Vascular Protection with Candesartan after Experimental Acute Stroke in Hypertensive Rats: A Dose-Response Study

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ABSTRACT

We have shown that candesartan decreases the acute stroke-induced elevation of mean arterial blood pressure (MAP) in Wistar rats and improves functional outcome. The aim of the present study was to determine whether the same benefit could be achieved in spontaneously hypertensive rats (SHR). Animals were subjected to middle cerebral artery occlusion (MCAO) or sham for 3 h followed by reperfusion. Either candesartan (0.1, 0.3, or 1.0 mg/kg) or saline was administered. MAP of the rats was monitored by means of telemetry, and neurological function was assessed. Infarct size, edema formation, and hemoglobin content in the ischemic hemisphere were evaluated 24 h after the stroke. MAP of SHR increased immediately upon MCAO from 135 (baseline) to 189 mm Hg, and it remained elevated until reperfusion. Candesartan decreased MAP in a dose-dependent manner, with a drop below baseline after a dose of 1.0 mg/kg. SHRs experienced greater blood pressure (BP)-lowering effects of candesartan after stroke compared with a sham condition (p < 0.0001). Neurological deficit after stroke was reduced in candesartan-treated animals, revealing a dose-dependent effect (p < 0.01). Infarct size, edema formation, and hemoglobin content were all reduced by candesartan at doses of 0.1 and 0.3 mg/kg (p < 0.05 for all). Candesartan (1 mg/kg) was not different from saline. Low doses of candesartan provide neurovascular protection after stroke in SHRs. Caution is warranted because acute stroke increases the sensitivity to BP lowering, which, in turn, increases the likelihood of overshooting.

Hypertension is present in almost 80% of acute stroke patients, and it is known to increase the occurrence and severity of ischemic stroke (Li et al., 2005). The newest clinical guidelines for management of patients with acute ischemic stroke recommend only treating the most severely elevated pressures in the first 24 h and then only decreasing mean arterial blood pressure (MAP) by 15% (Adams et al., 2007). For patients with preexisting hypertension, there is support for restarting antihypertensive medications, but only after 24 h (Adams et al., 2007).

The consequences of hypertension before and after stroke can also be demonstrated in animals. Spontaneously hypertensive rats (SHRs) exhibit more severe neurological deficits and edema formation (Slivka et al., 1995), and they more frequently demonstrate extensive cerebral infarct damage than normotensive animals after permanent focal cerebral ischemia (Coyle, 1986; Barone et al., 1992). However, the studies using SHR have yielded contradictory results regarding the influence of blood pressure (BP) on ischemic injury and prognosis. It has been postulated that antihypertensive drugs may reduce the pressure-dependent cerebral blood flow to the ischemic penumbra, or conversely, poststroke hypertension may be deleterious and facilitate edema formation in the ischemic brain tissue (Harms et al., 2000). It is hypothesized that in the SHR, stimulation of brain and cerebrovascular angiotensin II (Ang II) systems contributes to vasoconstriction, increased expression of proinflammatory factors, and increased microvessel permeability (Ito et al., 2001). In support of these data, it has been demonstrated that Ang II AT1-receptor blockade reduces blood pressure, normalizes the brain microcirculation, and decreases the vulnerability to stroke in chronically hypertensive rats (Ito et al., 2002; Ando et al., 2004; Zhou et al., 2005). Acute and prolonged Ang II AT1-receptor blockade before the onset of experimental stroke may contribute to the protection against brain ischemia and inflammation in the SHR (Nishimura et al., 2000; Zhou et al., 2006). However, this protection with
AT$_1$ receptor inhibition as a pretreatment is not directly correlated with blood pressure reduction (Ito et al., 2002). Recent studies from our laboratory showed that candesartan (an AT$_1$ receptor blocker), administered after reperfusion in acute ischemic stroke, normalizes blood pressure, reduces neurovascular damage, and improves outcome in normotensive male Wistar rats (Fagan et al., 2006). The purpose of the present study was to assess whether candesartan at reperfusion will be similarly protective in SHRs, a model that is possibly more clinically relevant.

