Augmented Arterial Pressure Responses to Cyclosporine in Spontaneously Hypertensive Rats

Role of Cytochrome P-450 3A

Ashish K. Basu, Siddhartha Ghosh, Pramod K. Mohanty, Charles O. Watlington

Abstract Evidence to support a hypertensinogenic role of family 3A cytochrome P-450 (CYP3A) activity is that troleandomycin, a selective inhibitor of CYP3A, decreases both blood pressure and in vivo corticosterone 6b-hydroxylation in spontaneously hypertensive rats (SHR). Renal CYP3A activity is markedly increased in SHR compared with Wistar-Kyoto (WKY) rats. Cyclosporine acutely increases both systolic blood pressure and renal total cytochrome P-450 in SHR. We tested the hypothesis that the augmentation of blood pressure by cyclosporine is mediated by a further increase in renal CYP3A activity. Accordingly, we assessed the effect of troleandomycin administration on cyclosporine-induced systolic blood pressure increase and renal and hepatic microsomal CYP3A activity in SHR. Cyclosporine (5 mg/kg SC) given daily in 11-week-old SHR resulted in substantial augmentation of blood pressure after 6 days. This blood pressure increase was attenuated by troleandomycin (40 mg/kg) given either during or after development of hypertension. Cyclosporine increased renal (60%) but decreased hepatic (25%) microsomal CYP3A activity in SHR. In contrast, cyclosporine failed to produce any detectable increase in either blood pressure or renal CYP3A activity in WKY rats. Troleandomycin completely inhibited renal CYP3A activity measured after cyclosporine treatment of SHR, which correlated with its attenuation of the cyclosporine-induced blood pressure increase. These findings suggest that renal CYP3A could play an important role in acute cyclosporine-induced hypertension.

Key Words • rats, inbred SHR • cyclosporine • blood pressure • corticosterone • kidney

Cyclosporine (CSA) has significantly enhanced long-term survival after organ transplantation.1 However, hypertension has emerged as a universal side effect of immunosuppression with CSA. This has been most vividly shown in heart transplant recipients, in whom the incidence of hypertension has increased from less than 20% in the pre-CSA era to more than 90% currently.2-4 Hypertension has been noted to occur as early as a few weeks after transplantation in 10% to 80% of patients.5 Thus, hypertension has become one of the major problems in the clinical care of heart transplant recipients. Although various mechanisms have been advanced2-5 to explain CSA-induced hypertension in both humans and experimental animals,6 the role of the family 3A cytochrome P-450 (CYP3A) system in an experimental model has not been studied. The spontaneously hypertensive rat (SHR) is noteworthy in this regard because it is the only rat model reported to exhibit this phenomenon of a short-term CSA-induced augmentation in arterial pressure.6 Earlier work done by Schwartzman and coworkers (Sacerdoti et al56) has contributed to our understanding of the sensitivity of SHR to the hypertensigenic effect of CSA. They first demonstrated that both renal and hepatic total cytochrome P-450 (CYP) is increased in SHR compared with normotensive Wistar-Kyoto (WKY) rats.7,8 The administration of stannous chloride decreased systolic blood pressure (SBP) in SHR and was associated with the depletion of renal but not hepatic CYP.9 This hypotensive effect was attributed to an associated decrease in the elevated vasoactive arachidonate metabolites produced by renal CYP4A, 6 and 9 hydroxylases.7,8 Another important observation in this regard is that CSA increases total renal CYP but decreases total hepatic CYP in SHR.9 These latter findings raise the possibility that renal CYPs not only may account for a component of the basal blood pressure elevation in SHR but also may play a major role in the augmentation induced by CSA administration.

