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The effects of nifedipine on cyclosporine nephrotoxicity in rats

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Abstract. We have investigated the effect of nifedipine on cyclosporine nephrotoxicity in the Sprague-Dawley rat employing a repeatable, single-shot, isotopic technique of measuring the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF). Groups of 10 rats received either cyclosporine 5 mg/kg daily or cremaphor with either nifedipine 0.5 mg/kg daily or its vehicle for 14 days. In the cyclosporine group the GFR ($P < 0.001$, paired t-test), ERPF and filtration fraction (FF) ($P < 0.01$) all fell significantly. The cyclosporine plus nifedipine group underwent an increase in the ERPF ($P < 0.01$), the GFR remained unchanged, and the FF fell significantly ($P < 0.0001$). In this model, nifedipine completely abolished the renal arteriolar vasospasm produced by cyclosporine. That the FF fell in the cyclosporine plus nifedipine-treated animals indicates that cyclosporine has an effect which is not mediated by arteriolar vasoconstriction. This action may be at the glomerular level and is resistant to calcium channel blockade.

Key words: Cyclosporine nephrotoxicity – Nifedipine – Calcium channel blockade – Renal haemodynamics

Cyclosporine remains the mainstay of clinical immunosuppression for both solid organ and bone marrow transplantation. The associated nephrotoxicity, however, has limited its clinical use and has imposed a narrow therapeutic window for clinicians. There is mounting evidence that long-term use of cyclosporine results in chronic renal damage in both renal allografts [7, 24] and in patients with functioning native kidneys [14, 15]. The future of cyclosporine may therefore heavily depend on the results of investigation of the nature of its nephrotoxic action and the development of therapeutic intervention strategies to limit this effect.

Mechanistically speaking, the organic calcium channel blocking group of drugs might counter cyclosporine nephrotoxicity. Most of the experimental evidence of a beneficial effect, however, has come from studies on animal models which poorly represent the clinical situation, in that the administration of cyclosporine was acute, and the animals underwent extensive surgical preparation in order to measure renal function. As a result, clinicians have generally remained unconvinced of their efficacy, and few routinely employ calcium channel blockers for this purpose.

We have developed a new rat model of cyclosporine nephrotoxicity which closely represents the clinical situation in terms of the dose of cyclosporine used, the decrement in renal function observed and the duration of administration of the drug. This study describes the effects of nifedipine on this model.

Materials and methods

Male Sprague-Dawley rats weighing 180–320 g were used. The animals were housed 5 per cage and were kept at a constant temperature of 21 °C with 12-h cycles of light and dark. They had free access to water and were fed on a standard diet (Pilsbury's modified rat and mouse breeding diet).

Technique of measurement. The method used to use to measure the glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) was a single injection and single blood sample isotopic technique using chromium-51-labelled ethylene diamine tetra-acetic acid (^{51}Cr-EDTA) and iodine-125-labelled Hippuran (^{125}I-Hippuran) [4, 18, 21].

The animals were lightly anaesthetised in an ether chamber. Vascular access was obtained by puncture of the ventral tail vein with a 23 gauge butterfly needle. Providing there was good blood flow indicating that a clean puncture of the vessel had been obtained, the isotopes were injected. The isotopes were delivered in a volume of 0.4 ml 0.9% NaCl, and the activity of each was approximately 0.5 becquerel. The animals were allowed to recover from the anaesthesia and re-anaesthetised 1 h later when 1–2 ml of blood was taken from the tail vein at a site distant from the injection site. The blood was anticoagulated with lithium heparin and the plasma separated. The dose of radioactivity administered, the specimens and the residual radioactivity in the syringe used for the injection were counted on a Scaler Timer ST7 scintillation counter (Nuclear Enterprises, Thorn EMI).

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Table 1. GFR, ERPF and FF before the institution of the various treatment regimens and after 14 days of treatment

Group	Day 1	Day 14
(A) Cyclosporine ($n = 8$)		
GFR (ml/min)	2.39 (0.4)	1.31 (0.33)[3*]
ERPF (ml/min)	6.48 (0.66)	4.86 (0.78)[2*]
FF	36.8 (4.67)	26.9 (4.7)2*
(B) Nifedipine and cyclosporine		
GFR (ml/min)	2.03 (0.28)	2.03 (0.26)
ERPF (ml/min)	5.14 (0.75)	5.87 (0.54)[1*]
FF	39.5 (1.9)	34.5 (1.7)[4*]
(C) Double vehicle		
GFR (ml/min)	2.24 (0.3)	2.51 (0.23)[1*]
ERPF (ml/min)	5.72 (0.4)	6.35 (0.53)[2*]
FF	39 (3.8)	39 (2.1)
(D) Nifedipine		2.12 (0.27)
GFR (ml/min)	2.02 (0.24)	5.71 (0.61)
ERPF (ml/(min)	5.51 (0.5)	37 (2.6)
FF	37 (2.1)	

