Study of Arterial and Autonomic Effects of Cyclosporine in Humans
Daniela Lucini, Richard V. Milani, Hector O. Ventura, Mandeep R. Mehra, Franz Messerli, Massimo Pagani

Abstract—Altered sympathetic activity and peripheral vascular function are suspected as a mechanism of the development of arterial hypertension in organ transplantation recipients treated with cyclosporine. We assessed whether cyclosporine might alter peripheral vascular properties or autonomic modulation of the sinus node and the vasculature during rest and standing. We examined 17 orthotopic heart transplantation recipients, 8 solid organ transplantation recipients, 17 patients with essential hypertension, and 42 normotensive control subjects. All except the normotensive control subjects were treated with a long-acting dihydropyridine calcium entry blocker; transplantation recipients also received cyclosporine-based immunosuppression. Radial artery compliance was reduced in patients with essential hypertension and in patients with heart and solid organ transplantation as compared with normotensive control subjects, with this reduction being more marked in heart transplantation recipients. At rest, R-R variance was lowest in heart transplantation recipients, denoting denervation. The spectral profile of both R-R and systolic blood pressure variability as well as the index of baroreflex gain was normal at rest in patients with solid organ transplantation. On standing, both transplantation groups demonstrated reduced responsiveness in markers of autonomic modulation. The decrease in arterial compliance in cyclosporine-induced hypertension seems to imply a degree of ventricular vascular uncoupling more apparent in heart transplantation recipients. These changes are associated with alterations in autonomic modulation that are evidenced by an orthostatic stimulus. (Hypertension. 2000;35:1258-1263.)

Key Words: autonomic nervous system ▪ arterial mechanics ▪ baroreflex ▪ heart transplantation ▪ compliance, arterial

The mechanisms by which cyclosporine can induce hypertension are still under active scrutiny.1,2 The most commonly investigated mechanisms include alterations in the sympathetic nervous system activity3 and peripheral vascular function.4 Studies that have addressed sympathetic nervous system activation have provided disparate results. Experimental studies in animals have demonstrated that the acute administration of cyclosporine increased renal and lumbar sympathetic nerve activity5 and that the increase in blood pressure is related to the activation of excitatory neural reflexes arising from subdiaphragmatic regions.6 Moreover, in a clinical study of heart transplantation recipients with cyclosporine-induced hypertension, Scherrer et al3 proved an increase in sympathetic activity measuring peroneal nerve discharges.

Other studies have shown that cyclosporine does not affect sympathetic nervous system activity. Stein et al7 and Kaye et al8 demonstrated normal norepinephrine spillover and normal muscle sympathetic discharges in patients receiving cyclosporine compared with control subjects. In addition, plasma and urinary catecholamines have been reported to be within normal ranges in heart transplantation recipients treated with cyclosporine.9 We have previously shown by using spectral analysis of heart rate and systolic arterial pressure variability the maintenance of baroreflex circulatory control in patients with organ transplantation treated with cyclosporine.10 The latter suggested that cyclosporine does not markedly alter the autonomic control of the sinus node nor of the peripheral vasculature at rest. In addition, these findings also reinforced the concept that cyclosporine might induce hypertension through an impairment in peripheral vasodilation or changes in vascular mechanics largely independent of the sympathetic nervous system. This hypothesis of vascular alterations in the pathogenesis of cyclosporine-induced hypertension is gaining increasing credibility, as indicated by several recent studies underlying a pivotal role of this mechanism in the development of this disease.11-14

The purpose of this study was first to assess the effects of cyclosporine-induced hypertension on arterial vascular mechanics and second to assess the autonomic modulation of the
sinus node and the vasculature on standing in cohorts of patients with solid organ transplantation, heart transplantation, and essential hypertension.

Methods

Study Population
Four groups of patients were included in the study (Table 1). Group 1 consisted of 42 normal control subjects, group 2 included 17 patients with essential hypertension, group 3 included 17 hypertensive patients with orthotopic heart transplantation (time elapsed since transplantation 10±3 months), and group 4 consisted of 9 hypertensive patients with other solid organ (kidney or liver) transplantation (time elapsed since transplantation 22±18 months). Clinical evaluation and definition of hypertension (diastolic blood pressure >90 mm Hg measured by cuff method) followed established guidelines.

All patients who underwent transplantation received the same triple immunosuppressive therapy, including cyclosporine (4 mg/kg per day) to achieve a blood level of 183±20 ng/mL in patients with heart transplantation and of 178±16 in patients with solid organ transplantation, respectively, prednisone (0.1 mg/kg per day), and azathioprine (2 mg/kg per day); all were free of acute allograft rejection at the time of the study. In addition, patients with essential hypertension or cyclosporine-induced hypertension were receiving the same dose of a long-acting dihydropyridine calcium channel blocker at the time of the study.

