The Effect of Nifedipine on Renal Function in Normotensive Cyclosporin-A-Treated Renal Allograft Recipients

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Abstract. Intrarenal vasoconstriction is a characteristic feature of CsA nephrotoxicity. The influence of nifedipine, a dihydropyridine calcium channel blocker and potent renal vasodilator, on renal haemodynamics was investigated in 11 cyclosporin A (CsA)- and 9 azathioprine (Aza)-treated normotensive long-term renal allograft recipients. Baseline Cr²⁵¹-EDTA clearance and effective renal plasma flow (ERPF) were similar in both groups. Nifedipine 20 mg twice daily for 28 days significantly increased Cr²⁵¹-EDTA clearance (+14.8%) in the CsA group; however, ERPF, renal vascular resistance (RVR), and filtration fraction did not change. Nifedipine did not influence renal haemodynamics in the azathioprine group. The increase in Cr²⁵¹-EDTA clearance in the CsA group did not correlate with baseline renal function, CsA dose or whole blood levels, donor age, duration of graft, or renal functional reserve capacity. This study suggests that nifedipine confers a beneficial effect on renal haemodynamics in long-term CsA-treated renal allograft recipients and appears to improve renal function by a non-haemodynamic mechanism.

Key words: Cyclosporin nephrotoxicity; Nifedipine; Renal allograft recipients

Introduction

The introduction of cyclosporin A (CsA) into organ transplantation has improved graft survival rates [1]. However, it was realised from the first clinical trials that its major limiting side-effect was nephrotoxicity. Although the acute nephrotoxic effects are rapidly reversible with a reduction in the daily CsA dose [2], concern has recently been expressed regarding the reversibility of the long-term effects of CsA on the kidney [3,4]. In cardiac allograft recipients treated with CsA for 12 months or more, Myers et al have documented progressive renal microvascular damage, tubulointerstitial scarring, and irreversible loss of renal function [3,4].

The precise pathophysiological mechanisms underlying CsA nephrotoxicity remain unclear. However, experimental [5,6] and clinical [7] studies have suggested that haemodynamic factors play a pivotal role. In rats acute and chronic CsA administration induces a marked increase in renal vascular resistance (RVR) due to intrarenal vasoconstriction, with a concomitant reduction in renal blood flow and glomerular filtration rate (GFR) [5,6]. Increased activity of the renin–angiotension system [8], altered eicosanoid production [9] and excessive sympathetic nerve stimulation [5] have been implicated as potential mediators for the renal vasoconstrictor effects of CsA.

Although low-dose CsA protocols have recently been advocated to minimise the nephrotoxic effects of long-term therapy, a comparison of high- and low-dose protocols in cardiac allografts reported that low-dose CsA conferred only marginal protection against the development of microvascular damage [4]. Pharmacological intervention with renal vasodilators may, however, improve renal function and potentially prolong graft survival. Although several different classes of vasodilator, including prostaglandins, converting enzyme inhibitors,
and α-adrenoreceptor blockers have been shown to prevent the reduction in GFR and renal blood flow following acute CsA infusions [5,9-11], calcium channel blockers have proved most beneficial in experimental models of chronic CsA nephrotoxicity [6,12]. By contrast, manipulation of eicosanoid production in man with fish oil containing omega-3 polyunsaturated fatty acids [13] or vasodilator prostaglandins of the E series [14] have recently been reported to confer a beneficial effect on renal function in renal allograft recipients receiving CsA. To date no prospective clinical studies have been reported in similar patients administered calcium channel blockers, although a retrospective study of CsA-treated renal allografts documented better renal function in patients receiving nifedipine, a dihydropyridine calcium channel blocker, for CsA-related hypertension, than an equivalent group treated with other antihypertensive agents [15].

The present study investigates the effect of short-term nifedipine administration on renal haemodynamics in long-term CsA-treated renal allograft recipients, compared to a group of long-term azathioprine-treated recipients.

