Vasomotor Responses in Cyclosporin A–Treated Rats After Chronic Angiotensin Blockade

Wolfgang Auch-Schwelk, Ellen Duske, Ulrich Hink, Mathias Betz, Manfred Unkelbach, Eckart Fleck

Abstract Chronic angiotensin-converting enzyme (ACE) inhibition prevents endothelial dysfunction in animal models with hypertension and hypercholesterolemia. Long-term treatment with cyclosporin A impairs endothelium-dependent relaxations and augments contractions to angiotensin II in the rat aorta. The present study compares vasomotor responses to several vasoconstrictor and dilator stimuli after 6 weeks of oral treatment with either the angiotensin-converting enzyme inhibitor lisinopril (10 mg/kg per day), the angiotensin subtype 1 receptor antagonist D 8731 (10 mg/kg per day), cyclosporin A (15 mg/kg per day), or a combination of cyclosporin A with lisinopril or D 8731 (n=15 rats per group). Twenty-four hours after the last treatment, aortic rings were mounted in organ chambers for measurement of isometric force. Endothelium-dependent relaxations to acetylcholine and calcium ionophore were impaired by cyclosporin A but not affected by the vasodilators. Cyclosporin A–induced endothelial dysfunction was prevented by cotreatment with lisinopril or D 8731.

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hronic angiotensin-converting enzyme (ACE) inhibition prevents endothelial dysfunction in animal models with hypertension and hypercholesterolemia. Even in hypertensive animals the modulation of endothelial function is not exclusively explained by the reduction of blood pressure, since treatment with hydralazine does not modify endothelium-dependent relaxations to the same extent, if at all. Considering additional studies showing reduced intima formation after endothelial injury and reduced development of atherosclerosis during ACE inhibition, it has been hypothesized that chronic ACE inhibition protects or restores endothelial function. Both reduced angiotensin II (Ang II) formation and bradykinin accumulation during ACE inhibition have been discussed as mechanisms contributing to these effects.

Long-term treatment with cyclosporin A (CsA) impairs endothelium-dependent relaxations in rats and humans. In the rat this effect is not a consequence of increased blood pressure, since CsA does not significantly increase blood pressure in normotensive rats. We chose this model to determine possible protective effects of chronic ACE inhibition on endothelial function, since CsA has been shown to augment selectively the effects of Ang II in rat vascular smooth muscle. Chronic ACE inhibition was compared with selective angiotensin subtype 1 (AT1) receptor blockade to elucidate the contribution of nitroglycerin, SIN-1, and forskolin were not affected by any treatment. Contractions to phenylephrine and serotonin were reduced by lisinopril but not by D 8731. In contrast, contractions to angiotensin II were augmented by cyclosporin A, lisinopril, and the combination of both but not by D 8731 or D 8731 plus cyclosporin A. The data suggest a role for angiotensin II in cyclosporin A–induced endothelial dysfunction. Chronic ACE inhibition reduces overall smooth muscle contractility. The selective augmentation of angiotensin II effects by ACE inhibition and cyclosporin A suggests upregulation of angiotensin receptor in the aortic smooth muscle by these treatments. Chronic angiotensin subtype 1 receptor blockade does not appear to affect angiotensin receptor function. (Hypertension. 1994;23[part 2]:832–837.)

Key Words • angiotensin II • receptors, angiotensin • angiotensin-converting enzyme inhibitors • cyclosporine • endothelium • nitric oxide • serotonin • aorta