**Materials and Methods**

**Animals.** Nine-week-old male SHRs, weighing approximately 200 g, were purchased from Charles River Breeding Laboratories, Inc. (Wilmington, MA). After shipping, the animals were housed in individual plastic cages for 5 to 6 days before the surgery and experimentation. The rats were given food and water ad libitum on a 12:12-h light/dark cycle at 24°C in a 30 to 40% humid atmosphere. Rats were weighed and randomly assigned to each experimental group. When the rats reached a body weight of approximately 240 g, they were instrumented for telemetry blood pressure monitoring, and when they gained additional body mass of 275 to 300 g, they were subjected to the experimental protocol. In general, the rats were 12 to 13 weeks old at the time of the experimental cerebral ischemia or sham.

The Institutional Animal Care and Use Committee of the Charlie Norwood VA Medical Center (Augusta, GA) approved all procedures, in accordance with the Institute of Laboratory Animal Resources (1996).

**Blood Pressure Telemetry.** Telemetry transmitters (Data Sciences International, St. Paul, MN) were implanted according to the manufacturer’s specifications as described elsewhere (Fagan et al., 2006). In brief, the rats were anesthetized with sodium pentobarbital (65 mg/kg i.p.; Abbott Laboratories, Chicago, IL), and a midline incision was performed to expose the abdominal aorta. The exposed vessel was shortly occluded to allow insertion of the transmitter catheter into the abdominal aorta. Using tissue glue, the catheter was secured in place, and the incision (abdominal muscle and skin) was sutured. Rats returned to their individual cages and were allowed to recover from surgery for 10 days. By placing rats on top of the telemetry receivers, arterial pressure waveforms were continuously recorded throughout the study. Data were recorded every 10 min for several days before the stroke (or sham) and until the sacrifice at 24 h after the onset of stroke.

**Experimental Cerebral Ischemia.** Animals were anesthetized with 2% isoflurane via inhalation. Cerebral ischemia was induced using the intraluminal suture middle cerebral artery occlusion (MCAO) model (Zea Longa et al., 1989). The right middle cerebral
artery was occluded with 19 to 21-mm 3-0 surgical nylon filament, which was introduced from the external carotid artery lumen into the internal carotid artery to block the origin of the right middle cerebral artery. The animals were kept under anesthesia for only 10 min for the surgical procedure. The suture was removed after 3 h of occlusion, and the animals were returned to their cages.

Before reperfusion, the animals were subjected to the test for assessment of neurological function. Immediately after reperfusion, either saline or 0.1, 0.3, and 1.0 mg/kg doses of candesartan cilexetil (AstraZeneca Pharmaceuticals LP; Wilmington, DE), as indicated in the figures, was administrated i.v. by tail vein at an injection volume of 1 ml/kg. A group of 12 SHRs and 12 Wistars were shams for the same doses as described above.

Perimed Laser Doppler Perfusion Imaging hardware and software system (PeriScan PIM 3 System; Perimed AB, Stockholm, Sweden) was used to document reduction of flow due to MCAO. In a subset of animals (n = 5), the skin was reflected, the bone was cleaned, and scans were performed at baseline, 5 min after the MCAO, 5 min after reperfusion and inject.

**Fig. 2.** MAP (mm Hg) after acute stroke in SHRs. MAP was recorded every 10 min (telemetry) before stroke (baseline between 6:00 and 9:00 AM), during the onset of ischemia (at 10:00 AM; left arrow), reperfusion (at 1:00 PM; right arrow), and then during the after 21 h until sacrifice next day. At reperfusion, the animals received i.v. injections with saline (n = 8) and/or with candesartan at the doses as shown. The black horizontal bar indicates night-time in the light/dark cycles. Values shown are 1-h averages ± S.E.M.