[1*] $P < 0.05$, [2*] $P < 0.01$, [3*] $P < 0.001$, [4*] $P < 0.0001$, by paired t-test
Mean and SD
Cyclosporine (5 mg/kg daily) causes a marked reduction in all parameters. Nifedipine (0.5 mg/kg daily) administration with cyclosporine causes reversal of the effect of cyclosporine on the ERPF and significant amelioration of the effect on the GFR. The FF remains depressed despite nifedipine
Filtration fraction (FF) = GFR/ERPF × 100; GFR, glomerular filtration rate; ERPF, effective renal plasma flow

Calculation of renal haemodynamics. Assuming instantaneous mixing of the clearance substances in the intravascular compartment and excretion exclusively via the glomerulus, the clearance substances will disappear from the plasma in a monoexponential fashion. Thus, the logarithm of the concentration plotted against time will be a straight line.

The initial plasma concentrtion (P_0) can be calculated from the injected dose (I) and the volume of distribution (V) in the animal (P0 = I/V). From the line drawn between these points, the decay constant (k) can be calculated.

$$k = \ln(P_0/T_t)/t$$

$$\text{Clearance} = V \cdot k$$

$$\text{Clearance} = V \cdot \ln(P_0/P_t)/t$$

where t is the time from injection and
P_t the plasma concentration at time t.

V has already been calculated in the rat over a wide range of body weights for both ^{51}Cr-EDTA and ^{125}I-Hippuran. The published data have been employed in our calculations [4, 18].

Blood pressure. Systolic blood pressure was measured using a tail blood pressure cuff, a pulse sensor attached to a piezoelectric crystal and an automated electrosphygmomanometer (Narco Biosystems). The animals were lightly sedated using Hypnorm (fentanyl 3.15 g and fluanisone 0.1 mg). Each measurement was a mean of 3 estimations.

Cyclosporine 5 mg/kg daily dissolved in cremaphor (polyethoxylated castor oil) was administered by intraperitoneal injection at the same time every morning except for the day of measurement of GFR and ERPF.

Nifedipine 0.5 mg/kg · day was administered as a twice daily subcutaneous injection. Nifedipine was kindly supplied by Bayer U.K. (Newbury) in an injectable formulation.

Statistical methods. Data from other experiments using this model have shown a normal distribution for the parameters measured, therefore parametric statistical analysis was used. The paired t-test was employed to compare data within each group, and comparisons between groups have been made using the unpaired t-test.

Study plan. Measurement of GFR and ERPF was made before and after 14 days of administration of the various drug regimens. Weight and blood pressure measurement was carried out throughout the study.

There were 4 study groups according to treatment: (A) cyclosporine, (B) cyclosporine and nifedipine, (C) both vehicles and (D) nifedipine. Groups A and D also received the vehicle for the other agent.

Results

In the cyclosporine group A ($n = 8$) there was a significant fall in GFR ($P < 0.001$), ERPF ($P < 0.01$) and filtration fraction (FF) ($P < 0.01$) over the study period (Table 1).

In the nifedipine and cyclosporine group B, in contrast to group A, the addition of nifedipine prevented a fall in GFR. In addition, there was a significant rise in ERPF ($P < 0.05$). The relative difference in change in ERPF and GFR resulted in a fall in FF ($P < 0.0001$). This fall in FF (5.04 ± 2.06; mean and SD) was significantly less ($P < 0.02$, unpaired t-test) than that seen in group A (9.94 ± 5.3).

In the control, double vehicle group C there was a significant increase in both GFR ($P < 0.05$) and ERPF ($P < 0.01$) during the 14 day period of the study. The FF remained unchanged.

In the nifedipine group D, there were no significant changes in any of the renal functional parameters. The GFR and ERPF both rose slightly, and the FF remained unchanged.

When the results at day 14 of the study were compared between groups, there was no significant difference in the percentage change in GFR or ERPF for groups B, C and D (unpaired t-test) (Figs. 1–3).

Systolic blood pressure. There were not significant changes in blood pressure in any of the groups (Table 2).