Methods Ninety male Wistar rats (body weight at the beginning of the study, 200 to 250 g) received six different treatments for a period of 6 weeks. After 1 week of acclimatization the animals were fed daily through an oral gastric tube with either placebo (1 mL olive oil per day); CsA (15 mg/kg per day dissolved in 1 mL olive oil); the ACE inhibitor lisinopril (10 mg/kg per day); D 8731, a selective nonpeptide AT1 receptor antagonist (10 mg/kg per day); or a combination of CsA with either lisinopril or D 8731 in the same dosage. Lisinopril and D 8731 in these high doses prevent blood pressure responses to Ang I or Ang II in rats, respectively, for more than 6 hours, whereas no accumulation was observed even with higher doses. Two animals per day were enrolled in the study and randomly assigned to one of the treatment groups. The animals were housed under comparable conditions and had free access to water and standard chow. Twenty-four hours after the last application of the treatment the rats were anesthetized with pentobarbital (50 mg/kg), and 5 to 8 mL of blood was withdrawn from the abdominal aorta for laboratory tests. Whole blood levels of CsA were measured by a monoclonal fluorescence-polarizing immunoassay used in routine drug monitoring of heart transplant patients (Abbott Laboratories). Laboratory tests for renal and liver function in the plasma were performed with an automatic analyzer (BM/Hitachi System 717, Boehringer Mannheim).
The heart and the aorta were rapidly excised; the heart wet weight was determined, and the aorta was placed into modified Krebs-Henseleit bicarbonate buffer of the following composition (mmol/L): NaCl 118, KCl 4.7, CaCl₂ 2.5, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, edetate calcium disodium 0.026, and glucose 11.1 (pH 7.4).

The thoracic part of the aorta was cut into rings 4 mm in length. The rings were mounted in organ chambers between a clip and a force transducer by two stainless steel wires inserted into the lumen of the vessels. The organ chambers were filled with 10 mL control solution, kept at 37°C, and aerated with a 95% O₂/5% CO₂ gas mixture. Changes in isometric force were measured. The preparations were set individually at the optimal point of their length-tension relation (5 g in all groups) as determined from the contractions to repeated exposure to KCl (40 mmol/L).

Endothelium-dependent relaxations to acetylcholine (in the presence and absence of 10⁻⁵ mol/L indomethacin) and thrombin were tested at the beginning of the experiments during contraction to phenylephrine (3 x 10⁻⁴ mol/L). After 1 hour of equilibration, contractions to the cumulative addition of Ang II and serotonin were registered; the effects of vasoconstrictors were studied in the presence or absence of the nitric oxide synthase inhibitor nitro-L-arginine (10⁻⁴ mol/L) to assess the modulatory role of basal nitric oxide release. The responses to the endothelium-dependent vasodilator calcium ionophore A 23187 and the endothelium-independent vasodilators SIN-1, nitroglycerin, and forskolin (the latter in the presence of 5 x 10⁻⁴ mol/L propranolol to prevent adenylyl cyclase stimulation by β-receptors) were tested in rings contracted by the cumulative addition of phenylephrine (10⁻⁴ to 10⁻² mol/L). The protocol for the application of Ang II (cumulative addition in 10-minute intervals) does not cause significant tachyphylaxis, as shown in a pilot study. Indomethacin (10⁻⁵ mol/L) was present throughout the experiments to prevent the generation of vasoactive prostaglandins except one ring exposed to acetylcholine.

The acute effects of D 8731 and lisinopril were determined in untreated rats from the same strain. The same protocol as in the chronic experiments was applied in rings treated for at least 30 minutes with either D 8731 (10⁻⁶ mmol) or lisinopril (10⁻⁵ mmol) and compared with controls studied in parallel.

All procedures were in accordance with institutional guidelines for animal experimentation.

Drugs

Acetylcholine, Ang II, the calcium ionophore A 23187, forskolin, indomethacin, nitro-L-arginine, propranolol, phenylephrine, serotonin, and thrombin were purchased from Sigma Chemical Co. CsA was obtained from Sandoz. Nitroglycerin (Pohl GmbH), SIN-1 (Casella-Riedel), lisinopril (ZENECA GmbH), and D 8731 (2-ethyl-4-[2-(1H-1,2,3,4-tetrazol-5-yl)biphenyl-4-yl]methoxy]quinoline hydrochloride; ZENECA GmbH) were generously provided by these companies; the drugs were prepared daily in distilled water except for indomethacin and calcium ionophore A 23187, which were dissolved in Na₂CO₃ (10⁻⁴ mol/L) and dimethyl sulfoxide (1%), respectively. The concentrations of the drugs are expressed as final molar bath concentrations.

