ABSTRACT

To evaluate the association of cyclosporine (CsA)-
related nephrotoxicity with nitric oxide (NO) and endo-
thelin, the effects of L-arginine (LIA) and branched-
chain amino acid (BCAA) infusions on renal hemody-
namics in 5 normal volunteers and 12 renal trans-
plant recipients were assessed. In normal hu-
mans, LIA, but not BCAA, reduced mean arterial pres-
sure and renal vascular resistance while increasing
RPF and urinary nitrate (NO3−) excretion. Group 1
included six transplant recipients not on CsA; Group 2
subjects (N = 6) were receiving CsA. In both groups,
mean arterial pressure declined during the infusion of
LIA (116 ± 4 to 109 ± 4 mm Hg; P < 0.001) but not BCAA
(116 ± 3 to 115 ± 3; P = not significant). In Group 1, LIA
increased RPF 33 ± 13% (329 ± 48 to 436 ± 77 mL/min
per 1.73 m²; P = 0.01) and GFR 37 ± 16% (95 ± 7 to
130 ± 18 mL/min per 1.73 m²; P = 0.01); renal vascular
resistance declined 27 ± 6%. In Group 2, LIA did not
affect renal hemodynamics. No changes occurred
with BCAA in either group. LA increased urinary NO3−
excretion by 27 ± 17% in Group 1 (P < 0.05), but only
by 16 ± 13% in Group 2 (P = not significant). Urinary
endothelin excretion was higher in Group 2 subjects
(10.1 ± 1.3 versus 5.3 ± 0.8 pg/mL of GFR, P < 0.01).
LIA-induced renal vasodilation is associated with the
increased urinary excretion of NO3−. The impaired
response noted in the presence of CsA could reflect
attenuated NO production and/or its local antago-
nism by a vasoconstrictor such as endothelin.

1 Received June 1, 1994. Accepted September 8, 1994.
2 Portions of the study were presented at the annual meeting of the American
354A), and at the 39th Annual Meeting of the American Society of Nephrology,
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Tower, University of Alabama at Birmingham, Birmingham, AL 35294.

Key Words: Hypertension, amino acid, renal hemodynamics,
nitric oxide, endothelin

During the past decade, cyclosporine (CsA) has
become the cornerstone of immunosuppressive
therapy in renal transplantation (1,2). However, the
utility of CsA is compromised by hypertension and
nephrotoxicity, the pathophysiology of which remain
incompletely understood (3,4). CsA is a potent vaso-
constrictor (5). In humans, CsA decreases RBF, in-
creases urea and sodium reabsorption, and impairs
renal functional reserve, all consistent with a mecha-
nism involving preglomerular vasoconstriction (6–10).

Although numerous vasoactive substances have
been postulated to mediate CsA-induced vasocon-
striction, experimental data obtained from animals
and humans are inconsistent (11). Recent studies
have implicated a newly described vasoconstrictor,
endothelin, in the pathogenesis of CsA nephrotoxicity.
Originating in epithelial, mesangial, and vascular en-
dotheolin cells, endothelin levels have been found to be
elevated in animals receiving CsA (12–14). Conversely,
endothelin-dependent relaxation, a vascular change
that reflects the local production of nitric oxide (NO), is
also impaired by CsA (15).

Vascular endothelial cells require L-arginine (LIA) to
produce NO via the action of the constitutive cytoplas-
mic enzyme NO synthase (16,17). In vivo, the resultant
NO diffuses into adjacent vascular smooth muscle,
and via a cGMP-dependent mechanism, induces va-
sodilation (18). LIA analogs, such as nitro-L-argi-
rine methyl ester and N’-methyl-L-arginine, when admin-
istered to animals, inhibit NO synthase, resulting in
elevated systemic blood pressure and renal vasocon-
striction (19,20). Alternatively, the chronic admin-
istration of LA stimulates NO production and attenu-
ares salt-sensitive hypertension and renal failure in
the Dahl/Rapp rat, perhaps via the activation of a
second, inducible NO synthase (21,22). To assess the
potential roles of altered endothelin and NO produc-
tion in CsA nephrotoxicity, we administered LA to
human renal allograft recipients, postulating that
CsA-based immunosuppression might influence renal
hemodynamic responses.

