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).

METHODS. Animals were subjected to middle cerebral artery occlusion (MCAO) or sham for 3 hours followed by reperfusion. Either candesartan 0.1 mg/kg, 0.3 mg/kg, 1.0 mg/kg or saline were administered. MAP of the rats was monitored by means of telemetry and neurologic function was assessed. Infarct size, edema formation and hemoglobin content in the ischemic hemisphere were evaluated 24 h after the stroke. RESULTS. MAP of SHR increased immediately upon MCAO from 135 (baseline) to 189 mmHg, and 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 were experienced greater BP lowering effects of candesartan after stroke compared with a sham condition (p<0.0001). Neurologic 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. CONCLUSION. Low doses of candesartan provide neurovascular protection after stroke in SHRs. Caution is warranted since acute stroke increases the sensitivity to BP lowering, increasing the likelihood of overshooting.
Introduction

Hypertension is present in almost 80% of acute stroke patients and 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 hours and then only decreasing MAP by 15% (Adams et al., 2007). For patients with preexisting hypertension, there is support for restarting antihypertensive medications, but only after 24 hours (Adams et al, 2007).

The consequences of hypertension before and after stroke can also be demonstrated in animals. Spontaneously hypertensive rats (SHR) exhibit more severe neurological deficits and edema formation (Slivka et al, 1995) and more frequently demonstrate extensive cerebral infarct damage than normotensive animals following 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 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, post-stroke 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, 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 prior to 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 AT₁ 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 AT₁ 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 or not candesartan at reperfusion will be similarly protective in SHRs, a model that is likely to be more clinically relevant.
Methods

Animals

Male spontaneously hypertensive rats (SHR) 9-week-old, weighing ca. 200 g, were purchased from Charles River Breeding Company (Wilmington, Massachusetts, USA). After shipping, the animals were housed in individual plastic cages for 5-6 days prior to the surgery and experimentation. The rats were provided food and water *ad libitum*, a 12:12-h light/dark cycle at 24°C, and 30–40% humidity. Rats were weighed and randomly assigned to each experimental group. When the rats reached body weight of ca. 240 g, they were instrumented for telemetry blood pressure monitoring, and when they further gained body mass to 275–300 g they were subjected to the experimental protocol. Usually, the rats were at 12–13 week of age at the time of the experimental cerebral ischemia or sham.

The Institutional Animal Care and Use Committee (IACUC) of the Charlie Norwood VA Medical Center in Augusta, Georgia approved all procedures, in accordance with the Guide for the Care and Use of Laboratory Animals from the National Institutes of Health.

Blood pressure telemetry

Telemetry transmitters (Data Sciences International, St. Paul, Minnesota, USA) were implanted according to the manufacturer’s specifications as described elsewhere (Fagan et al., 2006). Briefly, the rats were anesthetized with sodium
pentobarbital (65 mg/Kg, i.p.; Abbott Laboratories, Chicago, Illinois, USA) 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 minutes for several days before the stroke (or sham) and until the sacrifice at 24 hours 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 (MCA) was occluded with 19-21 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 MCA. The animals were kept under anesthesia for only 10 minutes for the surgical procedure. The suture was removed after 3 hours of occlusion and the animals were returned to their cages.