### TABLE 1

MAP for each group at different time points—mean (S.D.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Prestroke</th>
<th>MCAO</th>
<th>2-h Postperfusion*</th>
<th>Poststroke*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7</td>
<td>135.3 (7.5)</td>
<td>188.6 (7.3)</td>
<td>173.5 (10.0)a</td>
<td>160.2 (6.4)a</td>
</tr>
<tr>
<td>Candesartan 0.1</td>
<td>7</td>
<td>146.2 (8.2)</td>
<td>187.4 (8.3)</td>
<td>166.2 (9.8)b</td>
<td>160.6 (12.5) b</td>
</tr>
<tr>
<td>Candesartan 0.3</td>
<td>7</td>
<td>146.0 (7.5)</td>
<td>189.4 (8.1)</td>
<td>151.2 (10.6)bc</td>
<td>148.2 (12.7)bc</td>
</tr>
<tr>
<td>Candesartan 1</td>
<td>7</td>
<td>139.1 (8.2)</td>
<td>186.3 (8.0)</td>
<td>145.0 (7.6)bc</td>
<td>133.2 (10.4)bc</td>
</tr>
</tbody>
</table>

* Means with the same letter are not significantly different.

**TABLE 2**

AUC, MIN, and MAX values for each group—mean (S.D.)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>AUC*</th>
<th>MIN</th>
<th>MAX</th>
<th>Change Pre to Post, 6–9 AM*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>7</td>
<td>4340 (145)a</td>
<td>128.0 (6.4)a</td>
<td>193.2 (7.5)</td>
<td>22.7 (9.5)a</td>
</tr>
<tr>
<td>Candesartan 0.1</td>
<td>7</td>
<td>4382 (286)a</td>
<td>138.8 (7.6)a</td>
<td>192.2 (8.2)</td>
<td>7.2 (11.1)b</td>
</tr>
<tr>
<td>Candesartan 0.3</td>
<td>7</td>
<td>4123 (239)b</td>
<td>124.1 (10.5)bc</td>
<td>192.8 (9.0)</td>
<td>–1.4 (8.7)bc</td>
</tr>
<tr>
<td>Candesartan 1</td>
<td>7</td>
<td>3904 (228)b</td>
<td>120.3 (9.6)b</td>
<td>189.5 (8.1)</td>
<td>–9.3 (10.0)c</td>
</tr>
</tbody>
</table>

p value 0.0007 0.0036 0.84 <0.0001

* Means with the same letter are not significantly different.
Fig. 3. Effect of candesartan on ischemia-induced infarct size (A) and edema (B) in SHRs. Doses of candesartan, or saline as control, were injected intravenously at the time of reperfusion during MCAO, as shown in Fig. 2. *, p < 0.05 compared with control.
reperfusion (before drug administration), and before sacrifice at 24 h. Images were saved, and average perfusion units were documented in both hemispheres at each time point. All endpoints were assessed in a blinded fashion.

Neurological Assessment. Neurological function was measured before reperfusion and at 24 h (just before sacrifice) using the Bederson score (Bederson et al., 1986). An animal with no apparent deficits obtained a 0; the presence of forelimb flexion = 1; decreased resistance to push = 2; and circling = 3. A score of 3 is consistent with a middle cerebral artery occlusion. Only animals with a score of 3 before reperfusion were included in the analysis of infarct size, hemoglobin, and neurological function. In addition to the Bederson score, several other behavioral tests such as beam walk, paw grasp, and elevated body swing test were performed, and neurobehavioral deficit was assessed as described elsewhere (Wahl et al., 1992; Borlongan and Sanberg, 1995).

Assessment of Infarct Size, Edema, and Hemoglobin Content. At 24 h after the onset of MCAO, anesthesia was performed with 44 mg/kg ketamine and 13 mg/kg xylazine administered intramuscularly. Animals were then perfused with saline, sacrificed, and their brains were removed. The brain tissue was sliced into seven 2-mm-thick slices in the coronal plane and stained with a 2% solution of 2,3,5-triphenyltetrazolium chloride (Sigma-Aldrich, St. Louis, MO) for 15 to 20 min. Images of the stained sections were taken. Using image analysis software (Zeiss-KS300; Carl Zeiss, Oberkochen, Germany), infarction zones were measured, and percentage infarct size corrected for edema was calculated. Edema was quantified as the difference in area between the hemispheres, expressed as a percentage of the contralateral hemisphere. The ischemic and nonischemic hemispheres of the slices for the enzyme-linked immunosorbent assay were separated and processed, using the nonischemic side as a control. After homogenizing the 2,3,5-triphenyltetrazolium chloride-stained slices in the core of the infarct and collecting the supernatants, enzyme-linked immunosorbent assay was performed to measure the hemoglobin content of the brain tissue (Hilali et al., 2004).