CYP3As are the dominant steroid 6b-hydroxylases. 6b-OH-corticosterone is an agonist for transepithelial active Na+ transport stimulation in cultured kidney cells (A6) and seems to mediate the stimulation of Na+ transport by corticosterone, possibly by a nonclassic mechanism.10,11 We speculate that in the presence of increased CYP3A activity, when the metabolite is generated in excess, it might produce a defect in renal Na+ excretion. Previous studies from our laboratory have suggested that increased CYP3A activity could contribute to the blood pressure elevation in SHR. The proportion of injected [3H]corticosterone excreted in urine as [3H]6b-OH-corticosterone was fourfold to fivefold higher in SHR compared with WKY rats, indicating enhanced CYP3A activity in SHR.12 Moreover, the administration of the macrolide antibiotic troleandomycin (TAO), a putative selective inhibitor of CYP3A,13,14 decreased this in vivo index of CYP3A activity as well as

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SBP in SHR. We further demonstrated that renal CYP3A catalytic activity and CYP3A protein were increased fivefold or more in SHR compared with WKY rats, whereas there was less than a onefold increase in the hepatic catalytic activity in SHR. These latter findings support a role for enhanced renal CYP3A in the basal blood pressure elevation in SHR compared with WKY rats. The purpose of this study was to test the hypothesis that CSA augments SBP in SHR by further increasing renal CYP3A activity.

Methods

Experimental Protocol

CSA was a gift from Sandoz Research Institute (East Hanover, NJ). It was administered to 11-week-old rats at a dose of 5 mg/kg body wt, as this dose was shown to increase blood pressure in SHR in the absence of significant changes in creatinine clearance; a higher CSA dose caused renal function impairment after 7 and 28 days of drug administration. CSA and TAO (40 mg/kg body wt SC) were administered in 0.5 mL/kg dimethyl sulfoxide as diluent. Ten-day and 6-day protocols were used. In the 10-day protocol, two groups of SHR were used: one (9 rats) received CSA for 10 days, and the other (14 rats) received TAO for 4 days (days 6 through 9) in addition to CSA. SBP was measured before the first dose of drug at time zero, on day 6, and 24 hours after the 10th consecutive dose of the drug (day 10). In the 6-day protocol, one group of WKY rats (6 rats received CSA) and four groups of SHR were used. The SHR groups were group 1 (9 rats, control), group 2 (9 rats, CSA-treated), group 3 (8 rats, CSA+TAO-treated), and group 4 (9 rats, TAO-treated). Basal SBP was measured just before the first dose of drug was injected at time zero. The rats received the drug for 6 consecutive days. The second SBP was determined 24 hours after the sixth dose of drug (day 6). TAO was given for 4 days (days 2 through 5).

SBP Measurements

Male SHR and WKY rats were obtained from Taconic Farms (Germantown, NY) at 10 weeks of age. SBP in awake rats was measured by the tail-cuff method using a photoelectric sensor and pulse amplifier (ITTC Life Science) connected to a two-channel recorder. Before the experiment, animals were maintained in a quiet room for 4 to 5 days, during which they were trained twice by measurement of SBP. For each measurement, rats were placed in a restraining cage for 5 minutes, after which successive readings were taken until four to five sequential readings were within a range of 15 mm Hg. The average was used as the SBP value.

Measurement of CYP3A Catalytic Activity

Liver and kidney from 11-week-old male SHR and WKY rats were homogenized in 100 mmol/L Tris-EDTA buffer (pH 7.4) in the presence of protease inhibitors (2 mmol/L hydrocinnamic acid, 0.5 mmol/L benzyl arginine, and 5 mmol/L benzamidine) and microsomes prepared as described by Grogan et al. Corticosterone 6β-hydroxylase activity was measured by a radiometric method. Typically, 0.5 mg of microsomes was incubated for 30 minutes at 37°C in 50 mmol/L K2PO4 buffer, 5 mmol/L MgCl2, 10 mmol/L glucose-6-phosphate, 1 U/mL glucose-6-phosphate dehydrogenase, 0.5 mmol/L NADP+, and 3 μCi [3H]corticosterone (104 mol/L) in a total volume of 1.5 mL. Unlabeled corticosterone was obtained from Sigma Chemical Co, and [1,2-3H]corticosterone was obtained from New England Nuclear. The reaction was terminated by placing the mixture on ice followed by reversed solid-phase extraction, elution, and reversed-phase high-performance liquid chromatography (HPLC) as described below.

