Effect of pentoxifylline on cyclosporine-induced nephrotoxicity in rats

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Effect of unique hemorheologic agent pentoxifylline (PTX) was investigated on cyclosporine (CsA) induced nephrotoxicity in rats. Compared to saline control, CsA produced significant increase in blood urea and serum creatinine. Pentoxifylline treatment prevented the CsA-induced rise in blood urea and serum creatinine. Creatinine clearance (Ccr) and lithium clearance (Licr) was decreased with CsA. PTX treatment prevented the CsA-induced decrease in Ccr and Licr. Malondialdehyde (MDA) was increased with CsA compared to saline treated animals. PTX prevented the CsA-induced MDA rise. Kidney form CsA treated rat showed marked vascular degeneration of tubular epithelium with excess of microcalcification. Severity of the lesions was markedly reduced in rats treated with PTX plus CsA. The results indicate that PTX reduces CsA-induced renal toxicity in rats.

Advances in immunosuppressive therapy have resulted in significantly improved patient and graft survival after solid organ transplantation. The use of cyclosporine (CsA) as the mainstay of immunosuppressive therapy in often associated with short and long terms nephrotoxicity. CsA-induced renal impairment is often managed through pharmacokinetic dosing strategies as well as pharmacological intervention. Ability of pharmacological antagonists of mediators to reverse CsA-induced vascular toxicitv, endothelial dysfunction and functional changes in smooth muscle cells11. The aim of this study is to assess the effects of PTX on the nephrotoxicity elicited by CsA in rats.

Materials and Methods

Animals and treatment- Male wistar rats (mean weight 200-250 g) were housed under conditions of controlled temperature and 12 hour lighting cycle and fed standard rat chow. The animals were divided into 4 groups of 6 animals each. Animals of group one, which received normal saline, ip, 12 hourly for 14 days were used as control. The second group received PTX 100 mg/kg (obtained from Hoechst India Ltd), ip, 12 hourly daily for 14 days. The animals belonging to the third group received CsA 50 mg/kg body weight (obtained from Sandoz Pharma Ltd, Switzerland) po, daily for 14 days and fourth group was given the same dose of cyclosporine for 14 days po along with PTX 100 mg/kg body weight, ip 12 hourly daily, 3 days before and 14 days concurrently with CsA treatment.

Biochemical assays — At the end of the 14th day of CsA administration, the animals were kept in individual metabolic cages for 24-hr urine collection. Blood was collected by ocular puncture method.

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Estimation of urinary sodium and potassium were carried out using flame photometer. Concentration of chloride in urine was determined by mercury (II) nitrate method13. Blood urea concentration was determined by GLDH-kinetic method13. Creatinine clearance was calculated after estimating the serum and urinary creatinine by alkaline picrote method14, with Beckman Spectrophotometer DU62.

Lithium clearance studies — Through out the experiment, 5 mmol/l of lithium chloride was added to the freely accessible drinking water of all the four groups as described15. Preliminary studies had shown that such lithium exposure has no effect on glomerular or tubular function or renal structure. At the end of the 14th day of CsA treatment, serum lithium and 24-hr urinary lithium were estimated by using flame photometer and lithium clearance was calculated as described by Whitling and Simpson15. Ccr-Licr reflects the absolute (iso-osmotic) reabsorption of lithium and water in the proximal tubule, while the proximal fractional reabsorption (proximal tubular reabsorption as fraction of GFR), expressed as 1-(Licr/Ccr), was calculated. The basis of these calculations had been earlier validated in experimental animals15.

Lipid peroxidation products — To investigate the possible involvement of free radicals, product of free radicals, lipid peroxide was estimated in plasma by using thiobarbituric acid (TBA) method16. Since this method measures the malondialdehyde (MDA) reactive products, the final result obtained is referred as MDA-equivalents (MDA-eq).

Histopathological examination — Kidney from all the four treated groups were fixed in 10% buffered formalin and processed with paraffin wax. Sections (5 μm thick) stained with hematoxylin and eosin, periodic acid Schiff’s stain and Von Kossa’s stain was used to detect calcification. The slides were coded and were examined by a histopathologist who was ignorant about the treatment group.

Statistics — The statistical significance of differences among values of individual parameters was evaluated by using Student’s t test. All values are expressed as mean ± SD.

Results and Discussion

In the present study CsA (50 mg/kg) caused significant functional and histological changes in kidney. CsA caused dose-related decrease in renal function in experimental animals17 and humans18,19. Renal dysfunction observed after CsA administration in the present study was characterized by increase in blood urea, serum creatinine (Fig. 1), reduced Ccr, Licr, increased urine flow rate (Table 1) and histopathological damage similar to that reported in other studies15,20. An CsA-induced increase in blood urea, serum creatinine and fall in Ccr and Licr was significantly prevented PTX. Proximal tubular reabsorption (1-Licr/Ccr) was increased with CsA, but absolute iso-osmotic reabsorption in the proximal tubule (Ccr-Licr) remain unaltered. PTX blocked the CsA-induced increase in proximal tubule fractional reabsorption (Table 1). There was an increase in urine volume, sodium excretion and Licr with PTX

![Fig. 1—Effect of cyclosporine and pentoxifylline on blood urea, serum creatinine and plasma malondialdehyde (MDA). (All values are expressed as mean ± SD from 6 animals. P: * < 0.05 compared to control; ** < 0.05 compared to cyclosporine).](image-url)
(Table 2). Xanthine derivatives, theophylline, caffeine have mild diuretic and natriuretic effects. Similar increase in urine flow and sodium excretion with PTX has been reported in rats. This may explain the increase in Licr in PTX and PTX+CsA groups. Creatinine clearance did not alter with PTX in the present study, this in accordance with previous studies in rats.