Patients and Methods

Patients

Twenty renal allograft recipients with stable renal function were studied. Eleven were receiving CsA and prednisolone and nine azathioprine and prednisolone maintenance immunosuppression. All were at least 6 months post-transplant and normotensive (<140/90 mmHg) without antihypertensive therapy, nor were they receiving other vasoactive therapy. Daily CsA dose and whole blood CsA values were stable. All patients had a stable protein intake for at least 6 months prior to the study (19 normal-protein and 1 low-protein diet). All studies were performed with informed consent and Local Ethical Committee approval.

Renal Function Studies

Study 1. The effect of nifedipine on renal function. All studies were performed in the morning following a light breakfast (<10 g protein). Patients attended with a baseline 24-h urine collection to measure urinary sodium, urea, and protein excretion. Lying and standing blood pressure was recorded in the non-fistula arm, after 15-min rest (mean of three readings). Baseline GFR was measured using chromium\textsuperscript{51}-labelled ethylenediaminetetra-acetate (Cr\textsuperscript{51}-EDTA) and ERPF measured using \textsuperscript{125}I-hippuran. Clearances were estimated using the methods of Chantler and Barratt [16] and Wagoner et al [17] respectively. A baseline serum biochemical profile and 12-h trough CsA level were taken before injecting a single bolus dose of Cr\textsuperscript{51}-EDTA (5 MBq) and \textsuperscript{125}I-hippuran (5 MBq) intravenously. Serial blood samples were taken after 10, 20, 30, 40, 50, and 60 min, and hourly thereafter for 4 h from an indwelling cannula in the opposite limb. Height (m) and weight (kg) were recorded and the results corrected for body surface area (1.73 m\textsuperscript{2}). Renal blood flow (RBF) was calculated as ERPF/(1 - haematocrit) and FF as the ratio of GFR/ERPF. Renal vascular resistance (RVR) was calculated using Gomez's formula [18]: \( RVR = \text{mean arterial pressure/RBF x 80,000 and expressed in dynes/s/cm}^{-5}/10^3/1.73\text{m}^2 \). Mean arterial pressure was calculated from diastolic pressure plus one-third of the pulse pressure.

After baseline studies patients were commenced on nifedipine SR 20 mg twice daily for 28 days and instructed to remain on the same diet for the duration of the study. Clearance studies were repeated at a second visit and trough whole blood CsA levels and serum nifedipine and its major metabolite M-I measured (2 h after the morning dose).

Study 2. Measurement of renal functional reserve capacity (RFRC). Eighteen patients were studied (10 CsA and 8 azathioprine treated). RFRC was assessed using a 80-g oral protein load in the form of cooked red meat. Studies were performed in the morning following an overnight fast. On arrival patients were water loaded orally (20 ml/kg) over 30 min, to achieve a urine output between 3 and 10 ml/minute. Hydration was maintained by replacing urine losses with an equivalent volume of water for the duration of the experiment. Following loading doses of Cr\textsuperscript{51}-EDTA (1 MBq) and \textsuperscript{125}I-hippuran (1 MBq) a sustaining infusion of both isotopes was maintained for 6 h (0.053–0.48 MBq/h and 0.25–1.23 MBq/h respectively). Infusion doses were calculated individually from baseline Cr\textsuperscript{51}-EDTA clearances obtained in study 1 by the method of Liedtke and Duarte [19]. After a 60-min equilibrium period two baseline 45-min urine collections were performed. Complete bladder emptying was confirmed by assessing the radioactivity (using a gamma counter) over the bladder region before and after voiding and comparing the radioactivity to that obtained over the cardiac region (background level). The patient was instructed to void again if the radioactivity remained high over the bladder region. The volume of urine passed was recorded and a 20-ml aliquot saved for counting. Blood samples were taken at the beginning and end of each collection period and the plasma saved for counting. Baseline clearances were derived from the mean count of the two 45-min urine collections and mean count of the two plasma samples using the standard clearance formula. Clearance periods with urinary flow rates outside the range 3–10 ml/min were discarded because of the effects of dehydration and overhydration to renal function.
After baseline collections patients ingested an 80-g protein meal and a further 60-min equilibrium period was observed. Three further 45-min urine collections were performed with blood sampling before and after each urine sample. The results were corrected for body surface area (1.73 m$^2$) and expressed as a percentage of the baseline renal function.