METHODS

Subjects

This study was performed in two phases. Initially, five
healthy, normotensive volunteers were recruited to estab-
lish the feasibility of the protocol. These subjects consisted of
three men and two women, aged 25 to 41 yr.

Subsequently, 12 male renal allograft recipients were re-
cruited from the kidney transplant clinic at the University of
Protocol

The protocol was approved by the University of Alabama at Birmingham Institutional Review Board for Human Use, and written, informed consent was obtained from all patients at the time of recruitment. Subjects were admitted to the GCRC and were placed on a diet containing 150 mg/day of sodium. Baseline laboratory values and a 24-h urine collection for creatinine clearance were obtained. Antihypertensive medications were discontinued after admission. In four subjects who were also receiving a single daily dose of diuretics (one receiving thiazide and three receiving furosemide), their administration was delayed each day until after amino acid infusion. Group 2 subjects continued to receive CsA at their previously stable dosage every 24 h.

After a 36-h equilibration period, subjects received, on consecutive days in crossover fashion, an infusion of 4% LA (30 g [360 ± 4 mg/kg or 3 ± 1 per minute] in 750 mL of 5% dextrose) (Kabi Pharmacia, Inc., Clayton, NC) and an equimolar, 4% solution of branched-chain amino acids (BCAA: BranchAmin; Clintec Nutrition Co., Deerfield, IL) containing leucine (1.38 g/dL), isoleucine (1.38 g/dL), and valine (1.24 g/dL). Six subjects received t-arginine before BCAA; in six the order was reversed. The washout period in each subject between amino acid infusions was 20 h. In Group 2 recipients, infusions occurred 18 to 22 h after the previous CsA dose. After the completion of the second amino acid infusion on Day 3, subjects were observed for 2 to 4 h and then discharged.

Two hours before the study infusions, subjects were given a 20 mL/kg oral water load. An IV catheter was placed in each arm, one for infusion and the other for blood sampling. Subjects then received 4 priming boluses of isothalamate (500 mg) (Conray 50; Mallinckrodt Medical, Inc., St. Louis, MO) and para-aminobiphenyl (PAH, 8 mg/kg) (Merck Research Laboratories, West Point, PA), followed by the continuous infusion of both isothalamate (100 mg/h) and PAH (700 mg/h) over 5 h. After 60 min of equilibration, urine was collected at 30-min intervals and output was replaced orally with water. In the initial normotensive subjects, after three 30-min periods to establish a baseline, a 90-min (three 30-min intervals) continuous infusion of either LA or BCAA was begun. In order to lengthen the observation period for transplant recipients, the amino acid infusion was continued for an additional 30 min (total, 2 h). During baseline and infusion periods, blood was collected at the midpoint of each 30-min interval. Blood pressure was measured by electronic sphygmomanometer every 10 min throughout the study.

Laboratory Analysis

Blood was collected in both heparinized tubes, with and without isobutylmethylxanthine, and calcium-EDTA-treated tubes, with and without aprotinin, and was immediately centrifuged. Plasma and urine were frozen at −70°C and then thawed for analysis.

Clearances of isothalamate and PAH were determined by the use of reverse-phase, high-performance liquid chromatography (HPLC) (Waters Division of Millipore, Milford, MA), as described previously (23,24). Two hundred microliters of acetonitrile (J.T. Baker, Inc., Phillipsburg, NJ) containing 0.035% HPLC-grade phosphoric acid (Fisher Scientific, Atlanta, GA) was added to 200 μL of plasma and urine samples. The samples were vortexed and then centrifuged at 3,200 × g for 10 min. Isothalamate and PAH in these samples were separated and quantitated by the injection of 5 μL of the supernatant onto a 30-cm μBondapak C18 reverse-phase column (particle size, 10 μm) (Waters). Standards prepared from stock solutions of isothalamate and PAH were run simultaneously. The mobile phase consisted of a mixture of HPLC-grade NH4H2PO4 (Fisher Scientific Atlanta, Georgia), 5 mM, and HPLC-grade acetonitrile (J.T. Baker) (98.5:1.5), pH 2.6 ± 0.01. The flow rate was 1.0 mL/min, resulting in an average pressure of approximately 1,000 psi. The detector was set at 236 nm for the analysis of both plasma and urine samples. All HPLC experiments were performed at 30°C with a column heater (Waters). The output detector and pumps were controlled by computer software (Baseline 810 Chromatography Workstation; Dynamic Solutions, Ventura, CA). Standard curves correlating peak height with the concentration of isothalamate and PAH were used to determine the concentrations in plasma and urine. Samples were run in duplicate, and the results were averaged. Isothalamate and PAH clearances were calculated as follows.