Prior to reperfusion, the animals were subjected to the test for assessment of neurological function. Immediately after reperfusion, either saline or doses of
candesartan cilexitil (Astra-Zeneca; 0.1 mg/Kg, 0.3 mg/Kg, and 1.0 mg/Kg), as indicated in the figures, were administrated intravenously 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 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 cleaned and scans were performed at baseline, 5 minutes after the MCAO, 5 minutes after reperfusion (before drug administration) and prior to sacrifice at 24 hours. All using the same scale, 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 prior to reperfusion and at 24 hours (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 prior to 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 hours after the onset of MCAO, anesthesia was performed with Ketamine 44 mg/Kg and Xylazine 13 mg/Kg administered intra-muscularly, 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 (TTC) (Sigma Chemical Co., St. Louis, Missouri, USA) for 15-20 minutes. Images of the stained sections were taken. Using image analysis software (Zeiss-KS300, 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 percent of the contralateral hemisphere. The ischemic and non-ischemic hemispheres of the slices for the enzyme-linked immunosorbent assay (ELISA) were separated and processed, using the non-ischemic side as a control. After homogenizing the TTC-stained slices in the core of the infarct and collecting the supernatants, ELISA 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 prior to MCAO was the pre-stroke value. Values obtained during the 3 hours of MCAO were
averaged for the estimate of BP during stroke, the values for the first 2 hours post-reperfusion were averaged for an estimate of the immediate effects of the drugs, and all values 5 hours 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 vs SHR) by 2 condition (stroke vs. no stroke) factorial analysis of covariance was used to analyze differences in MAP within the time periods above, using baseline MAP as a covariate. A 2-way mixed model repeated measures analysis of variance (ANOVA) was used to determine treatment (saline, candesartan 0.1mg/Kg, candesartan 0.3mg/Kg and candesartan 1 mg/Kg), time (pre-stroke, MCAO, 2hr post-perfusion, post-stroke), and treatment by time differences in mean arterial pressure (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. Maximum (MAX) and minimum (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, hemoglobin content, and post-perfusion 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, North Carolina, USA). Statistical significance was measured at an alpha level of 0.05. All values are represented as mean ± standard deviation (SD).
Results

*Changes of blood pressure in the SHR upon brain ischemia, reperfusion and candesartan treatment*

Changes in mean arterial pressure (MAP; 1-h averages) over time by treatment are illustrated in Figs 1 and 2. Of note, we tested the effectiveness of candesartan in reduction of MAP in the sham SHR. Data shown in Figure 1 demonstrates that all three doses of candesartan used (0.1, 0.3, and 1 mg/Kg) reduced MAP within two hours of injection from a baseline (approximately 140 mmHg) 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 mg/Kg and 1.0 mg/Kg of candesartan returned to the pre-injection levels after 48 h and 72 h, respectively, whereas MAP of the rats injected 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 to the sham situation (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 (ca. 140 mmHg) to approximately 190 mmHg, and 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 pre-stroke and the MCAO values (Table 1).
For the two hours post-reperfusion the candesartan 0.3 mg/Kg and candesartan 1 mg/Kg groups lowered BP the most whereas candesartan 0.1 mg/Kg and the saline groups were not different. For the 19 hours during the post-stroke period the candesartan 1 mg/Kg group had MAP that was significantly lower than saline and the candesartan 0.3 mg/Kg group was intermediate. The post-stroke means for candesartan 0.1 mg/Kg and the saline groups were significantly higher than their pre-stroke 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 candesartan 1 mg/Kg group was significantly lower than the saline group and the lowest dose of candesartan but not different from the candesartan 0.3 mg/Kg group. A dose of 0.3 mg/kg of candesartan following reperfusion caused a decrease of the MAP to the pre-stroke baseline, while a dose of 1.0 mg/kg provoked a drop of MAP below baseline. Post-reperfusion MAP following the injection of a dose of 0.1 mg/Kg of candesartan also decreased, however to a lesser extent and remained above baseline until sacrifice. It did not differ significantly from that seen in saline group (control), except during the first hour following 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 SHR treated with candesartan. This reduction, however, was dose-related within a narrow range of candesartan doses (a range between 0.1 – 0.3 mg/Kg in our
The greatest effects on infarct size and edema were observed in the rats treated with a dose of 0.3 mg/kg of candesartan. Infarct size in the saline-treated group (n = 16) was 62.3% compared to 50.7% of 0.3 mg/Kg candesartan group. Calculated edema revealed a similar reduction (23.3% in saline group vs. 18.3% in 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 candesartan 0.3 mg/Kg group had the lowest infarct size and edema, and 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 neurologic function prior to 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 candesartan 0.3 mg/Kg and 1 mg/kg 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
can be seen, doses of 0.1 mg/Kg and 0.3 mg/Kg of candesartan reduced hemoglobin content in a dose-dependent manner (8.3 ng/mg and 4.0 ng/mg for doses of 0.1 and 0.3 mg/kg of candesartan, respectively, compared to 9.7 ng/mg in saline-treated rats). Only the 0.3 mg/kg group was significantly different from control (p=0.0092). Hemoglobin content in the ischemic brain tissue of rats treated with a dose of 1.0 mg/Kg of candesartan, however, did not differ from that seen in the saline-treated group (10.0 ng/mg in candesartan-treated vs. 9.7 ng/mg in saline-treated rats).