All patients provided informed consent to a protocol approved by the Ochsner Medical Institutions Review Board.

Recording Procedure
Recordings were performed in a quiet room, with a comfortable temperature (22° to 24°C), always in the same time window (between 7 AM and 1 PM). On the morning of the study, every subject had a light breakfast, with no caffeinated beverages (coffee or tea) that might produce long-lasting autonomic effects.

Each participant was connected to a 2-channel telemetry system (Marazza) that provided continuous ECG and respiratory signals (obtained with a piezoelectric transducer). Arterial pressure was continuously estimated with a noninvasive device (Finapres, Ohmeda). After a 10-minute period allowed for stabilization, a control recording of 10 minutes was obtained in the supine position, to be followed by a further period of 7 minutes of recording during active standing, leading to a shift of the sympathovagal balance toward sympathetic predominance.15

Spectral Analysis of Heart Rate and Blood Pressure Variability
With the use of a PC with a D/A board from the ECG signal,16,17 a continuous R-R interval series (ie, tachogram) was obtained, as previously reported with the use of an autoregressive algorithm; the power and frequency of every spectral component were computed both in absolute (ie, s²) and normalized units (ie, μθ) (see References 16 and 17 for details). Spectral analysis was also performed on the systolic arterial pressure and the respiratory signals with the use of a similar procedure.17

Baroreflex Gain
From the simultaneous analysis of arterial pressure and R-R interval variability, a frequency domain index α can be derived,18,19 which is a measure of the overall gain of the arterial pressure heart period relation and provides results similar to those obtained with the phenylephrine slope approach, as already described.18

Arterial Compliance
The arterial pressure wave contour was obtained noninvasively from the right brachial artery with a hand-held 7-mm-diameter pencil-type probe incorporating a micromanometer (Millar Instruments Inc). The brachial artery waveform was digitized at a rate of 250 Hz and stored on a PC computer for off-line analysis. Two to 5 digitized signals were signal-averaged and analyzed, excluding premature and post-premature beats from analysis. Arterial compliance was determined by means of the area method,20,21 which is based on the 2-element Windkessel model of the arterial circulation according to Yin’s approach,20 in which

$$C = \frac{A}{R (P_1 - P_2)}$$

This model relates arterial compliance (C) directly to the diastolic pressure-time integral (A) and inversely to the product of local vascular resistance (R) and the difference between end-systolic pressure and end-diastolic pressure (P₁ and P₂). Vascular resistance was calculated as the ratio of mean arterial pressure to mean nominal flow.

Statistics
Data that were not normally distributed are presented as median±semi-interquartile. Two-way ANOVA for repeated measures, with the Geisser-Greenhouse conservative test and Bonferroni correction, was used to compare the 4 groups. A level of P<0.05 was considered significant.

Results

Blood Pressure
Systolic and diastolic blood pressures were significantly elevated at rest in patients with cyclosporine-induced hypertension or essential hypertension compared with normal control subjects (Table 2 and Figure 1). On standing, only patients with essential hypertension demonstrated a higher level of systolic and diastolic blood pressures compared with normal subjects (Table 2).

Indexes of Autonomic Nervous System Activity
As a result of the effects of cardiac denervation, patients with orthotopic heart transplantation had a significantly lower R-R interval, both at rest and standing, compared with the other 3 groups.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>NO.</th>
<th>AGE, Y</th>
<th>NORMOTENSIVE</th>
<th>HYPERTENSIVE</th>
<th>ORTHOTOPIC HEART TRANSPLANTATION</th>
<th>ORTHOTOPIC SOLID ORGAN TRANSPLANTATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normotensive subjects</td>
<td>42</td>
<td>45.8±1</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Hypertensive patients</td>
<td>17</td>
<td>52±2</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Orthotopic heart transplantation</td>
<td>17</td>
<td>48.2±3</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td></td>
</tr>
<tr>
<td>Orthotopic solid organ transplantation</td>
<td>9</td>
<td>43.5±4</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td></td>
</tr>
</tbody>
</table>
The LFRR components (in nu) at rest are shown in all groups in Figure 1 and Table 2. Heart transplantation recipients displayed very small values of R-R low-frequency components but with a high degree of variability (Figure 2). The latter was related to the presence of an LFRR component in 9 heart transplantation recipients (18.8 nu, time elapsed after heart transplantation 10 months, range 4 to 46 months) compared with 8 in whom LFRR component was not observed (time elapsed after heart transplantation 6 months, range 1 to 14 months). During standing (Table 2), patients with essential hypertension and normal subjects demonstrated an increase in LFRR compared with solid organ or heart transplantation recipients. This spectral component in heart transplantation recipients displayed very small values on standing.