\[ C_{\text{IOH}} = \frac{(\text{urine flow rate} \times [\text{ISOH}]_{\text{urine}})}{[\text{ISOH}]_{\text{plasma}}} \]

and

\[ C_{\text{PAH}} = \frac{(\text{urine flow rate} \times [\text{PAH}]_{\text{urine}})}{[\text{PAH}]_{\text{plasma}}} \]

The mean coefficients of variation of these tests of GFR and RPF averaged 8.6 ± 1.8 and 8.2 ± 0.9%, respectively.

Renal vascular resistance (RVR) was calculated as mean arterial pressure (MAP/RPF) × 100 and is reported in resist-

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**TABLE 1. Characteristics of renal transplant recipients comprising Groups 1 and 2**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group 1</th>
<th>Group 2</th>
</tr>
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<tbody>
<tr>
<td>N</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>44 ± 5</td>
<td>40 ± 6</td>
</tr>
<tr>
<td>Race (Black:White)</td>
<td>0.6</td>
<td>3.3</td>
</tr>
<tr>
<td>Donor Source (Cadaver:Living)</td>
<td>1:5</td>
<td>5:1</td>
</tr>
<tr>
<td>Time Since Transplant (months)</td>
<td>94 ± 25</td>
<td>22 ± 5</td>
</tr>
<tr>
<td>Serum Creatinine (mmol/L)</td>
<td>1.6 ± 0.1</td>
<td>1.8 ± 0.1</td>
</tr>
<tr>
<td>Creatinine Clearance (mL/min)</td>
<td>81 ± 6</td>
<td>68 ± 4</td>
</tr>
</tbody>
</table>

* P < 0.01.
ance units (millimeters of mercury per milliliter per minute × 100). RPF, GFR, and RVR are reported as values per 1.73 m² body surface area. Urinary sodium concentration was measured by flame photometry. Plasma and urine uric acid concentrations were measured with an autoanalyzer (Ektachem 700; Eastman-Kodak, Rochester, NY).

Urinary endothenin was measured with a commercially available, specific enzyme immunometric assay (Cayman Chemical, Ann Arbor, MI), which recognizes endothenin-1, endothenin-2, and endothenin-3, but not big endothenin. Urine samples were prepared by passage over a 1-ml C18 reverse-phase cartridge (Bondapak), followed by elution with a methanol-water-trifluoroacetic acid mixture. The sample was then dried by vacuum centrifugation and reconstituted with enzyme immunometric assay buffer. Absorbance was then assayed in an automated plate reader (Thermo, Molecular Devices Corp., Menlo Park, CA) and compared with a standard curve to determine endothenin concentrations.

Urinary nitrite (NO₂⁻) and nitrate (NO₃⁻) excretion rates were determined as described previously (25,26). Urine samples and sodium nitrate standards (0 to 200 μM) were simultaneously reduced for 1 h at 37°C by Escherichia coli (ATCC 25922; American Type Culture Collection, Rockville, MD) that had been grown under anaerobic conditions. After centrifugation for 10 min at 1,000 × g, 50 μL of supernatant was added to 50 μL of 1.0% sulfanilamide in 30% acetic acid and 50 μL of 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride in 60% acetic acid (Griess reagent). After mixing, the optical density was read at 540 nm with a microplate reader (Thermo). To determine nitrite concentration, the protocol was repeated with the same samples, except the E. coli were not added and sodium nitrate, 0 to 300 μM, served as the standard. As expected, urinary nitrite (NO₂⁻) levels were undetectable. The mean coefficient of variation was 5.2 ± 1.2% for the nitrate assay. Urinary nitrate and endothenin excretion are reported as per 1.73 m² body surface area.