**Cerebral Perfusion**

In all 5 animals tested, MCAO resulted in consistent reductions in average perfusion units ± standard error (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 five minutes after occlusion. Perfusion was restored to relative hyperemia (521 ± 25.8; average of 13.5% higher than contralateral) in the five minutes after reperfusion (Figure 7A). Hyperemia was bilateral prior to sacrifice in the saline treated (no asymmetry) but asymmetry was maintained in the candesartan 0.3 mg/kg animals (Figure 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 neurologic 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 SHR treated with a dose of 1.0 mg/kg eliminated this protective effect. In fact, the animals treated with candesartan at a dose of 1.0 mg/kg revealed neurological and vascular damage resembling that seen in rats treated with saline. It is of particular importance that hypertensive rats appeared more sensitive to candesartan after stroke than normotensive rats, since normotensive Wistar rats given a dose of 1.0 mg/kg immediately after reperfusion decreased MAP to the baseline, and showed improvement in neurologic outcome (Fagan et al., 2006).

Our data suggest that in the hypertensive rats, lowering acutely increased blood pressure may be beneficial. However, extension of the decrease of pressure to below SHR baseline values with candesartan can be deleterious. It indicates that post-stroke recovery and homeostatic defense processes against post-ischemic brain damage are indeed pressure-dependent. Thus, our present results add to the discussion regarding the management of blood pressure after
stroke. Presumably, there is a feedback and fine inter-relationship among blood pressure, processes limiting the infarct size, and mechanisms involved in the recovery of ischemic tissue.

It is possible, however, that the improved outcomes with candesartan occurred despite of reducing blood pressure. It is known that apart from blood pressure reduction, Ang II AT₁-receptor antagonism also inhibits inflammation (Ando, 2004), normalizes cerebrovascular autoregulation (Nishimura, 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-AT₁-mediated mechanisms involved in the protective effects of candesartan. Of note, it has been postulated that stimulation of the Ang II AT₂ receptor may be protective in focal cerebral ischemia (Iwai et al., 2004).

In line with the above, Engelhorn et al. (2004) found that post-ischemia 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. Also, 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 (Brdon 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 neurologic function with candesartan 1 mg/kg 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 hours), 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 pre-stroke doses are used. More research is necessary to identify patients most likely to benefit from blood pressure lowering with candesartan after stroke.
Acknowledgments

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References


Footnotes

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Legends for Figures

Figure 1.
Changes of mean arterial pressure (MAP; mmHg) of non-stroke SHR injected intravenously (arrow) in the separate groups with 3 doses of candesartan (n=4 per group). MAP values are 1-h means ± SEM. Black horizontal bars indicate a night-time in the 12:12 h light/dark cycles.

Figure 2.
Mean arterial blood pressure (MAP; mmHg) after acute stroke in the SHR. MAP was recorded every 10 min (telemetry) prior to stroke (baseline between 6 and 9 AM), during the onset of ischemia (at 10 AM; left arrow), reperfusion (at 1 PM; right arrow) and then during the following 21 hrs until sacrifice next day. At reperfusion, the animals were intravenously injected 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 ± SEM.