On standing, the HFRR component was significantly reduced in normal subjects and only slightly diminished in patients with essential hypertension; this index was un-

| TABLE 2. Descriptive Statistics of R-R and Systolic Arterial Pressure Variabilities in Normotensive Subjects, Hypertensive Patients, Heart Transplantation Recipients, and Solid Organ Transplantation Recipients During Rest and Standing |
|----------------------------------|------------------|------------------|------------------|
|                                  | NT               | HT               | HTX              |
| R-R, ms                          |                  |                  |                  |
| Rest                             | 873±94           | 853±104          | 604±27†§         | 928±170          |
| Standing                         | 772±49*          | 767±81*          | 561±48†§         | 761±99*          |
| VARRR, ms²                       |                  |                  |                  |
| Rest                             | 1323±1186        | 843±343          | 33±16†§          | 1243±1259        |
| Standing                         | 1347±729         | 955±1061         | 9±5†§            | 781±517†         |
| LFRR, nu                         |                  |                  |                  |
| Rest                             | 53.6±11.4        | 48.4±27.9        | 0.1±6.5†§        | 49.3±22.7        |
| HFRR, nu                         |                  |                  |                  |
| Rest                             | 38.7±7.4         | 34.3±24.7        | 30.3±20.5        | 28.9±16.5        |
| Standing                         | 14.1±8.7*        | 20.3±11.2        | 39.7±20.0†       | 29.8±14.2†       |
| LF/HF                            |                  |                  |                  |
| Rest                             | 1.3±0.6          | 1.4±1.9          | 0.08±0.1†‡§      | 1.8±1.7          |
| Standing                         | 5.6±4.0          | 3.5±2.5          | 0.00±0.1†‡§      | 1.6±1.1†         |
| SAP, mm Hg                       |                  |                  |                  |
| Rest                             | 121±11           | 138±10†          | 142±13†          | 142±21†          |
| Standing                         | 125±7            | 138±7†           | 131±14           | 129±11†          |
| DAP, mm Hg                       |                  |                  |                  |
| Rest                             | 79±10            | 90±10†           | 90±10†           | 80±13            |
| Standing                         | 80±20            | 90±4†            | 84±9             | 74±4             |
| VARSAP, mm Hg²                   |                  |                  |                  |
| Rest                             | 22.1±10.1        | 23.8±9.7         | 23.1±22.1        | 23.9±3.7         |
| Standing                         | 27.3±10.9        | 39.7±37.4        | 34.0±15.7        | 20.0±8.8         |
| LFSPAP, mm Hg²                   |                  |                  |                  |
| Rest                             | 2.7±1.9          | 3.3±2.6          | 1.6±0.6          | 1.8±1.7          |
| Standing                         | 6.8±3.2*         | 10.2±3.2*        | 6.8±7.0*         | 3.02±2.0†        |
| HFSPAP, mm Hg²                   |                  |                  |                  |
| Rest                             | 0.8±0.5          | 1.6±1.5          | 2.4±2.0†§        | 0.8±0.4          |
| Standing                         | 1.3±0.8          | 2.1±1.5          | 4.7±2.6†§        | 1.1±0.4          |
| α ms/mm Hg                       |                  |                  |                  |
| Rest                             | 13.3±3.5         | 7.5±5.5†         | 0.3±0.6†‡§       | 12.3±3.9‡        |
| Standing                         | 8.1±2.4*         | 5.0±3.5†         | 0.1±0.3†‡§       | 8.8±2.7          |

NT indicates normotensive subjects; HT, hypertensive patients; HTX, heart transplantation recipients; and OTX, solid organ transplantation recipients.

Two-way repeated-measures nonparametric ANOVA according to Koch demonstrated a significant interaction (patient group and posture) in all variables (except VARRR and HFSPAP).

Data are presented as median±semi-interquartile.

*P<0.05 vs rest.
†P<0.05 vs NT.
‡P<0.05 vs HT.
§P<0.05 vs OTX.
changed in the transplantation groups. Heart transplantation recipients had an HFRR component in 16 of 17 subjects.

The LF SAP component was present in all subjects in all groups. Patients with essential hypertension (Figure 1 and Table 2) had the greatest LFSAP both at rest and during standing up. In all groups, the power of LF SAP increased from supine to upright positions; the greatest value being observed in hypertensive patients and the smallest in patients with solid organ transplantation ($P < 0.05$).

**Baroreflex Gain**
The gain of the heart period–arterial baroreflex, as measured by the index $\alpha$, was lowest in orthotopic heart transplantation recipients. In addition, whereas the index $\alpha$ was similar in patients with solid organ transplantation and normal control subjects, patients with essential hypertension had a lower index $\alpha$ compared with that in normal subjects (Table 2 and Figure 1). On standing, a significant reduction of the index $\alpha$ was observed in normal control subjects, and a slight decrease was observed in patients with essential hypertension. Conversely, patients with solid organ transplantation had a very small reduction of the index $\alpha$ (Table 2).