Statistical Analysis. For blood pressure data, the average of all measurements before MCAO was the prestroke value. Values obtained during the 3 h of MCAO were averaged for the estimate of BP during stroke, the values for the first 2 h postreperfusion were averaged for an estimate of the immediate effects of the drugs, and all values 5 h after the onset of ischemia were averaged for the post-stroke value. For assessment of the effect of the stroke on the response to BP lowering, a two-strain (Wistar versus SHR) by two-condition (stroke versus no stroke) factorial analysis of covariance was used to analyze differences in MAP within the time periods described above, using baseline MAP as a covariate. A two-way mixed model repeated measures analysis of variance (ANOVA) was

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Infarct Size*</th>
<th>Edema*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>16</td>
<td>62.3 (6.3)</td>
<td>23.3 (3.0)</td>
</tr>
<tr>
<td>Candesartan 0.1</td>
<td>7</td>
<td>58.0 (3.5)</td>
<td>21.2 (2.4)</td>
</tr>
<tr>
<td>Candesartan 0.3</td>
<td>11</td>
<td>50.7 (6.7)</td>
<td>18.3 (5.0)</td>
</tr>
<tr>
<td>Candesartan 1</td>
<td>11</td>
<td>61.8 (6.8)</td>
<td>22.8 (5.5)</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td>0.0001</td>
<td>0.023</td>
</tr>
</tbody>
</table>

* Means with the same letter are not significantly different.

Fig. 4. Effect of candesartan on Bederson score in SHRs subjected to MCAO. The animals were tested before reperfusion and treatment (white bars), and only animals with a score of 3 were further examined. Dashed bars represent the scores (means ± S.E.M.) assessed 24 h after the reperfusion and injections, before sacrifice. *, p < 0.05 compared with control.
used to determine treatment (saline or 0.1, 0.3, 1 mg/kg candesartan), time (prestroke, MCAO, 2-h postperfusion, poststroke), and treatment by time differences in MAP. The area under the curve (AUC) for the entire duration of the experiment was also calculated for each rat where baseline was considered to be zero. Maximal (MAX) and minimal (MIN) MAP were also determined. Differences among different treatments and control were determined by one-way ANOVA for AUC, MAX, MIN, average infarct size, edema, hemoglo-
bin content, and postperfusion values of the Bederson, beam walk, and paw grasp scores. A Tukey-Kramer adjustment for multiple comparisons was used for all post hoc mean comparisons. All analyses were performed using SAS 9.1.3 (SAS Institute Inc., Cary, NC). Statistical significance was measured at an alpha level of 0.05. All values are represented as mean ± S.D.

Results

Changes of Blood Pressure in the SHR upon Brain Ischemia, Reperfusion, and Candesartan Treatment. Changes in MAP (1-h averages) over time by treatment are illustrated in Figs. 1 and 2. It is noteworthy that we tested the effectiveness of candesartan in reduction of MAP in the sham SHR. Data shown in Fig. 1 demonstrate that all three doses of candesartan used (0.1, 0.3, and 1 mg/kg) reduced MAP within 2 h of injection from a baseline (approximately 140 mm Hg) to below baseline. Reduction was dose-dependent, with the most pronounced and long-lasting effect seen for a dose of 1.0 mg/kg. MAP of animals treated with doses of 0.3 and 1.0 mg/kg candesartan returned to the preinjection levels after 48 and 72 h, respectively, whereas MAP of the rats that received injections with a dose of 0.1 mg/kg returned to baseline within 24 h of the treatment. SHRs experienced greater BP-lowering effects of candesartan after stroke compared with the sham condition (p < 0.0001) and their Wistar counterparts (p = 0.014) (Wistar data not shown).