**Laboratory Methods**

Serum and urinary electrolytes, creatinine, urinary urea, and protein were measured using standard laboratory techniques. Whole blood CsA levels were measured by high-performance liquid chromatography (HPLC; mean of three measurements). Serum nifedipine and its major metabolite M-I were measured by automated electron-capture capillary gas chromatography [20]. Radioactivity was counted in duplicate 2-ml aliquots of serum and urine, using a Philips PW4800 automatic gamma counter.

**Statistics**

Results are expressed as mean ±SEM. Statistical comparisons were made using Wilcoxon matched pairs signed rank test and Mann-Whitney U test for paired and unpaired data respectively. Correlation was assessed using Spearman’s rank correlation test. Statistical significance was taken as $P<0.05$.

**Results**

Nineteen patients (10 CsA and 9 azathioprine) completed the study (one patient on CsA was withdrawn because of an unrelated pyrexial illness during the study period). The mean age of the patients was similar in both groups (Table 1). However, duration of graft was significantly longer in the azathioprine-treated group. The mean daily CsA dose and CsA whole blood level at baseline are shown in Table 1. All patients were receiving 10mg of prednisolone on alternate days at the time of the study. At baseline, serum creatinine, Cr$^{51}$-EDTA clearance and ERPF were not significantly different, although FF was significantly lower in the CsA-treated patients (Table 2). Twenty-four-hour urinary urea did not change throughout the study period in either the azathioprine or CsA-treated patients (66.9 ± 14.8 vs 66.9 ± 13.2 and 35.1 ± 5.6 vs 27.7 ± 7.3 ng/ml respectively).

Serum creatinine concentration did not change after treatment in the CsA-treated patients. However, a poor correlation between the prevailing serum creatinine concentration and the Cr$^{51}$-EDTA clearance was demonstrated in this group ($r_s = 0.06$, n.s.), as opposed to the azathioprine group ($r_s = -0.83$, $P<0.001$). The percentage increase in Cr$^{51}$-EDTA clearance in the CsA-treated patients following nifedipine therapy did not correlate with baseline Cr$^{51}$-EDTA clearance (Fig. 1), CsA daily dose or whole blood level, donor age, duration of graft, or number of rejection episodes.

**Systemic and Renal Haemodynamic Response to Nifedipine**

**Isotopic studies.** Table 2 documents the effect of nifedipine on mean arterial pressure, serum creatinine, renal haemodynamics, and FF for both groups. Mean arterial pressure was not influenced by nifedipine in either the azathioprine- or CsA-treated patients. There was an increase in Cr$^{51}$-EDTA clearance in CsA-treated patients (mean; +14.8% (range; -4 to 38%), $P<0.02$), although ERPF, RVR, and FF did not change. In azathioprine patients, Cr$^{51}$-EDTA clearance, ERPF, RVR, and FF were unchanged. Serum creatinine concentration did not change after treatment in the CsA-treated patients. However, a poor correlation between the prevailing serum creatinine concentration and the Cr$^{51}$-EDTA clearance was demonstrated in this group ($r_s = 0.06$, n.s.), as opposed to the azathioprine group ($r_s = -0.83$, $P<0.001$). The percentage increase in Cr$^{51}$-EDTA clearance in the CsA-treated patients following nifedipine therapy did not correlate with baseline Cr$^{51}$-EDTA clearance (Fig. 1), CsA daily dose or whole blood level, donor age, duration of graft, or number of rejection episodes.