The present data clearly demonstrate protective effect of PTX against CsA-induced nephrotoxicity. Pentoxifylline prevents nephrotoxicity induced by mercuric chloride, gliceryl, amphotericin B and cadmium chloride in rats. Methylxanthine derivative (HWA-448), structurally similar to PTX, showed dose dependent protection in rat model of candidiasis against amphotericin B renal toxicity. It has been postulated that various manifestations of CsA renal toxicity can be attributed to its effects on renal vessel. In animal models of hemorrhagic shock, PTX leads to an immediate hyperemic response with an increase in microvascular blood flow that protects tissues from ischemic damage.

In vivo studies have evaluated the ability of pharmacological antagonists to reverse CsA-induced vasoconstriction. In vitro pretreatment with theophylline could reduce the number of mesangial cells contracting with CsA. On isolated perfused rat kidney PTX attenuated the endothelial dysfunction and the functional changes induced in smooth muscle cell by CsA. Clinical benefit reported with the use of PTX on patients chronically exposed to CsA, may partially be due to improvement of vascular endothelial function. Endothelin receptor blockade inhibits afferent arteriolar vasoconstriction-induced by CsA and reduces renal toxicity. Oral pretreatment with PTX significantly reduces renal vasoconstriction and endothelin release due to CsA administration in dog.

Malondialdehyde (MDA) was elevated in plasma with CsA treatment; PTX significantly prevented CsA-induced MDA increase (Fig.1). A role of reactive oxygen formation in CsA mediated impairment of renal functions has been suggested. It has been demonstrated that CsA induces lipid peroxidation in cell culture. The protection provided by allopurinol against CsA-induced deleterious effects of lipid peroxidation may be due to reduced formation of oxygen radicals. Histologically, kidney specimen of CsA treated rat showed corticomedullary calcification (Fig.2B). Nephrocalcinosis at the corticomedullary junction, calcified laminated concentrations in tubules and microcalcification are seen with CsA. A specific effect of CsA on mitochondrial calcium release has been suggested as the basis of CsA nephrotoxicity. In rats a decrease in calbindin-D-38kD protein levels by CsA has been shown to increase intratubular kidney calcification. Animals treated with PTX did not show any

Table 1—Effect of cyclosporine and pentoxifylline on renal functions in rats
[Values expressed as ml/hr/kg are mean ± SD from 6 rats]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CsA</th>
<th>PTX</th>
<th>PTX + CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFR</td>
<td>3.5 ± 1.63</td>
<td>5.5 ± 0.6*</td>
<td>6.23 ± 1.9*</td>
<td>7.83 ± 2.86*</td>
</tr>
<tr>
<td>Cr.clea rance</td>
<td>234 ± 40</td>
<td>137 ± 25*</td>
<td>218 ± 56</td>
<td>279 ± 49*</td>
</tr>
<tr>
<td>Li.clea rance</td>
<td>99 ± 19</td>
<td>57 ± 6.7*</td>
<td>191 ± 52*</td>
<td>215 ± 43*</td>
</tr>
<tr>
<td>Ccr-Licr</td>
<td>112 ± 50</td>
<td>93 ± 33</td>
<td>97 ± 38</td>
<td>122 ± 27</td>
</tr>
<tr>
<td>1-(Licr/Ccr)</td>
<td>0.4 ± 0.18</td>
<td>0.60 ± 0.07*</td>
<td>0.089 ± 0.038*</td>
<td>0.498 ± 0.284*</td>
</tr>
</tbody>
</table>

P values: *< 0.05 compared to control ; + < 0.05 compared to cyclosporine treated group.
CsA = cyclosporine; PTX = pentoxifylline; UFR = urine flow rats; Cr = creatinine clearance; Licr = lithium clearance; Ccr – Licr = absolute iso-osmotic reabsorption of the proximal tubule; 1-(Licr/Ccr) = proximal tubule fractional reabsorption.

Table 2—Effect of cyclosporine and pentoxifylline on renal functions in rats
[Values expressed as mmoles/kg/day are mean ± SD from 6 rats]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>CsA</th>
<th>PTX</th>
<th>PTX + CsA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary Sodium</td>
<td>8.92 ± 3.3</td>
<td>16 ± 1.9*</td>
<td>21.1 ± 4.9*</td>
<td>26.6 ± 13.2</td>
</tr>
<tr>
<td>Urinary Potassium</td>
<td>4.8 ± 2.2</td>
<td>7.34 ± 0.94*</td>
<td>6.80 ± 1.58</td>
<td>10.48 ± 3.6*</td>
</tr>
<tr>
<td>Urinary Chloride</td>
<td>4.7 ± 1.34</td>
<td>7.67 ± 2.37</td>
<td>4.18 ± 0.7</td>
<td>7.79 ± 4.16</td>
</tr>
</tbody>
</table>

P value: *< 0.05 compared to control
CsA = cyclosporine; PTX = pentoxifylline
Fig. 2A—Photomicrograph of kidney from control rat treated with only normal saline (H&E 600). B — Kidney from rat treated with cyclosporine showing a) marked calcification, b) widening of interstitium, c) vacuolar degeneration of tubular epithelium (H&E×600). C — Kidney from rat treated with pentoxifylline and cyclosporine shows patchy epithelial damage of proximal tubular cells, obliteration of the lumen in the focal areas with scattered infiltration of mononuclear cells, glomeruli are essentially normal (H&E×600).
Renal histology was normal in animals treated with PTX plus CsA (Fig. 2C). CsA induced clinical use of calcification in mesangial and glomerular contraction through xanthine derivatives. Present study suggests that supplementation was shown to prevent CsA-induced arteriopathy in a rat model of chronic cyclosporine nephropathy, Kidney Int, 48 (1995) 431.

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References