Within 1 h of ischemia, MAP of the rats increased from baseline (approximately 140 mm Hg) to approximately 190 mm Hg, and it remained at that level until reperfusion (Fig. 2). Reperfusion provoked a drop of MAP, and it was dramatically enhanced by candesartan treatment. The repeated measures ANOVA showed a significant interaction between treatment and time (p < 0.0001). There were no significant differences among the groups for the prestroke and the MCAO values (Table 1). For the 2-h postreperfusion, the 0.3 and 1 mg/kg candesartan groups lowered BP the most, whereas the 0.1 mg/kg candesartan and saline groups were not different. For the 19 h during the poststroke period, the 1 mg/kg candesartan group had MAP that was significantly lower than saline, and the 0.3 mg/kg candesartan group was intermediate. The poststroke means for the 0.1 mg/kg candesartan and saline groups were significantly higher than their prestroke values (all p < 0.001). There was a significant difference among the groups for AUC (p = 0.0007) and MIN (p = 0.0036) (Table 2). The 1 mg/kg candesartan group was significantly lower than the saline group and the lowest dose of candesartan, but not different from the 0.3 mg/kg candesartan group. A dose of 0.3 mg/kg candesartan after reperfusion caused a decrease of the MAP to the prestroke baseline, whereas a dose of 1.0 mg/kg candesartan also decreased, however to a lesser extent, and it remained above baseline until sacrifice. It did not differ significantly from that seen in the saline group (control), except during the 1st h after reperfusion. There were no differences among the groups for the MAX BP values during the duration of the experiment.

Infarct Size, Hemispheric Edema, and Behavior. Infarct size (Fig. 3A) and brain edema (Fig. 3B) were diminished in the SHRs treated with candesartan. However, this reduction was dose-related within a narrow range of candesartan doses (a range between 0.1–0.3 mg/kg in our study). The greatest effects on infarct size and edema were observed.
in the rats treated with a dose of 0.3 mg/kg candesartan. Infarct size in the saline-treated group \( (n=16) \) was 62.3% compared with 50.7% of the 0.3 mg/kg candesartan group. Calculated edema revealed a similar reduction (23.3% in the saline group versus 18.3% in the 0.3 mg/kg candesartan group). Table 3 shows that there are significant differences among the groups for infarct size \( (p = 0.0004) \) and edema \( (p = 0.023) \). The 0.3 mg/kg candesartan group had the lowest infarct size and edema, and it was significantly different from the control group. Neither of the other two candesartan doses were different from the saline group.

Animals receiving candesartan showed significantly better neurological function before sacrifice, assessed in the Bederson score (Fig. 4) as well as in beam walk (Fig. 5A) and paw grasp (Fig. 5B) tests. In the elevated body swing test, all animals subjected to ischemia, regardless of the treatment upon reperfusion, revealed a consistent left-oriented circling. There were significant differences among the groups for post-stroke values of Bederson scores \( (p = 0.0012) \) and paw grasp \( (p = 0.005) \). Both the 0.3 and 1 mg/kg candesartan groups had Bederson and beam walk scores that were significantly better than the control group.
Effect of Candesartan on Hemoglobin Content in the Ischemic Brain Tissue. Figure 6 shows the effect of three doses of candesartan on hemoglobin content in the ischemic brain tissue collected 24 h after the onset of stroke. As shown, doses of 0.1 and 0.3 mg/kg candesartan reduced hemoglobin content in a dose-dependent manner (8.3 and 4.0 ng/mg for doses of 0.1 and 0.3 mg/kg candesartan, respectively, compared with 9.7 ng/mg in saline-treated rats). Only the 0.3 mg/kg candesartan group was significantly different from control ($p = 0.0092$). However, hemoglobin content in the ischemic brain tissue of rats treated with a dose of 1.0 mg/kg candesartan did not differ from that seen in the saline-treated group (10.0 ng/mg in candesartan-treated versus 9.7 ng/mg in saline-treated rats).