**Study 2.** To investigate whether the increase in Cr$^{51}$-EDTA clearance following nifedipine therapy in the CsA-treated patients related to RFRC, the renal response to an 80-g oral protein load was documented. Eighteen patients were studied (10 CsA and 8 azathioprine). Cr$^{51}$-EDTA clearance and ERPF did not change following the

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Table 1. Characteristics of renal transplant recipients studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>Azathioprine group (n = 9)</th>
<th>CsA group (n = 10)</th>
</tr>
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<tbody>
<tr>
<td>M:F ratio</td>
<td>5:4</td>
<td>5:5</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.4 ± 3.7</td>
<td>54.3 ± 3.0</td>
</tr>
<tr>
<td>Duration of graft (months)</td>
<td>79.2 ± 12.9$^1$</td>
<td>23.4 ± 4.5</td>
</tr>
<tr>
<td>Mean daily CsA dose (mg/kg per day) (range)</td>
<td>—</td>
<td>5.1</td>
</tr>
<tr>
<td>CsA whole blood level (ng/ml)</td>
<td>—</td>
<td>(2.8–6.6)</td>
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$^1P<0.001$ compared to CsA group
Table 2. Effect of nifedipine on serum creatinine, creatinine clearance, Cr<sub>51</sub>-EDTA clearance, ERPF, RVR, and FF in Aza- and CsA-treated patients

<table>
<thead>
<tr>
<th></th>
<th>Azathioprine (n = 9)</th>
<th>CsA (n = 10)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>94.1 ± 2.7</td>
<td>93.3 ± 2.2</td>
</tr>
<tr>
<td>Serum creatinine (µmol/l)</td>
<td>156 ± 40</td>
<td>156 ± 40</td>
</tr>
<tr>
<td>Cr&lt;sub&gt;51&lt;/sub&gt;-EDTA clearance (ml/min/1.73 m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>58.5 ± 10.3</td>
<td>56.5 ± 9.3</td>
</tr>
<tr>
<td>ERPF (ml/min/1.73 m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>262.4 ± 42.9</td>
<td>274.9 ± 48.9</td>
</tr>
<tr>
<td>RVR (dynes/cm&lt;sup&gt;-5&lt;/sup&gt;/10&lt;sup&gt;3&lt;/sup&gt;/1.73 m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>23.5 ± 7.0</td>
<td>23.3 ± 6.9</td>
</tr>
<tr>
<td>FF (%)</td>
<td>22 ± 1</td>
<td>21 ± 1</td>
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</table>

<sup>1</sup>P<0.02 compared to baseline CsA Cr<sub>51</sub>-EDTA clearance

<sup>2</sup>P<0.05 compared to baseline Aza FF

![Graph](image-url)

Fig. 1. Percentage change in Cr<sub>51</sub>-EDTA clearance from baseline versus baseline Cr<sub>51</sub>-EDTA clearance following nifedipine in Aza- and CsA-treated recipients.

![Graph](image-url)

Fig. 2. Percentage change (maximum) in Cr<sub>51</sub>-EDTA clearance from baseline following an 80-g protein load in Aza- and CsA-treated patients.

Discussion

The administration of nifedipine (20 mg twice daily) for 28 days to long-term normotensive CsA-treated renal protein load in the CsA-treated patients. However, there was a small but significant increase in both Cr<sub>51</sub>-EDTA clearance and ERPF in the azathioprine-treated patients (12.1 ± 3.4%, P<0.02 and 13.1 ± 3.3%, P<0.02 respectively). Individual patients in the CsA group did, however, demonstrate a RFRC (Fig. 2). The maximum change in Cr<sub>51</sub>-EDTA clearance and ERPF in CsA patients did not correlate with baseline Cr<sub>51</sub>-EDTA clearance, CsA daily dose or whole blood levels, donor age, duration of graft, or the number of rejection episodes.
allograft recipients led to a significant increase in Cr\textsuperscript{+1}. EDTA clearance (+14.8%). This increase was not associated with a parallel increase in ERPF, or a reduction in either mean arterial pressure or RVR, suggesting a non-haemodynamic mechanism may be implicated. Although the pathophysiological mechanism underlying CsA-induced nephrotoxicity is uncertain, experimental studies suggest that CsA acts primarily on the renal arteriolar vessels [21] and/or the mesangial cell [22,23].