**Statistical Analysis**

For statistical analysis, the mean value of each parameter during baseline and infusion was determined. Each patient served as his own control. When data for a given parameter were normally distributed, a t test was used to compare means. For other data, the Wilcoxon signed rank test was used to compare baseline with infusion values within groups for both LA and BCAA, as well as to compare mean values during LA infusion with those obtained during BCAA infusion. The Mann-Whitney U test was used for intergroup comparisons. Data are presented as mean ± SE. A P value ≤0.05 was interpreted as statistically significant.

**RESULTS**

In the five normotensive controls, the protocol was well tolerated and without adverse effects. As noted in Table 2 and Figure 1, MAP declined with LA infusion, but did not change with BCAA. Likewise, LA infusion increased RPF and reduced RVR. No change in renal hemodynamics occurred with BCAA infusion. Mean GFR, reflecting wide variability in the data (declines in three subjects and increases in two) remained constant with both infusions. In all five control subjects, urinary NO₃⁻ excretion increased with LA relative to BCAA infusion (mean percent change: 41 ± 15 versus −1 ± 7; P ≤ 0.05).

Table 3 summarizes data from renal transplant recipients during L-arginine and BCAA infusions. As noted in Table 1, serum creatinine concentration and creatinine clearances did not differ between groups after admission to the GCRC. However, after water loading, baseline GFR was higher in Group 1 than in Group 2 patients (101 ± 7 versus 78 ± 3 mL/min per 1.73 m²; P < 0.01). Although blood pressure was higher in Group 2 patients at baseline, MAP declined significantly in all subjects with LA infusion; BCAA did not affect systemic pressure.

As depicted in Figure 2a, Group 1 subjects responded to LA infusion in a fashion similar to that noted in normal controls. A drop in MAP was accompanied by increased RPF and reduced RVR. Subjects in Group 1 also demonstrated a 37% increase in GFR during LA infusion. As in the normal controls, BCAA produced no changes in renal hemodynamics. The urinary excretion of NO₃⁻ was higher in transplanted patients than in normal controls and exhibited marked variability. In Group 1, LA infusion increased NO₃⁻ excretion in five of six subjects by 27 ± 17% (median Δ = 0.73 μmol/min; P < 0.05). BCAA infusion was associated with a 10% decline in urinary NO₃⁻ excretion.

In Group 2 patients, the decline in systemic blood pressure with LA infusion was not accompanied by changes in renal hemodynamics (Figure 2b).

**Table 2. Renal and hemodynamic parameters during the infusion of LA and BCAA in normotensive humans**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LA</th>
<th>BCAA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Infusion</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>91 ± 5</td>
<td>81 ± 7</td>
</tr>
<tr>
<td>RPF (mL/min per 1.73 m²)</td>
<td>575 ± 50</td>
<td>733 ± 44</td>
</tr>
<tr>
<td>RVR (mm Hg/mL per min × 100)</td>
<td>16 ± 1</td>
<td>11 ± 1</td>
</tr>
<tr>
<td>GFR (mL/min per 1.73 m²)</td>
<td>181 ± 18</td>
<td>120 ± 15</td>
</tr>
<tr>
<td>Urine flow rate (mL/min)</td>
<td>18 ± 2</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Urinary NO₃⁻ excretion (μmol/min per 1.73 m²)</td>
<td>0.43 ± 0.13</td>
<td>0.55 ± 0.15</td>
</tr>
<tr>
<td>Urinary endothenin excretion (pg/mL GFR)</td>
<td>3.5 ± 1.1</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

* P < 0.05, baseline versus infusion.
These changes were accompanied by a modest increase in RPF, RVR, and GFR that did not change with either LA or BCAA. Likewise, the increased NO₃⁻ excretion noted in Group 1 subjects with LA infusion was attenuated in Group 2 patients (median Δ = 0.23 μmol/min; 16 ± 19%). The urinary excretion of endothelin was higher in Group 2 than in Group 1 patients (Table 2) at baseline. Endothelin excretion did not change with either amino acid infusion.

At baseline, there was no difference in urinary sodium excretion between groups. However, LA, but not BCAA, increased sodium excretion in both groups (P < 0.05). Plasma uric acid levels were higher at baseline in Group 2 subjects (6.7 ± 0.4 versus 4.7 ± 0.3 mg/dL; P = 0.001), although uric acid clearance did not differ between the two groups. Subsequently, urate clearance increased by 60% in Group 1 subjects receiving LA (5.7 ± 0.9 to 9.1 ± 1.8 mL/min per 1.73 m²; P < 0.05), whereas no change occurred in Group 2 subjects or in patients from either group receiving BCAA.