Calculations and Statistical Analysis

Increases in isometric force in response to contracting agents are expressed in grams. Relaxations are expressed in percentage of the previous contraction induced by phenylephrine. Results are given as mean ± SEM; n refers to the number of rats from which the vessels were taken. ED₅₀ values, representing the concentrations (log molar) that cause 50% of the maximal effect of a drug, were obtained by interpolation in each individual experiment. Statistical comparisons between the six groups were performed by one-way ANOVA and Scheffe's test. Student's t test for paired observations was applied to detect differences in the responses within the same group. The means were considered statistically different at P < 0.05.

Results

Endothelium-dependent relaxations to acetylcholine (10⁻⁸ to 10⁻⁶ mol/L) and calcium ionophore A 23187 (10⁻⁷ to 10⁻⁵ mol/L) were significantly reduced in CsA-treated animals (Fig 1). The impaired response to acetylcholine was observed whether or not cyclooxynase was inhibited in these rings with indomethacin (10⁻⁵ mol/L). Indomethacin did not affect the relaxations to acetylcholine in any group (data not shown).

Long-term treatment with CsA alone did not affect the response to acetylcholine or A 23187. Combined treatment with CsA and either lisinopril or D 8731 resulted in responses similar to those observed in controls (Fig 1).

Endothelium-dependent relaxations to thrombin (0.3 U/mL) were smaller in CsA-treated rats, but the difference did not reach statistical significance.
with lisinopril or D 8731 either alone or in combination with CsA resulted in relaxations comparable to the response in the control group (Table).

Contraction responses to potassium chloride (40 mmol/L), phenylephrine (10^-6 mol/L), and serotonin (10^-8 to 10^-5 mol/L) were not significantly affected by treatment with CsA. The potentiation of the contractions to phenylephrine and serotonin after inhibition with nitro-L-arginine (10^-4 mol/L) was similar in controls and CsA-treated rats (Table). Lisinopril, alone and in combination with CsA, reduced the maximal responses to these vasoconstrictors. The modulation of contractions to potassium chloride did not reach statistical significance (Table). Reductions in contractile responses were not observed with the angiotensin antagonist D 8731 either alone or in combination with CsA.

**Acute Effects of D 8731 and Lisinopril**

Incubation with lisinopril and D 8731 (both 10^-6 mol/L) did not significantly affect endothelium-mediated relaxations to acetylcholine (maximal relaxation at 10^-5 mol/L: placebo, 78±10%; lisinopril, 78±9%; D

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**Table:**

<table>
<thead>
<tr>
<th>Contractions</th>
<th>Placebo</th>
<th>CsA</th>
<th>Lisinopril</th>
<th>Lisinopril+CsA</th>
<th>D 8731</th>
<th>D 8731+CsA</th>
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<tr>
<td>KCl (40 mmol/L), g</td>
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<td>0.9±0.1</td>
<td>0.9±0.1</td>
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<td>Max, g</td>
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<td>1.7±0.2</td>
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<td>Max, g</td>
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<td>2.3±0.1</td>
<td>1.7±0.1*</td>
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<td>1.3±0.1*</td>
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<td>Thrombin (0.3 U/mL), %</td>
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<td>41±7</td>
<td>60±6</td>
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<td>57±7</td>
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<tr>
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</table>

CsA indicates cyclosporin A; Max, maximal response; EDso, median effective dose; and NLA, nitro-L-arginine. Values are mean±SEM. *P<.05 compared with placebo.
Weight Gain, Heart Weight, and Laboratory Tests

Long-term treatment with CsA did not significantly affect weight gain (placebo, +44±5%; CsA, +39±4%). Weight gain, however, was reduced by the combination of lisinopril plus CsA (+21±4%, P<.05) but not by the other treatments (lisinopril, +38±4%; D 8731, +44±4%; CsA plus D 8731, +34±4%). Heart wet weight was unaffected by CsA (placebo, 1.23±0.05 g; CsA, 1.21±0.03 g) and D 8731 (1.11±0.04 g; CsA plus D 8731, 1.05±0.03 g) but was significantly reduced after treatment with lisinopril (0.94±0.04 g) and CsA plus lisinopril (0.97±0.04 g).

CsA whole blood levels 24 hours after the last administration of the oral treatment were 506±96, 431±50, and 242±36 ng/mL (P<.05) in CsA-, CsA plus lisinopril-, and CsA plus D 8731-treated rats, respectively.

