Comparative interaction of few antihypertensive drugs with Cyclosporine-A in rats

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The maximal endothelial dependent relaxation of isolated aortic rings to cumulative doses of acetylcholine was significantly decreased in the Cyclosporine-A (CSA, 20 mg kg$^{-1}$ day$^{-1}$) treated animals compared to olive oil (CSA vehicle) treated control. Administration of antihypertensive drugs like diltiazem, enalapril or propranolol to CSA treated animals augmented the endothelial damage induced by CSA. These drugs also increased the bioavailability of CSA. However, administration of losartan to CSA treated animals produced a significant increase in endothelial dependent relaxation as compared to CSA treated control but did not affect the bioavailability of CSA significantly. The results suggest that losartan is safer compared to other antihypertensives for the treatment of CSA induced hypertension.

Keywords: Cyclosporin-A, Diltiazem, Enalapril, Hypertension, Losartan, Propranolol, Thoracic aorta

Hypertension is a serious problem in most patients who have undergone renal transplantation and receiving Cyclosporine-A (CSA) as the immunosuppressant. The pathogenesis appears to be multifactorial but vasoconstrictor tone in renal and systemic vascular beds is one of the main outcomes. Long-term CSA therapy, even at low doses is associated with high incidence of hypertension.

The angiotensin II levels are increased by CSA in rats. Further, it is known that the rennin-angiotensin but not the kallikrein-kinin system plays a crucial role in CSA-toxicity. The increase in angiotensin II effect after treatment with angiotensin converting enzyme inhibitor or CSA is reported to be due to upregulation of angiotensin receptors in the aortic smooth muscle. In early stages of CSA toxicity, the predominant functional alteration occurs at the endothelium level. The abnormal vascular function is characterized by impaired prostacyclin and endothelium dependent relaxing factor.

Medical management of the transplant recipient requires effective treatment of hypertension, avoiding interactions between antihypertensive regimens and CSA. Calcium channel blockers have achieved the status of “preferred” drugs in CSA induced hypertension but several calcium channel blockers like verapamil, diltiazem, and nicardipine are likely to interfere with CSA metabolism leading to an increase in its plasma concentration. The objective of the present is to compare the possible drug-drug interaction between certain antihypertensive drug and CSA and to identify the better antihypertensive drug that can be used to treat CSA induced hypertension.

Materials and Methods

Chemicals and drugs — Cyclosporine-A (Strides Arco Labs, Bangalore, India), phenylephrine (BDH Chemicals Ltd, Poole, England), acetylcholine (Kochlight Laboratories Ltd, Poole, England), enalapril (Cadila Healthcare, India), diltiazem (Torrent Pharmaceuticals, India), propranolol (ICI, India), losartan (Unichem, India) were obtained in pure form and for HPLC estimation, reagents used were of HPLC grade.

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Animals — Adult Wistar rats (250-300 g) were used for the study. The institutional Animal Ethics Committee approved the experimental procedures. Animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Method — The animals were divided into 6 groups of 8 each. The first group served as control and received only vehicle (olive oil, 1 ml/kg, ip). The rats in other five groups received CSA (20 mg/kg, ip) dissolved in olive oil for 9 days. The second group served as CSA treated control while the animals in group III, IV, V and VI received diltiazem (60 mg/kg, po)\textsuperscript{19}, enalapril (10 mg/kg, po)\textsuperscript{20}, losartan (6.0 mg/kg, po)\textsuperscript{21} and propranolol (1.0 mg/kg, po)\textsuperscript{22} respectively from 6\textsuperscript{th} to 9\textsuperscript{th} day of CSA treatment. On the 6\textsuperscript{th} and 10\textsuperscript{th} day, blood was drawn 2 hr after dosing. The plasma was separated and was used for estimation of CSA by high performance liquid chromatography (HPLC) method. On the 10\textsuperscript{th} day, animals were sacrificed under light ether anaesthesia and a mid-sternal dissection was performed to cut open the thorax so as to expose the thoracic contents and the arch of aorta along with the thoracic aorta was carefully dissected out.