Results

Blood Pressure Responses to CSA and TAO

Daily CSA administration alone (Fig 1, Table 1) produced an increase in SBP from 155±1.27 to 178±2.06 mm Hg at day 6. SBP was essentially the same at day 10 (180±3.28 mm Hg). Thus, the maximal effect
Table 1. Effect of Cyclosporine and Troleandomycin on Systolic Blood Pressure In 11-Week-Old SHR and WKY Rats

<table>
<thead>
<tr>
<th>Drug</th>
<th>Day 0</th>
<th>Day 6</th>
<th>Day 10</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CSA (9)</td>
<td>155±1.27</td>
<td>178±2.06</td>
<td>180±3.28</td>
<td>...</td>
</tr>
<tr>
<td>CSA+TAO (14)</td>
<td>156±1.40</td>
<td>179±2.72</td>
<td>139±1.66*</td>
<td>...</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (9)</td>
<td>150±1.28</td>
<td>149±1.66</td>
<td>...</td>
<td>NS</td>
</tr>
<tr>
<td>CSA (9)</td>
<td>146±1.70</td>
<td>178±2.63</td>
<td>...</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CSA+TAO (8)</td>
<td>155±2.68</td>
<td>140±3.78</td>
<td>...</td>
<td>&lt;.001</td>
</tr>
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<td>TAO (9)</td>
<td>154±2.65</td>
<td>128±1.82</td>
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<td>&lt;.001</td>
</tr>
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<td>WKY</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>CSA (6)</td>
<td>105±1.79</td>
<td>106±1.86</td>
<td>...</td>
<td>NS</td>
</tr>
</tbody>
</table>

SHR indicates spontaneously hypertensive rats; WKY, Wistar-Kyoto; CSA, cyclosporine; and TAO, troleandomycin. Values are mean±SEM. Group A followed an 11-day protocol; group B, a 6-day protocol. Numbers in parentheses indicate number of animals used in each treatment schedule.

*P<.001, CSA- vs CSA+TAO-treated series in Group A using two-tailed t test; in group B, results of paired t test are shown comparing day 0 with day 6 SBP in each series.

CYP3A Activity

Corticosterone 6β-hydroxylase activity (CYP3A) in the renal microsomes of SHR treated with CSA was 60% greater than the value found in control kidneys (P<.05, Table 2). Concomitant TAO administration (CSA+TAO series) reduced renal catalytic activity in SHR to nondetectable levels (P<.05). In contrast to kidney, liver microsomes from CSA-treated SHR had 30% lower catalytic activity compared with those from control rats (P=NS). On simultaneous TAO administration, CYP3A activity was further reduced in liver compared with control (P<.05, Table 2).

CYP3A enzyme activity was not detectable in kidney of control or CSA-treated WKY rats (Table 2), and thus no effect of CSA was discerned. Undetectable CYP3A activity is often the case in WKY kidney (the lower limit of detection of the catalytic assay is 0.05 pmol·min⁻¹).

Fig 2. Plot shows effect of cyclosporine (CSA), troleandomycin (TAO), and CSA+TAO on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. SBP is shown as mean±SEM. Number of rats in each group is shown in parentheses. See Table 1 for results of statistical evaluation.

Fig 3. Bar graph shows percent change in systolic blood pressure (SBP) (±SEM) from day 0 to day 6 in rat groups shown in Fig 2. Spontaneously hypertensive rats were divided into control (C), cyclosporine (CSA), troleandomycin (TAO), and CSA+TAO groups. Wistar-Kyoto (WKY) rats were treated with CSA only. *P<.05 by ANOVA.
CSA decreased CYP3A activity by 33% in WKY liver, which was similar to the change in SHR liver and was not statistically significant.