Murray et al [5] demonstrated rapid changes in renal haemodynamics in rats infused with CsA, including a reduction in renal blood flow and GFR, due to a marked increase in RVR. Recent observations indicate that the vasoconstrictor effects of CsA are predominantly limited to the preglomerular circulation [21,24]. Although calcium channel blockers, including nifedipine, are potent renal vasodilators [25], their capacity to induce renal arteriolar vasodilatation is dependent on the prevailing level of vascular tone. In studies performed with the isolated perfused kidney preparation calcium channel blockers induce vasodilatation only in vessels that have been previously exposed to vasoconstrictor agonists [25]. If nifedipine-induced afferent arteriolar vasodilatation is the principal mechanism responsible for the observed increase in GFR, then the predicted increase in glomerular filtration pressure would lead to a preferential increase in GFR, without necessarily altering renal blood flow. However, these changes in renal haemodynamics were not associated with a significant decrease in RVR, making it unlikely that nifedipine-induced afferent arteriolar vasodilatation is the major factor contributing to the increase in GFR.

Alternatively, the improvement in GFR induced by nifedipine may be secondary to an increase in the glomerular capillary ultrafiltration coefficient (Kf). Kf is determined predominantly by the available glomerular filtration surface area and is mainly regulated by mesangial cell contractility [26]. CsA-induced decreases in glomerular Kf have been reported [3,11]. Nifedipine could influence Kf by directly interfering with mesangial cell contractility or by antagonising the contractile response to angiotensin II, as both involve calcium-dependent mechanisms [26,27]. Experimental studies suggest that CsA is able to both induce mesangial cell contraction and augment contractility in the presence of agonists [22,23], with similar observations reported in vascular smooth muscle cells [28]. These effects may be secondary to CsA-enhanced transmembrane calcium influx and mobilisation from intracellular stores [23,28–30]. These changes in calcium homeostasis may potentiate mesangial contractility and thereby exaggerate the reduction in Kf and ultimately GFR. Nifedipine may potentially improve GFR by disrupting excitation–contraction coupling within the mesangial cell [27], although verapamil was recently shown to be only able to partially inhibit CsA-induced mesangial contraction [22]. Furthermore, the perturbations in cellular calcium kinetics induced by CsA were not abrogated by verapamil or nifedipine [23,29]. Thus, in-vitro studies infer that the improvement in GFR conferred by nifedipine and other calcium channel blockers may not be due entirely to a direct effect on mesangial cell contractility.

Nifedipine may operate by antagonising the actions of several vasoactive hormones acting on the mesangial cell, particularly angiotensin II and the vasoconstrictor prostaglandins. In both in-vivo and in-vitro studies CsA has been shown to stimulate angiotensin II and renin release [8,31], the effects of which may be blocked by nifedipine, as its actions are mediated by calcium-dependent pathways [32,33]. However, a major role for angiotensin II in CsA nephrotoxicity is doubted, as normal [34] or reduced activity [35] has been reported in clinical studies. Furthermore, conflicting results are reported with converting enzyme inhibition in experimental models of CsA nephrotoxicity, with both a complete reversal of CsA-induced vasoconstriction [11], or no response induced, despite documenting over-stimulation of the renin–angiotensin system [5,6].