Effect on acetylcholine induced endothelial dependent relaxation — The thoracic aorta was placed in a petridish containing warm physiological salt solution (PSS) maintained at 37±0.5°C (pH 7.4). The composition of PSS (mM) was: NaCl, 118; KCl, 4.7; CaCl\textsubscript{2}2H\textsubscript{2}O, 2.5; MgSO\textsubscript{4}.7 H\textsubscript{2}O, 0.5; NaH\textsubscript{2}PO\textsubscript{4}, 1.0; NaHCO\textsubscript{3}, 25; glucose, 11 and EDTA, 0.03; dissolved in double distilled water. A mixture of O\textsubscript{2} (95%) and CO\textsubscript{2} (5%) was bubbled through the PSS. Fat and connective tissues were carefully trimmed away without injuring the aorta. Aorta was cut into 5 mm wide transverse rings without damaging the endothelium\textsuperscript{23,24}. These rings were suspended in 10 ml jacketed tissue bath containing PSS. The tissue was connected via a silk suture to a force displacement transducer (Grass FT 03) coupled with the preamplifier of a Grass polygraph (Model 79) to record the tissue activity at the tension of 2 g. After 1 hr equilibration period, a submaximal dose of phenylephrine (30 nM) was added to prime the aorta for 1-2 min and washed. All the tissues were further allowed to equilibrate for at least 1.30 hr before drug application. During the total equilibration period of 2.30 hr, PSS was replaced several times\textsuperscript{25}. Using pre-contracted aorta with phenylephrine (10 nM), the dose response curves were recorded by cumulative addition of acetylcholine (Ach), followed by sodium nitroprusside (SNP) until complete relaxation.

Extraction of CSA from blood plasma — The plasma was separated and to 0.3 ml of plasma, 0.2 and 0.4 ml of the standard solution of cyclosporine-A (1000 μg/ml) were added. The plasma samples were lysed by rapid deep freezing and then thawed followed by addition of 0.3 ml of buffer solution (pH 10). The solutions were mixed for 5 sec with a vortex mixer and the final volume was adjusted to 2 ml, using diethyl ether. The tubes were shaken mechanically for 5 min on a horizontal shaker and centrifuged for 5 min at 800 g. An aliquot (1 ml) of the separated diethyl ether layer was then transferred to another conical centrifuge tube, which had been previously rinsed with diethyl ether. Another extraction was performed with addition of 1 ml of diethyl ether and then the two ether extracts were mixed and evaporated to dryness under vacuum at 40°C. The residue, concentrated at the bottom of the conical tube was dissolved in 1 ml of the mobile phase and injected into the chromatograph.

A reverse phase HPLC method for the determination of cyclosporine-A in blood plasma using Wakosil 5C–18 RS column was developed with mobile phase consisting of acetonitrile: ammonium acetate buffer (pH 4) in the ratio 90:10. The detector was set at 210 nm and the flow rate was maintained at 0.5 ml/min, retention time being 9.2 min that resulted in recovery data between 95.5 and 101% and a % RSD of 0.75\textsuperscript{26}.

Statistical analysis — Results are expressed as mean±SE. Statistical significance was determined using unpaired Student’s \textit{t}-test for all parameters. Statistical significance was considered when \( P \) value was less than 0.05.

Results
In CSA treated animals, the relaxant response of Ach (endothelium dependent) decreased when compared to tissues from untreated control (\( P < 0.001 \)). Ach induced relaxant responses in animals treated with both CSA and losartan were increased when compared with CSA treated group (\( P < 0.05 \); Fig. 1). The concomitant administration of CSA and different antihypertensives like diltiazem, enalapril
and propranolol increased the concentration of CSA by about 31, 24 and 64% respectively at the end of the treatment. However, concomitant administration of CSA and losartan produced only 5% increase in CSA concentration (Fig. 2).

Discussion

In the present study, administration of losartan to CSA treated animals reduced the CSA induced endothelial damage. The other antihypertensives tested viz. diltiazem; enalapril and propranolol did not show any significant effect on CSA induced endothelial damage. Concomitant administration of CSA with antihypertensives revealed that the bioavailability of CSA might be increased when it is administered along with diltiazem, enalapril and propranolol. About 80-90% of the patients receiving transplant develop hypertension when treated with CSA and they require antihypertensive therapy. Transplant recipient requires effective antihypertensive therapy that does not interact with the pharmacokinetics of CSA. A significant decrease in endothelium dependent relaxation to Ach correlates with concomitant increase in CSA concentration in the groups treated with CSA and antihypertensive drugs such as diltiazem, enalapril and propranolol. Thus, concomitant treatments with these antihypertensives may augment the level of CSA leading to toxicity in general. However; this was not seen when CSA was administrated with losartan.

Calcium channel blockers (diltiazem) are known as “preferred” drugs in CSA induced hypertension because of their efficacy in smooth muscle vasodilatation but these are reported to interact with CSA leading to increase in its bioavailability. The status of the calcium channel blockers depends on the advantage of vascular relaxation over CSA level in the plasma. CSA induces hyperreninemic hypoadosteronism and causes relative resistance of the adrenal zona glomerulosa to Ang II. Angiotensin II increases tissue endothelin and induces vascular hypertrophy. Chronic CSA administration, activates the rennin-angiotensin-axis system (RAAS), suppresses circulating atrial natriuretic peptide and results in chronic sodium retention. It also attenuates the natriuretic and diuretic response to acute volume expansion. The results of the present study suggest that losartan may be a better drug for the management of hypertension induced by CSA.

References