**CSA Blood Levels**

After 6 days of drug treatment, average CSA level in the group receiving CSA alone was 558±37.4 ng/mL; the level in the CSA+TAO-treated group was 399±94.7 ng/mL. The values between the two groups were not significantly different.

**Discussion**

The major new findings of this study are (1) CSA produces an augmentation in blood pressure in SHR but not in WKY rats; (2) an augmented blood pressure response is associated with a CSA-induced increase in renal CYP3A activity in SHR (but not in WKY rats), and hepatic CYP3A is, if anything, decreased by CSA in SHR; and (3) TAO, which is considered a selective inhibitor of CYP3A,1314 attenuated the CSA-induced augmentation of SBP, and this attenuated blood pressure response correlated with a marked reduction in renal CYP3A activity observed after CSA+TAO administration. The major new conclusion from this study is that a CSA-induced increase in renal CYP3A is of potential importance in CSA-induced hypertension. We believe that an understanding of such cellular mechanisms will be crucial to the design of future therapy to prevent CSA-induced hypertension in humans after heart and other solid organ transplantation.

Several potential etiologic factors contribute to CSA-induced hypertension. Indeed, evidence from our experiments indicates that more than one mechanism may be operative in SHR, as TAO did not abolish all of the CSA-induced blood pressure increase. Our reasoning is as follows: Previous work from our laboratory has demonstrated that TAO abolishes approximately 50% of the increment of SBP difference in SHR compared with WKY rats (50 mm Hg at 11 weeks) from a value of approximately 155 to 130 mm Hg.20 If all the SBP augmentation resulting from CSA administration to SHR were mediated by the concomitant increase in renal CYP3A, then TAO should decrease SBP in CSA-treated animals to the range of 130 mm Hg; ie, it should inhibit both the basal TAO-sensitive component and the CSA-stimulated component of blood pressure elevation. This was clearly not the case, because SBP fell only to 140±3.78 mm Hg in CSA+TAO-treated animals, a change that was significantly different from the effect of TAO alone (Fig 2, Table 1). In other words, if the effects on SBP of CSA (+25 mm Hg) and TAO (−25 mm Hg) are simply additive, then CSA+TAO treatment should produce no change in SBP, as observed in control animals given no drugs. However, the combined administration of the two drugs produced a significant decrease in SBP (Fig 2), indicating an inhibitory effect of TAO on the CSA-induced hypertension. We interpret these results to suggest that TAO inhibits a major component but not necessarily all of the CSA-induced augmentation in SBP. The absence of any hypertensive effect of CSA in WKY rats suggests an increased sensitivity of SHR to the hypertensigenic effect of CSA. The increased CYP3A activity in SHR could contribute to the enhanced short-term hypertensigenic response to CSA in this strain.

Two possibilities may explain the component of CSA-induced blood pressure increase that was not sensitive to TAO. First, there could be an element of renal failure produced by CSA administration, which accounts for a portion of SBP elevation, although the daily dose used here (5 mg/kg body wt) did not produce a detectable change in creatinine clearance.6 Controversy exists as to the role of Na+ balance in the nephrotoxicity produced by a CSA dose higher than used here.1617 We have made no attempt to control sodium intake or measure renal function in our studies. Second, the TAO-insensitive component could be due to an increase in CYP-dependent arachidonate metabolism to bioactive products, which are thought to contribute to basal blood pressure elevation in SHR compared with WKY rats.24 Not only are these metabolites increased in SHR compared with WKY kidney,24 but their production by SHR renal microsomes is further increased by CSA administration.21 Based on its apparent selective inhibitory effect of CYP3A,1314 TAO would not be expected to inhibit this other class of CYP (CYP4A) that metabolizes arachidonate.