**DISCUSSION**

In this controlled study, LA reduced MAP and RVR in both normotensive humans and a group of renal transplant patients not receiving CsA-based therapy. These changes were accompanied by a modest increase in urinary nitrate excretion, an increment rendered more significant when compared with the relative reduction in nitrate excretion noted when another nitrogen source (BCAA) was infused. However, in the presence of CsA-based immunosuppression, renal hemodynamics were unaffected by LA, and the increase in urinary nitrate excretion was attenuated. These data support a role for enhanced NO production in mediating the renal vasodilation that accompanies LA administration, a response that is preserved in renal allograft recipients but blunted in the presence of CsA.

A decided but transient increase in RPF and GFR in response to amino acid infusions is a well-described phenomenon in both animals and humans 27–30). More recently, it has become evident that renal hyperemia and hyperfiltration may not represent a universal response to protein loading, but rather an effect of specific amino acids or their metabolites. Although alanine and BCAA do not elicit such changes (31,32), the administration of either L-glycine or LA alone results in renal hemodynamic alterations identical to those previously described with mixed infusions (33–35). Furthermore, Nakaki et al. (36) have documented the renal vasodilation that accompanies LA in humans. In the normotensive subjects we studied, LA, but not BCAA, reproduced these findings: reduced systemic blood pressure was accompanied by increased RBF and reduced RVR. Unlike previous studies, GFR did not change in these subjects. This result may be artifactual, reflecting the small number studied and the relatively wide variability in measured GFR responses. Alternatively, the LA solution administered to our subjects was isotonic rather than hypertonic; in addition, it was at a dose (3 mg/kg per minute) that was lower than that used by Hirschkop and Kopple to elicit an increase in GFR (6.7 mg/kg per minute), yet higher than that associated with no response (1.7 mg/kg per minute) (33). Although it is possible that such a "midrange" dose might elicit a selective vasodilatory response, the former explanation seems more likely, especially in light of the GFR response noted in the Group 1 transplanted patients.

Several recent studies have linked amino acid-induced renal hyperemia and hyperfiltration to NO generation. King and coworkers found that N-methyl-L-arginine abolished the renal response to L-glycine and mixed amino acids in the Munich-Wistar rat, a finding corroborated by Tolins and Raji (37,38). Cernadas et al. reported data confirming the abrogation of the renal response to both LA and L-glycine in the rat by pretreatment with N-nitro-L-arginine, another inhibitor of NO synthase (34). Those studies support an essential role for NO generation in the renal vasodilation that accompanies amino acid infusion, reflecting either a specific effect of LA as substrate for NO synthesis or the activation of NO synthase by another amino acid (glycine) or group of amino acids.

In this study, LA-stimulated NO production appears to have contributed to the renal vasodilation noted in both normotensive humans and Group 1 transplant recipients. In the salt-sensitive Dahl-Rapp rat, LA abrogates hypertensive renal disease, a response that correlates with enhanced urinary nitrate excretion (22). Hlbers and coworkers have shown increased incorporation of radiolabeled guanido nitrogen atoms of LA into urinary NO₃⁻ during cancer therapy with interleukin-2 (26). At the same time, the labeled nitrogen did not appear in urea, suggesting that urinary NO₃⁻ was a specific marker for LA-induced NO production. In our normal subjects, baseline urinary NO₃⁻ levels approximated those noted by Hibbs and associates and increased substantially in all five (mean change of 41%) with LA relative to BCAA infusion. LA induced similar increases in five of six transplanted Group 1 subjects, versus a 10% decline in...
These findings, as well as the marked increase in RPF vasodilatory response to L-glycine, a response only present in CsA recipients and lograft transplanted controls during a 2-h infusion of mixed amino acids (9), GFR and RPF increased by approximately 20% in the non-CsA group and did not change in the CsA group. These findings were corroborated by Rondeau and coworkers using a similar protocol and by Nunley et al. with oral protein loading (40,41). In this study, the NO precursor LA induced renal vasodilatation only in those transplanted patients not receiving CsA (Group 1); the increase in RPF was similar to that noted in normal humans. Alternatively, in CsA-treated patients, neither LA nor BCAA altered renal hemodynamics: both the renal vasodilatory effect of LA and the enhanced urinary nitrate excretion were blunted in the presence of CsA.