Laboratory screening for renal and hepatic toxicity revealed the following changes: All rats receiving CsA showed hyperbilirubinemia (total bilirubin: placebo, 1.2±0.2 [0.07±0.01]; lisinopril, 1.0±0.2 [0.06±0.01]; D 8731, 1.0±0.2 [0.06±0.01]; CsA, 1.9±0.2 [0.11±0.01]; CsA plus lisinopril, 1.9±0.2 [0.11±0.01]; and CsA plus D 8731, 1.7±0.2 [0.1±0.01] μmol/L [mg/100 mL]). Blood urea was increased in rats treated with the combination of lisinopril plus CsA (34±2 mmol/L [95±7 mg/100 mL]) compared with controls (15±1 mmol/L [41±2 mg/100 mL]). This effect was observed neither after single treatment (CsA, 16±3 mmol/L [45±6 mg/100 mL]; lisinopril, 17±1 mmol/L [48±2 mg/100 mL]) nor with D 8731 (17±1 mmol/L [48±2 mg/100 mL]) or CsA plus CsA (17±2 mmol/L [48±5 mg/100 mL]). Serum creatinine was not affected by any treatment (data not shown).

Discussion

The present study demonstrates that chronic ACE inhibition and AT1 receptor antagonism can modulate the responsiveness of the rat aorta. Both inhibitors prevent the impairment of endothelium-dependent relaxations by CsA, whereas vasodilator treatment in control animals does not affect endothelial function. The treatments differ regarding the modulation of vascular smooth muscle reactivity. Chronic ACE inhibition, but not AT1 receptor blockade, reduces overall contractile capability of the rat aorta. Lisinopril, but not D 8731, selectively augments the ex vivo responsiveness to Ang II; D 8731 even prevents the chronic effects of CsA on the responsiveness to Ang II. Both drugs reduce the sensitivity to serotonin.

Chronic ACE inhibition was compared with chronic AT1 receptor blockade in rats chronically treated with CsA, since this model provides the unique combination of endothelial dysfunction and augmented angiotensin receptor function. It was not clear from previous studies whether these effects are secondary to reduced weight gain, are independent effects on the different cell types, or are linked to each other. The present data obtained with CsA blood levels close to clinical applications confirm previous observations regarding reduced endothelium-dependent relaxations obtained with high doses of CsA (30 mg/kg per day for 6 weeks). Neither reduced weight gain nor other major side effects (except mild hyperbilirubinemia) were observed with this dose. The alterations of angiotensin-induced contractions are highly selective with this treatment because CsA does not alter the response to any other vasoconstrictor, indicating that previously observed changes in contractions to phenylephrine may be due to the general toxicity of high doses or a consequence of reduced weight gain.
ble potentiation of angiotensin-induced contractions by nitro-L-arginine in controls and CsA-treated rats suggests that the effect of CsA on the response to Ang II is not explained by reduced basal release of nitric oxide from the dysfunctional endothelium. Thus, augmentation of angiotensin-induced contractions by CsA is most likely the result of altered angiotensin receptor function or expression on the vascular smooth muscle.

The reduction of endothelium-dependent relaxations must be due to an altered function of endothelial cells because endothelium-independent but nitric oxide–mediated vasodilator responses of the vascular smooth muscle to nitroglycerin and SIN-1 are not affected by CsA. The pattern of CsA-induced endothelial dysfunction differs from those described in the aorta of spontaneously hypertensive rats, in which the generation of the vasoconstrictor prostaglandin H₂ counteracts and inactivates endothelium-derived nitric oxide.21-23 There was no evidence for a modulatory effect of cyclooxygenase products in the responses to acetylcholine in any group. Basal release of nitric oxide, as judged from the effect of nitric oxide inhibition on contractions to Ang II, phenylephrine, and serotonin, was not affected by CsA.

Chronic ACE inhibition and AT₁ receptor blockade completely prevented impaired endothelium-dependent relaxations in this model as judged from the response to acetylcholine and the calcium ionophore A 23187. Endothelium-dependent relaxations to thrombin were reduced to the same extent by CsA and restored by cotreatment with the vasodilators. However, because of the high variation of thrombin responses the effects did not reach statistical significance.