**Artery Compliance**
All 3 groups of patients with hypertension, either cyclosporine-induced heart transplantation recipients and solid organ transplant recipients or patients with essential hypertension, had a significantly lower value of arterial compliance as compared with normal subjects (0.44±0.62, 2.15±1.66, and 2.34±1.95, respectively, vs 3.97±1.4 au) (See Figure 1). Among patients, heart transplantation recipients displayed the lowest value of arterial compliance (0.44±0.62 au).

**Discussion**
The present study shows that cyclosporine-induced hypertension in organ transplantation is associated with a decrease in radial artery compliance, particularly evident in orthotopic heart transplantation recipients. In addition, autonomic responsiveness to standing appeared to be reduced in transplantation recipients.

**Arterial Compliance**
Compared with control subjects, radial artery compliance was reduced in hypertensive subjects and in organ transplantation recipients. This reduction in hypertensive subjects has been demonstrated, and it is likely to be a reflection of the well-known widespread alterations in arterial mechanics.22 The impaired compliance observed in transplantation recipients might reflect the cyclosporine-induced hypertension, through an impairment in peripheral vasodilation or alterations in vascular mechanics.7,12 Although the direct vascular action by which cyclosporine causes hypertension is controversial, several studies, in addition to the present results, support this mechanism because it has been shown that cyclosporine might affect functional properties of the vasculature by an increase in endothelin production11 and reduction in nitric oxide synthesis. Thus, the significant decrease in regional arterial compliance observed in patients with solid organ or heart transplantation supports the notion that cyclosporine alters vascular mechanics. The more marked decrease in arterial compliance observed in heart transplantation recipients suggests that some factors other than the development of hypertension and the vascular effect of cyclosporine might be involved. An increased average sympathetic outflow to blood vessels, as occurs in heart transplantation recipients,23 might
lead to an increase in arterial smooth muscle tone and hence to a further increase in arterial stiffness.

**Autonomic Modulation of Sinoatrial Node and of the Vasculature**

An interpretation of the autonomic effects of cyclosporine must consider the complex, dual nature of cardiac innervation. Patients with solid organ transplantation had similar indexes either of the autonomic modulation of the sinoatrial (SA) node and vasculature at rest when they were compared with control subjects. These findings support the concept that these patients maintain resting oscillatory properties of autonomic circulatory control. In contrast, on standing, minimal changes were observed in oscillatory markers of the autonomic modulation of the SA node and of the vasculature, indicating a reduced responsiveness to excitatory stimuli. Heart transplantation recipients demonstrate markedly reduced heart rate variability secondary to the condition of cardiac denervation. Interestingly, the LF_RR components showed a great deal of variability because heart transplantation recipients with longer postoperative periods demonstrated the presence of LF_RR, a finding that supports previous studies suggesting reinnervation with time. Also of note is the small value of LF_RR in treated hypertensive subjects as studies suggesting reinnervation with time. Additionally, oscillatory properties of efferent sympathetic vasomotor control even in the near absence of LF_RR. These patients also had a clear increase in the LF_SAP components on standing. Heart transplantation recipients maintained a normal blood pressure variability at rest, suggesting a preservation of oscillatory properties of efferent sympathetic vasomotor control even in the near absence of LF_RR. These patients also had a clear increase in the LF_SAP components on standing. Thus, despite a possible increase in average resting sympathetic nerve activity in humans in a wide range of tonic sympathetic nerve activity in studies on untreated hypertensives, possibly reflecting some beneficial autonomic effects of long-acting dihydropyridine treatment.

**Limitations of the Study**

The method used to measure compliance, although sensitive and based on high-fidelity sensors measuring both local pressure and blood flow velocity, was limited to a single peripheral distal arterial bed and therefore cannot be considered an estimation of global vascular compliance. Spectral analysis of R-R interval and systolic arterial pressure variability provide indexes of the oscillatory properties of autonomic control of the SA node and of the vasculature and is not a measure of autonomic traffic. However, despite a debate about some aspects of interpretations, this approach has reached a large consensus and has been recently validated against direct measures of efferent sympathetic nerve activity in humans in a wide range of levels of sympathovagal balance with the use of vasoactive substances and muscarinic receptor blockade.

**Conclusions**

Cyclosporine-induced hypertension is associated with abnormal vascular mechanics, as evidenced by a decrease in arterial compliance. Heart transplantation recipients have a greater decrease in arterial compliance compared with patients with solid organ transplantation, possibly implying as well a greater degree in ventricular vascular uncoupling. In addition, the abnormality in peripheral vascular mechanics is associated in transplantation recipients with changes in autonomic modulation that are more apparent during an orthostatic stimulus.

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**References**


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