Body weight. The animals in the group A gained significantly less weight than in the other groups ($P < 0.005$, unpaired t-test). Group B gained weight at an equivalent rate to the control animals.

Discussion

This study has clearly demonstrated that nifedipine has a beneficial effect on cyclosporine nephrotoxicity in the Sprague-Dawley rat. Using a dosage of both drugs equivalent to those used in clinical practice, nifedipine completely abolished the effect of cyclosporine on the ERPF and markedly reduced its influence on the GFR.

The increase in the ERPF seen in group B was of the same order as that which occurred with increasing body weight in the double vehicle group (C), indicating that the arteriolar vasoconstriction associated with cyclosporine did not occur during co-treatment with nifedipine.

In addition, the GFR in group B remained unchanged over the 14-day treatment period, and its percentage change did not differ between groups C and D. This demonstrates that nifedipine produced a significant amelioration of the effect of cyclosporine on the GFR. The consquence of the difference in change between these

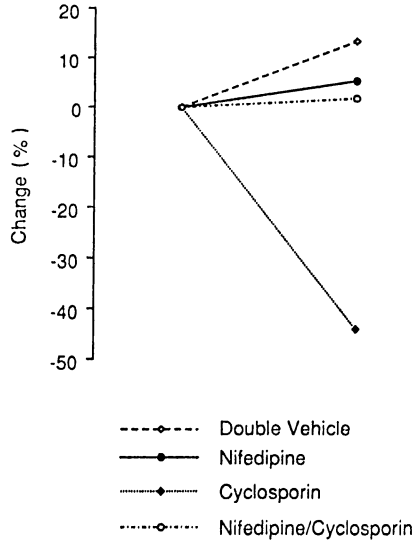

Fig. 1. Percentage change in GFR in each of the four groups. There was a significant fall in the cyclosporine-treated animals ($P < 0.001$, paired t-test) and a significant rise in the double vehicle group ($P < 0.05$, paired t-test), although when the percentage changes in GFR between the other three groups were compared, there was no significant difference between them

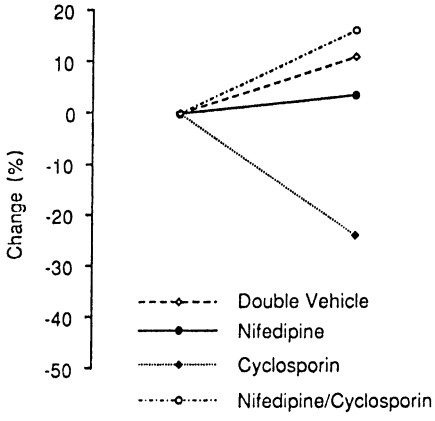

Fig. 2. Percentage change in ERPF in each of the groups. There was a significant fall in the cyclosporine-treated animals ($P < 0.01$, paired t-test) and a significant rise in the double vehicle and nifedipine/cyclosporine-treated gorups ($P < 0.01$ and 0.05, respectively, paired t-test)

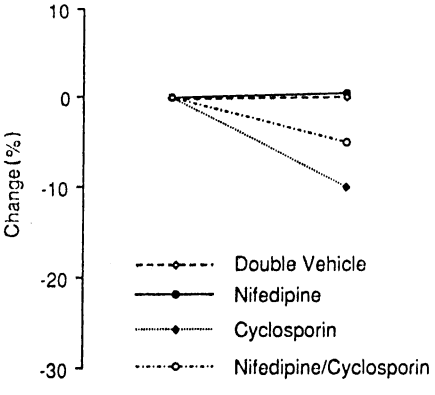

Fig. 3. Change in FF in each of the groups. There was a significant fall in both the cyclosporine-treated animals ($P < 0.01$, paired t-test) and the nifedipine/cyclosporine-treated group ($P < 0.0001$). The fall in FF in the cyclosporine-treated group was significantly larger than in the nifedipine/cyclosporine-treated group ($P < 0.05$, unpaired t-test)

Table 2. Systolic tail blood pressure for each group of rats before treatment and after 4 and 11 days of treatment. There were no significant changes in any of the groups showing that the changes observed in renal function were not a reflection of changes in systemic blood pressure

Group	Day 4	Day 11	Day 18
(A) Cyclosporine	133 (12)	136 (10.7)	131 (10.8)
(B) Nifedipine and cyclosporine	132 (6.3)	133 (10.9)	144 (16.8)
(C) Double vehicle	132 (23.5)	134 (14.8)	143 (20.2)
(D) Nifedipine	133 (16.4)	139 (17.3)	134 (14.6)

Treatment regimens instituted from day 7 to day 21 (mean and SD)
No significant differences by paired t-test

parameters was that the FF fell significantly, suggesting that arteriolar vasoconstriction is not the only mechanism by which cyclosporine is toxic to the renal microcirculation.