Although calcium channel blockers can influence mesangial cell prostaglandin production after stimulation with vasoactive hormones [36], a recent study by Bunke and Wilder [12] suggests that the beneficial effect of verapamil in CsA nephrotoxicity is not due to increased production of vasoconstrictory prostaglandins. A reduction in all three measured glomerular prostaglandins was documented in rats treated with CsA, including PGE\textsubscript{2}, 6-keto-PGF\textsubscript{1α}, and thromboxane B\textsubscript{2}, while the ratio PGE\textsubscript{2} to thromboxane B\textsubscript{2} decreased.

CsA is metabolised by the same discrete hepatic isozyme P-450PCN1 as nifedipine [37]. Nevertheless, neither trough whole blood CsA levels nor serum nifedipine and its major metabolite M-I levels changed. Thus the improvement in renal function is not attributable to a reduction in CsA concentrations. On the contrary calcium channel blockers, including verapamil [38] nicardipine [39], and diltiazem [40] are reported to increase CsA whole blood concentrations.

Adverse side-effects related to nifedipine were reported by five patients, although none was serious, unexpected, or necessitated cessation of therapy. These included headaches during the first few days of treatment (n = 3), mild ankle oedema (n = 3) and dizziness (n = 2). Significant hypotension was not induced, corroborating previous reports that normotensive individuals are relatively insensitive to the hypotensive effects of nifedipine, in contrast to hypertensive subjects [41]. Although gingival hyperplasia is a recognised complication of both CsA and nifedipine therapy alone, no cases developed over the duration of the study. However, a previous study from our unit reported that concurrent administration of
nifedipine and CsA resulted in an increased rate of gingival hyperplasia, compared to CsA alone [42]. Thus, nifedipine should be avoided in patients with pre-existing gingival hyperplasia receiving CsA, and cessation of nifedipine therapy considered in those developing the complication after treatment is initiated.

Most CsA-treated patients (9/10) responded with an increase in Cr\(^{51}\)-EDTA clearance, albeit small in four patients. However, it is unclear which factors predict a marked response to nifedipine, as the percentage increase in GFR did not correlate with baseline renal function, donor age, CsA daily dose, CsA whole blood concentrations or RFRC. Although the duration since transplantation was significantly longer in the azathioprine-treated patients it is unlikely that the failure to respond to nifedipine was related to chronic scarring or vascular damage preventing changes in renal vascular tone, since the azathioprine-treated patients were able to increase ERPF and GFR after the protein load. The response to the protein load in the CsA-treated patients was variable, with two patients demonstrating a significant renal reserve capacity. However, there were no features to distinguish these patients from the CsA-treated patients who did not have a RFRC. Cairns et al [43] also did not demonstrate a RFRC in CsA-treated recipients after infusion with an aminocaproic load, suggesting that the increase in afferent vascular tone induced by CsA impaired the vasodilatory response to a protein load.

The absence of a change in serum creatinine despite an increase in Cr\(^{51}\)-EDTA clearance highlights the inadequacy of a single serum creatinine value in quantifying the prevailing level of renal function. Furthermore, in common with other workers a poor correlation between the prevailing serum creatinine and Cr\(^{51}\)-EDTA clearance was documented [44]. Thus, markedly impaired renal function may be associated with normal or only mildly elevated serum creatinine concentration in CsA-treated patients. Augmented tubular secretion of creatinine has been suggested as a possible mechanism for the reduced serum creatinine values documented [44]. Nevertheless, it is probable that serial measurements in individual patients will allow changes in renal function to be recognised, although the degree of renal impairment may be underestimated.

In summary, this study suggests that nifedipine may confer a beneficial effect on renal function in certain CsA-treated renal allograft recipients even after long-term therapy. However, further studies are needed to identify those patients demonstrating a clinically relevant response and to determine whether prolonged concomitant therapy with CsA and nifedipine immediately post-transplant will preserve renal function and mitigate the pathological changes associated with chronic CsA therapy. The mechanism underlying the beneficial effect may involve vascular and non-vascular mechanisms, in view of the pivotal role that calcium plays in many physiological processes within the renal microcirculation.

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