Cerebral Perfusion. In all of the five animals tested, MCAO resulted in consistent reductions in average perfusion units ± S.E. (493.5 ± 25.7 baseline versus 352 ± 23.4 after occlusion) in the ischemic hemisphere, which ranged from 41.1 to 68% of the contralateral hemisphere 5 min after occlusion. Perfusion was restored to relative hyperemia (521 ± 25.8; average of 13.5% higher than contralateral) in the 5 min after reperfusion (Fig. 7A). Hyperemia was bilateral before sacrifice in the saline-treated group (no asymmetry), but asymmetry was maintained in the 0.3 mg/kg candesartan animals (Fig. 7B).

Discussion

The optimal approach to management of elevated blood pressure during the acute stroke period is unclear (Adams et al., 2007). Data presented here indicate that partial lowering of the pressure during reperfusion is beneficial in the SHR. It is represented by the improvement in neurological score, reduction of infarct size, and brain tissue damage, as demonstrated by decreased hemoglobin and edema formation in rats treated with candesartan at doses of 0.1 and 0.3 mg/kg immediately after reperfusion. On the other hand, reduction of blood pressure below baseline in the SHRs treated with a dose of 1.0 mg/kg candesartan eliminated this protective effect. In fact, the animals treated with 1.0 mg/kg candesartan revealed neurological and vascular damage resembling that seen in rats treated with saline. It is of particular importance that hypertensive rats seemed more sensitive to candesartan after stroke than normotensive rats, because normotensive Wistar rats given a dose of 1.0 mg/kg candesartan immediately after reperfusion decreased MAP to the normotensive Wistar rats given a dose of 1.0 mg/kg candesartan immediately after reperfusion decreased MAP to the normalized cerebrovascular autoregulation (Nishimura et al., 2000), promotes angiogenesis (Forder et al., 2005), reduces oxidative damage (Sugawara et al., 2005), and prevents apoptosis (Lou et al., 2004). Although these effects have been studied under chronic conditions, they may be beneficial in the event of an acute stroke. There may also be non-AT$_1$-mediated mechanisms involved in the protective effects of candesartan. Notably, it has been postulated that stimulation of the Ang II AT$_2$ receptor may be protective in focal cerebral ischemia (Iwai et al., 2004).

In line with the above, Engelhorn et al. (2004) found that postischemia treatment with candesartan (clinically relevant procedure similar to that applied in our present studies) at a dose that had no significant effect on blood pressure, reduced infarct size and improved neurological score. In addition, early administration of candesartan (3 h after onset of ischemia) to normotensive rats has been shown to be neuroprotective, but only when excessive BP lowering is avoided (Brodon et al., 2007). We are the first group to add the additional benefit of a reduction in vascular damage and hemorrhage to the benefits of candesartan in SHRs as well as identifying an interaction between preexisting hypertension and stroke in the response to BP lowering with candesartan. An interesting finding in our study of improved neurological function with 1 mg/kg candesartan despite no histologic neuro- or vascular protection, suggests an additional beneficial effect of the drug on recovery and perhaps brain plasticity.

Our findings are limited by the nature of the model (mechanical versus embolic; SHR versus other models of hypertension), the short duration of follow-up (24 h), and the inability to determine the mechanism of the protective effects seen. It is most likely that the protective effects of candesartan are multimodal, partly due to BP lowering and partly due to pleiotropic vascular protective effects. It is clear that blood pressure lowering after reperfusion protects the vasculature and is neuroprotective (Elewa et al., 2007). Translating this to human stroke patients is complicated by the fact that premorbid conditions, such as vascular disease due to chronic hypertension, changes the sensitivity to a given dose of antihypertensive and may make it more likely to overshoot baseline values and eliminate the benefit. Restarting blood pressure medications in patients with acute stroke, as has been recently recommended (Adams et al., 2007), may be perilous if the same prestroke doses are used. More research is needed to identify patients most likely to benefit from blood pressure lowering with candesartan after stroke.

Acknowledgments

Candesartan cilexetil was provided by AstraZeneca.

References