The mechanisms underlying the nephrotoxic effects of cyclosporine remain controversial and elusive. Cyclosporine nephrotoxicity is characterised by a reduction in the ERPF [8, 16, 22] and in the GFR [1, 5].

Current knowledge indicates that cyclosporine induces a reduction in renal plasma flow by causing constriction of the afferent glomerular arterioles. In a very elegant experiment using scanning electron micrographs of casts of the glomerular microcirculation, constriction of the afferent glomerular arterioles was demonstrated in Fischer rats which had received cyclosporine 50 mg/kg daily for 7 days [6]. The fall in GFR with cyclosporine may in part be a consequence of the afferent glomerular arteriolar constriction, which will cause a reduction in intraglomerular hydrostatic pressure.

The calcium channel blocking group of drugs are known to be potent renal vasodilators. They act via the voltage-sensitive calcium channels to reduce the calcium influx into the smooth muscle cell and thereby also the excitation-contraction coupling. It has been shown that these drugs are most active on the afferent glomerular arteriole [11], and this is the primary mechanism by which they could have a beneficial effect on cyclosporine toxicity.

Nifedipine was chosen for the present study as it is the only calcium channel blocking drug which has been shown not to interfere with the metabolism of cyclosporine [12, 23].

Recently, there has been some controversy as to whether cyclosporine causes a disturbance of glomerular filtration directly. Several groups of workers have demonstrated a reduction in the FF associated with cyclosporine in animal studies [3, 10, 17]. In human studies, a marked reduction in the FF in cardiac allograft recipients receiving cyclosporine was found [14], and in a study of 8 human volunteers receiving 4 mg/kg as an infusion over 6 h, a marked reduction in FF occurred [25].

Other workers appear to have found an opposite effect in animal [1, 19] and human [2] studies. In the rat studies that have not shown a decrease in FF, the renal functional parameters were measured after the acute administration of cyclosporine, and this may have influenced the results.

In our model, which closely represents the clinical situation, we have consistently shown a reduction in FF as a consequence of cyclosporine. As yet, there is no consensus, but overall it seems likely that cyclosporine does reduce the FF.

The most likely mechanism whereby cyclosporine could produce this fall in the FF is via contraction of the glomerular mesangial cells, resulting in a reduction in the surface area available for filtration within the glomerulus.

Using an isolated glomerular surface area model and cultured rat mesangial cells, cyclosporine has been shown to have a direct action on the contraction of these cells and to increase the contractile effect of other agents [13, 20]. This may be mediated via angiotensin II. It has been demonstrated in micropuncture studies that calcium channel blocking drugs reverse the effects of angiotensin II on the GFR and the ultrafiltration coefficient by preventing contraction of the glomerular mesangium [9].

That the FF remained reduced in the present study despite calcium channel blockade suggests that angiotensin II is not the sole mediator of the reduction in the FF in cyclosporine nephrotoxicity. This is in keeping with the in vitro studies which demonstrated that the mesangial cell contraction that occurred with cyclosporine was only partially reversed by verapamil and that it was more completely prevented by platelet activating factor antagonists [20].

The model of cyclosporine nephrotoxicity described here has several advantages over other models, particularly those which require surgical preparation of the animals in order to measure renal function. Our subjects undergo a minimum of stress and disruption to their physiological integrity. The ability to repeat the measurements in the same animals reduces the number of animals required as each acts as their own control, and the animals can be studied over a period of time that has relevance to the development of clinical cyclosporine nephrotoxicity. The techniques involved are also relatively easy to perform, resulting in a very low incidence of technical error.

In conclusion, it seems that the cyclosporine-induced vasoconstriction of the afferent glomerular arteriole is prevented by nifedipine; however, the effects of cyclosporine on the glomerular filtration are ameliorated, but not reversed. This may be cause cyclosporine has an action on the glomerular mesangium which is mediated by agents that are not countered by calcium channel blockade, possibly locally produced autocoids such as platelet activating factor or thromboxane A_2.

While the calcium channel blocking group of drugs do not offer a complete solution to cyclosporine nephrotoxicity, as demonstrated in this model, they do appear to provide useful therapy. It may be that this problem can be more completely solved by the use of calcium channel blockers in combination with other agents.

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