In normotensive rat strains long-term treatment with CsA does not raise blood pressure. In the present study the lack of any change in heart weight after treatment with CsA is in agreement with observations in previous studies.10,12 Thus, blood pressure is unlikely to be a pathogenetic factor for endothelial dysfunction in CsA-treated rats. Chronic ACE inhibition with high doses as well as AT₁ receptor blocker may lower blood pressure even in normotensive animals.16,24,25 The reduction of heart weight by lisinopril may be due to reduced blood pressure and altered hormonal influences. It was comparable in the single treatment and in the cotreatment group, suggesting that combined effects on blood pressure and hormonal influences were comparable in both groups. D 8731 had no significant effect. Since the vasodilators alone did not modify endothelial function, it is unlikely that a reduction in blood pressure is the only mechanism leading to the preservation of endothelial function after combined treatment. Although it cannot be ruled out that even normal blood pressure facilitates the effects of CsA on endothelial cells, the present data suggest that the vasodilators exhibit a protective effect regardless of changes in blood pressure.

CsA levels were unaffected by combining CsA with lisinopril, which excludes a pharmacokinetic explanation for the effects of lisinopril. Cotreatment with D 8731 reduces CsA blood levels, a phenomenon previously observed by combining CsA with other drugs.26 Lower CsA blood levels are considered one of the protective mechanisms after treatment with D 8731; however, in another series of experiments endothelium-dependent relaxations were reduced after treatment with CsA 10 mg/kg per day (blood levels, 262 ng/mL) for 6 weeks (calcium ionophore 10⁻⁵ mol/L, 69% versus 48% [E.D., W.A.-S., E.F., unpublished data, 1992]). Because both ACE inhibition and AT₁ receptor blockade protect endothelial function, it can be assumed that the drugs prevent the action of Ang II on endothelial cells during CsA treatment. Specific Ang II binding was demonstrated in endothelial cells in culture.27 The functional role of these receptors is thus far not known. It may be hypothesized from this study, as well as from those in hypertensive and atherosclerotic animals, that chronic stimulation of endothelial Ang II (subtype 1) receptors impairs the release of relaxing factors from the endothelium.

Long-term treatment with lisinopril reduces maximal contractions to phenylephrine and serotonin. Because this effect was observed with single treatment and in combination with CsA, it could be a consequence of reduced blood pressure during ACE inhibition. D 8731 did not affect these responses, which may indicate either less efficacy of AT₁ receptor blockade or a contribution of bradykinin to the effect of the ACE inhibitor. Both ACE inhibition and selective AT₁ receptor blockade lower the sensitivity to serotonin but not to phenylephrine, as judged from ED₅₀ values. This effect was independent of CsA treatment and reported in spontaneously hypertensive rats as well. It may represent chronic receptor modulation on vascular smooth muscle cells as a consequence of reduced blood pressure or chronically reduced Ang II exposure.

Chronic ACE inhibition with lisinopril either alone or in combination with CsA augments contractions to Ang II. Since contractions to other vasoconstrictors are unaffected or even reduced, angiotensin receptor function or its second messenger pathways must be selectively modulated. The combination of CsA and lisinopril does not result in an additive effect, suggesting that either both treatments cause maximal stimulation or they exhibit their effect by the same pathway. The reduction in CsA blood levels may contribute to but not totally explain the prevention of CsA effects on the Ang II response by D 8731. The mechanism of CsA-induced regulation of angiotensin receptors is not known. During ACE inhibition, upregulation of angiotensin receptors due to chronic withdrawal of the agonist would be a plausible hypothesis. One would expect a similar effect with the receptor antagonist, which was not the case. Theoretically, an acute effect of D 8731 remaining in the vascular tissue could have masked the upregulation by chronic blockade. However, adding the compound to the organ bath shifts the concentration response-curve to Ang II to the right, as expected from a competitive antagonist, which was not the case in long-term experiments. The reason for the differences in the regulation of angiotensin receptors during long-term treatment between ACE inhibition and angiotensin receptor blockade is not clear and requires further investigation.

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References


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