<table>
<thead>
<tr>
<th>TABLE 3. Renal and hemodynamic parameters during the Infusion of LA and BCAA in renal transplant recipients, by group</th>
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<tbody>
<tr>
<td><strong>Group</strong></td>
</tr>
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<td>------------------</td>
</tr>
<tr>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
</tr>
<tr>
<td>RPF (mL/min per 1.73 m²)</td>
</tr>
<tr>
<td>RVR (mm Hg/mL per min × 100)</td>
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<tr>
<td>GFR (mL/min per 1.73 m²)</td>
</tr>
<tr>
<td>Urine flow rate (mL/min)</td>
</tr>
<tr>
<td>Urinary sodium excretion (mEq/min)</td>
</tr>
<tr>
<td>Urinary NO&lt;sub&gt;3&lt;/sub&gt;- excretion (μmol/min per 1.73 m²)</td>
</tr>
<tr>
<td>Urinary endothelin excretion (pg/mL GFR)</td>
</tr>
<tr>
<td><strong>Group 2</strong></td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
</tr>
<tr>
<td>RPF (mL/min per 1.73 m²)</td>
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<tr>
<td>RVR (mm Hg/mL per min × 100)</td>
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<td>GFR (mL/min per 1.73 m²)</td>
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</tr>
<tr>
<td>Urinary endothelin excretion (pg/mL GFR)</td>
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</tbody>
</table>

<sup>a</sup>P<0.05, baseline versus infusion.
<sup>b</sup>P<0.05, LA infusion versus BCAA infusion.

urinary NO<sub>3</sub>-excretion with BCAA. The increased urinary excretion of NO<sub>3</sub>- accompanying the infusion of LA, but not another nitrogen source (BCAA), implies that the response to LA was specific and indicates that enhanced NO synthesis occurred. Although these data do not conclusively link renal vasodilation to NO production, they support findings of previously noted animal studies in this regard (34,37,38).

Additional hemodynamic effects of LA infusion occurred. In Group 1 subjects, LA reduced both MAP and RVR, with a relatively greater change in RVR. These findings, as well as the marked increase in RPF that accompanied the reduction in systemic pressure, suggest that renal afferent vasodilation exceeded systemic vasodilation. Group 2 subjects, although demonstrating a similar decline in systemic pressure, experienced no change in renal hemodynamics. Taken together, these data indicate that the response to LA may be quantitatively different in the renal and systemic circulations and that NO, as its mediator, may play a relatively greater role in modulating renal vascular tone. This mechanism remains intact in transplant recipients but may be locally impaired in the presence of CsA.

CsA attenuates the renal response to amino acid infusion in both animals and humans (9,39–41). In a rat model, pretreatment with CsA abrogated the renal vasodilatory response to L-glycine, a response only partially restored by LA (39). Cafruns et al. studied renal hemodynamics in nine CsA-treated renal allograft recipients and nine transplanted controls during undergoing a 2-h infusion of mixed amino acids (9). GFR and RPF increased by approximately 20% in the non-CsA group and did not change in the CsA group. These findings were corroborated by Rondeau and coworkers using a similar protocol and by Nunley et al. with oral protein loading (40,41). In this study, the NO precursor LA induced renal vasodilatation only in those transplanted patients not receiving CsA (Group 1); the increase in RPF was similar to that noted in normal humans. Alternatively, in CsA-treated patients, neither LA nor BCAA altered renal hemodynamics: both the renal vasodilatory effect of LA and the enhanced urinary nitrate excretion were blunted in the presence of CsA.

