treated animals, vacuolisation and PAS positive cytoplasmic inclusion bodies that correspond to possible mitochondrial damage. Since mitochondria are one of the main physiologic sources of ROS, we have also focused on their structural appearance. Ultrastructural studies showed swollen mitochondria that differ in size. Rupture of their cristae, inner and outer membrane was seen in some of them. Autolysosomes
containing mitochondrial material were also observed. Single necrotic cells were present. Similar observations were described in previous studies (15, 58, 59). Structural damage to mitochondria seems to be the result of their impaired function. De Hornedo et al. (60) documented that CsA produces a depolarization of mitochondrial membranes that parallels with ROS production. CsA promotes a caspase-independent release of proapoptotic cytochrome c and Smac/Diablo from mitochondria (61, 62). This hypothesis of mitochondrial degradation with release of proapoptotic cytochrome c could partially explain presence of single necrotic cells in our ultrastructural studies.

Lipid peroxidation products decrease ADP- and NADH-dependent mitochondrial respiratory chain (63, 64). This results in disturbances in membranous Na+K+ ATP-ase activity and decrease in the main mitochondrial product: ATP. To assess mitochondrial phosphorylation and red-ox state we examined ADP/ATP, NAD+/NADH and NADP+/NADPH ratios. CsA treated animals showed significant increase of ADP/ATP ratio when compared with the control group. The impairment of mitochondrial respiration might be one explanation. These findings correlated with decrease in NAD+/NADH ratio. Decreased regeneration of NAD+ and/or increased NADH might also result from respiratory chain impairment. Oxidative stress increases NAD+, ATP consumption and promotes PARP-1 (nuclear enzyme: poly, ADP-ribose, polymerase-1) dependent cell death (65). Decreased NAD+/NADH promotes further mitochondrial damage, which may lead to cell death. Increased NADP+/NADPH ratio was noticed in CsA group. It could be associated with the use of NADPH by antioxidative mechanisms. De Hornedo et al. (60) in their in vitro studies noticed that CsA causes dysfunction of enzymes responsible for NADPH production, such as mitochondrial dehydrogenases. NADPH is also substrate for NADPH oxidase in ROS production. CsA can stimulate NADPH oxidase by an increase in angioplastin II (66-68). We cannot exclude that increase in NADP+/NADPH ratio observed in this protocol is the result of NADPH oxidase stimulation and dysfunction of peroxisome dehydrogenases and in consequence decrease in NADPH. We cannot also exclude, that increase in NADP+/NADPH ratio might be associated with increased activity of glutathione reductase. Decrease in GSH and increase in GSSG levels seem to support this hypothesis.

PPAR-γ agonists are known to inhibit inflammatory processes, mainly by regulation of anti-and proinflammatory cytokines (45, 46). Miana et al. documented in their experimental study that mechanisms that cause the damage to the kidney in hypertension involve the down-regulation of PPAR-γ (70). The protective effect of PPAR-γ agonists on mitochondrial dysfunction was also documented in many studies. In experimental conditions TZDs’ decrease lipid peroxidation products and oxidative stress (71), protect mitochondrial membranes and decrease release of proapoptotic factors (40, 71-74). Positive effect of PGD2 was observed in experimental oxidative stress (75-78) and resulted in inhibition of ROS production and apoptosis. PPAR-γ agonists in in vitro studies decrease the effects of oxidative stress by inhibition of NADH and NADPH oxidases (77, 78). Cha et al. in their study carried out on the cell culture noticed that PGD2 decreased Helicobacter pylori dependent gastritis by NADPH oxidase inhibition (79). In both PPAR-γ agonist + CsA groups, we observed significant decrease of MDA + 4HAE when compared with CsA group. These animals presented also significant increase in GSH and decrease in GSSG levels when compared with CsA group. These results indicate that both PPAR-γ agonists used in this experiment reduced oxidative stress induced by CsA. Significant decrease of ADP/ATP and NADP+/NADPH ratios and increase of NAD+/NADH ratio were present in CsA + rosiglitazone and CsA + PGDJ2 animals, when compared with CsA group. Ultrastructural examination revealed in these animals very mild swelling of tubular epithelial cells mitochondria without desintegration of their membrane system. No necrotic cells were seen. All these results let us speculate that PPAR gamma agonists used in this experiment not only stabilized the mitochondrial function, but also protected these cytoplasmic structures from structural damage. These observations would be in agreement with results of other researchers (29, 70, 72-74) that carried out their experiments mainly on cell cultures. Results of our biochemical studies and ultrastructural examination of mitochondria let us conclude that PPAR γ agonists used in this experiment show the protective effect on CsA-induced damage of these cytoplasmic organelles.

The results of long-term clinical studies strongly suggest that cyclosporine treatment still remains one of the main choices in transplant medicine (74-76). The attempts to improve graft survival in kidney transplant recipients or function of own organs in nonrenal transplants are of great significance. Some of recent studies proved, that activation of PPAR-γ enhances immunosuppressant effect of CsA. Rampino et al. (77) in their in vitro experiment showed, that low-dose CsA treatment combined with rosiglitazone was significantly more powerful than either high-dose CsA alone or rosiglitazone alone in suppressing interleukin-2 release, arresting the T cell cycle, and blocking the growth of activated T cells. Tanaka et al. (78) using rat heterotopic heart transplantation model proved, that pioglitazone treatment with low-dose cyclosporine has synergistic protective effects for cardiac allografts and recipient kidneys, leading to improvement of graft survival with a minimal cyclosporine-induced nephrotoxicity.

Our study shows that CsA administration results in decreased renal function, pronounced oxidative stress and morphological damage to the kidney. PPAR-γ agonists ameliorated the renal dysfunction and parameters of redox state. Administration of PPAR-γ agonists to CsA treated rats prevented the morphological damage to the kidney. No significant differences were observed in comparison of results obtained from animals treated with exogenous agonist (CsA + rosiglitazone) and endogenous agonist (CsA + PGDJ2). The combined use of PPAR-γ ligands and CsA might represent a rationale therapeutic approach for the prevention of CsA nephrotoxicity. Further experimental studies and clinical trials should be considered to support this hypothesis.

Conflict of interests: None declared.

REFERENCES


Received: January 13, 2014
Accepted: October 16, 2014

Author’s address: Prof. Assoc. Agnieszka Korolczuk, Department of Clinical Pathomorphology, Medical University, 1 Ceramiczna Street, 20-150 Lublin, Poland.
Email: agnieszka.korolczuk@wp.pl