The mechanism by which enhanced 6β-OH-cortico-sterone production might exert a basal hypertensive effect in SHR, and its augmentation by CSA, is uncertain. However, we have shown that 6β-OH-corticosterone is an agonist for increased Na+ transport in cultured epithelia derived from amphibian kidney (A6 cells).10 Most of the active transepithelial Na+ transport stimulation produced by corticosterone in the cells appears to be mediated by 6β-OH-corticosterone,11

### Table 2. Effect of 6-Day Cyclosporine Administration, With or Without 4-Day Trolenamycin, on Kidney and Liver Microsomal CYP3A (Corticosterone 6β-Hydroxylase) Activity in 11-Week-Old SHR and WKY Rats

<table>
<thead>
<tr>
<th>Definitions</th>
<th>Control</th>
<th>CSA</th>
<th>CSA + TAO</th>
<th>Control</th>
<th>CSA</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.86±0.22</td>
<td>3.13±0.48*</td>
<td>ND*</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Liver</td>
<td>5.84±0.55</td>
<td>4.30±0.71</td>
<td>2.20±0.36*</td>
<td>2.93±0.33</td>
<td>1.95±0.32</td>
<td>NS</td>
</tr>
</tbody>
</table>

Definitions are as in Table 1; ND indicates not detectable (<0.05 pmol·min⁻¹·mg⁻¹ protein). Values are mean±SEM; n=4 for all series except SHR control (n=6).

*P<.05. ANOVA was used to compare SHR kidney and liver CSA and CSA+TAO series with control, respectively. In the WKY series, two-tailed t test was used to determine statistical significance between CSA treatment and diluent alone.
which is produced in the effector cell by CYP3A. We speculated that increased 6β-OH-corticosterone production in some segment of the nephron might cause a defect in renal Na⁺ excretion. Such a renal defect in Na⁺ excretion is postulated for essential hypertension in humans and in SHR. However, the renal Na⁺ retentive mechanism hypothesized may be necessary but not sufficient to produce a component of basal blood pressure elevation in SHR. It is also possible that excess renal CYP3A exerts a hypertensinogenic effect by some mechanism other than excess 6β-OH-corticosterone production.

Since pharmacokinetic interaction between CSA and TAO could occur, we measured blood levels of CSA at the end of drug treatment. CSA levels were not significantly different between the two groups of SHR (CSA-treated and CSA+TAO-treated), indicating that the effect of TAO on SBP was not simply the result of an alteration in CSA levels.

Experimental and clinical studies have shown that the most common mechanism of CSA-induced hypertension relates to renal vasoconstriction, a suppressed renin-angiotensin system, and a salt-dependent hypertension with a tendency toward extracellular fluid volume expansion. Nephrotic effects per se have been suggested as a cause of CSA-induced hypertension. Studies have also shown that CSA results in progressive increased peripheral vascular resistance leading to preglobular vasoconstriction, and it also causes a progressive narrowing of afferent arterioles secondary to smooth muscle proliferation, both resulting in diminished renal blood flow and increased mean arterial pressure. CSA-induced increased sympathetic tone also contributes to intrarenal vasoconstriction and proximal tubule sodium avidity in the innervated kidneys of extrarenal transplant recipients and those receiving CSA for autoimmune disorders. The renal vasoconstriction could also result from an imbalance in the production of vasoconstrictor eicosanoids and/or failure of vasodilator prostaglandin synthesis. The endothelin vasoconstrictor axis has recently been suggested to play a role in CSA-induced hypertension. CSA stimulates endothelial cell synthesis of endothelin, which may mediate renal vasoconstriction in CSA-treated rats. CSA activity and 6β-OH-corticosterone production may relate to the endothelin vasoconstrictor axis remains to be defined. In rats, CSA is metabolized by CYP3A in intestine and liver. CSA metabolism by these organs as well as by kidney has also been demonstrated in humans. Whether any of the CYP3A metabolites are hypertensinogenic is unknown.

Further investigation is needed to determine the exact mechanism of how the CSA increase of renal CYP3A in SHR might contribute to and interact with a host of vasoactive mechanisms influencing hypertension. However, our results add another dimension to the pathogenetic mechanisms of CSA-induced hypertension, suggesting that CYP3A may play a significant role in this disorder.

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