CsA-induced renal vasoconstriction has been well documented in previous studies (4,10–12). Pathogenetic roles for angiotensin and thromboxanes and increased sympathetic outflow in mediating CsA-related renal vasoconstriction have been postulated, but experimental data are inconsistent (11,42). Some might suggest that long-term CsA administration results in irreversible renal injury, limiting the ability of the kidney to vasodilate in response to any stimulus (43,44). However, other studies, in both animals and humans, have shown prolonged reversibility of CsA-induced vasoconstriction: the discontinuation of CsA and the administration of calcium antagonists or angiotensin-converting enzyme inhibitors reduce RVR (6,45,46). Our data indicate that, in humans, an aberrant response to endothelial mediators may contribute to clinical CsA nephrotoxicity: the failure of the...
kidney to vasodilate in CsA-treated recipients could reflect the inhibition of NO production (consistent with the work of Dinh-Xuan et al. [47]), the local antagonism of NO by a vasoconstrictor, or some combination of both. Recently, Bobadilla and coworkers administered extremely high doses of LA (15 mg/kg per minute) to CsA-treated rats, found increased urinary NO3-excretion along with evidence of renal vasodilation, and concluded that endothelial responsiveness to LA is preserved [48]. In this trial, we purposely chose an isotonic dose of 3 mg/kg per minute, along with a control infusion of a different nitrogen source, to detect specific effects of LA and to avoid inducing nonspecific changes due to osmotic gradients or the stimulation of atrial natriuretic peptide secretion. Nonetheless, it is possible that higher doses of LA might have overcome the attenuated effect noted in our CsA-treated subjects.

In vitro, human vascular endothelial cells secrete endothelin when incubated with CsA [13]. In animal models, CsA increases the urinary excretion of endothelin, and CsA-induced renal vasoconstriction can be abrogated by the administration of antiendothelin antibodies or an endothelin receptor antagonist, although conflicting data exist [12,14,49,50]. The increased urinary excretion of endothelin has also been reported in humans receiving CsA [51,52]. Perico and associates found that peak plasma CsA levels in renal allograft recipients (2 to 4 h after dosing) were accompanied by reduced GFR and RPF and increased urinary endothelin excretion relative to predosing values [52]. In this study, performed at trough CsA levels (20 h after the previous dose), RPF in CsA-treated patients approximated the baseline values reported by Perico et al., but was lower than in transplant recipients not on CsA. Likewise, urinary endothelin excretion, although not elevated to the extent noted by Perico and colleagues at peak CsA levels, was substantially greater in CsA-treated patients. Our data confirm that, even in the presence of nadir CsA concentrations, renal hemodynamics and endothelin excretion remain abnormal. Awazu and associates found CsA to preferentially up-regulate endothelin binding in renal tissue compared with hepatic tissue, providing a potential link between increased endothelin production and renal-specific effects [53].

Thus, these data are consistent with previous studies indicating a substantial role for NO in mediating the vasodilatory effects of LA. They likewise support the hypothesis that CsA nephrotoxicity in humans is a complex phenomenon that may reflect, at least in part, an adverse imbalance between the effects of the vasodilator NO and the vasoconstrictor endothelin within the renal circulation. Future studies using specific inhibitors of NO synthase and/or endothelin receptor antagonists may further characterize the relative contributions of each of these pathogenetic influences in humans.

ACKNOWLEDGMENTS

This study was supported in part by Grant 2 M01 RR00032 from the NIH (General Clinical Research Center) and by grants from Sandoz Pharmaceuticals, Inc. (East Hanover, NJ) and the Alabama Kidney Foundation (Birmingham, Al). We express appreciation to Kabil Pharmacies, Inc. (Clayton, NC) and Clinteck Nutrition Co. (Deerfield, IL) for providing L-arginine and BranchArm, respectively. We also thank Michael Allen, MD, for assistance in designing the protocol; Connie Gibson for assistance with laboratory assays; Jeanette Lee, PhD, for review of the statistical analyses; and Arnold G. Dietelhlm, MD, for his ongoing support.

REFERENCES

Cyclosporine and L-Arginine in Humans


Vepacian, one of the great schoolmasters of avarice, which could pick out profit of every thing (yea even of mens urine) taught his Scholars (I mean the whole court of covetous persons) this lesson ensuing:

\textit{Lucrbonus odor cace qualibet.}
\textit{Lucre is sweet, and hath a good savour,}
\textit{Though it come of urine, dirt or ordure.}

So that if there be any Physician so arrogant, that he will take upon him to tell all things alone and will not hear the Patient speak, specially not knowing the party before, neither seeing other signs but only the urine, as I dare boldly pronounce, that such a man is unworthy to be called a Physician.

Robert Record, The Urinal of Physick, printed by Gartrude Dawson, London, 1651. From the collection of the Clendening Library of the History of Medicine, University of Kansas.