Figure 3.
Effect of candesartan on ischemia-induced infarct size (panel A) and edema (panel B) in the SHR. 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 to control)
Figure 4.
Effect of candesartan on Bederson score in the SHR 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 ± SEM) assessed 24 h after the reperfusion and injections, prior to sacrifice. (∗ = p < 0.05 compared to control)

Figure 5.
Tests of beam walk (panel A) and paw grasp (panel B) of the SHR as described in Fig. 4. Rats were tested prior to sacrifice. (∗ = p < 0.05 compared to control)

Figure 6.
Effect of candesartan on hemoglobin content (Hb; ng/mg of protein) in the ischemic hemispheres of SHR. (∗ = p < 0.05 compared to control)

Figure 7.
Laser Doppler scan images (PIM 3) of tissue perfusion (top) and orientation (bottom). (A ) A representative animal where perfusion is symmetric at baseline, reduced (dark blue = 3.6 perfusion units) in the ischemic hemisphere at 5 minutes after MCAO (5'MCAO) and returns to relative hyperemia (dark red = 1295 perfusion units) in the ischemic hemisphere 5 minutes after reperfusion (5' after reperfusion). (B) The animal treated with saline demonstrated bilateral hyperemia prior to sacrifice and this was accompanied by a large infarct (white) with hemorrhage. In the animal treated with candesartan (cand) 0.3 mg/kg, the
hyperemia in the ischemic hemisphere was still present at 24 hours and this was associated with a smaller infarct size. The animal treated with candesartan (cand) 1 mg/kg had symmetric, lower perfusion prior to sacrifice and a larger infarct.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Pre-stroke</th>
<th>MCAO</th>
<th>2hr post-perfusion *</th>
<th>Post-stroke *</th>
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<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>
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<td>7</td>
<td>146.2 (8.2)</td>
<td>187.4 (8.3)</td>
<td>166.2 (9.8) a</td>
<td>160.6 (12.5) a</td>
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<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) b</td>
<td>148.2 (12.7) a,b</td>
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<tr>
<td>Candesartan 1</td>
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<td>139.1 (8.2)</td>
<td>186.3 (8.0)</td>
<td>145.6 (7.6) b</td>
<td>133.2 (10.4) b</td>
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</tbody>
</table>

* Means with the same letter are not significantly different
Table 2. AUC, MIN and MAX values for each group – Mean (SD)

<table>
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<tr>
<th>Treatment</th>
<th>N</th>
<th>AUC *</th>
<th>MIN, mmHg *</th>
<th>MAX, mmHg</th>
<th>Change pre to post, 6-9 am *</th>
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<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>
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<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 (293) a,b</td>
<td>134.1 (10.5) a,b</td>
<td>192.8 (9.0)</td>
<td>-1.4 (8.7) b,c</td>
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<tr>
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<td>7</td>
<td>3804 (228) b</td>
<td>120.3 (9.6) b</td>
<td>189.5 (8.1)</td>
<td>-9.3 (10.0) c</td>
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<tr>
<td>p-value</td>
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<td>0.0036</td>
<td>0.84</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

* Means with the same letter are not significantly different
Table 3. Infarct size and edema for each group – Mean (SD)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Infarct size *</th>
<th>Edema *</th>
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</thead>
<tbody>
<tr>
<td>Saline</td>
<td>16</td>
<td>62.3 (6.3) a</td>
<td>23.3 (3.0) a</td>
</tr>
<tr>
<td>Candesartan 0.1</td>
<td>7</td>
<td>58.0 (3.5) a,b</td>
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<tr>
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<td>11</td>
<td>50.7 (6.7) b</td>
<td>18.3 (5.0) b</td>
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<tr>
<td>Candesartan 1</td>
<td>11</td>
<td>61.8 (6.8) a</td>
<td>22.8 (5.5) a,b</td>
</tr>
</tbody>
</table>

p-value: 0.0001 0.023

* Means with the same letter are not significantly different
† Analysis performed on the ranks
Figure 2

MAP (mmHg)

Time (h)

- Saline (n=8)
- Candesartan 0.1mg/kg (n=7)
- Candesartan 0.3mg/kg (n=7)
- Candesartan 1mg/kg (n=7)

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Figure 3A

Infarct size average (%)

Saline (n=16) Candesartan 0.1mg/kg (n=7) Candesartan 0.3mg/kg (n=11) Candesartan 1mg/kg (n=11)

*
Figure 3B

Edema (% change from contralateral)

Saline (n=16)  
Candesartan 0.1mg/kg (n=7)  
Candesartan 0.3mg/kg (n=11)  
Candesartan 1mg/kg (n=11)
Figure 4

- **Saline (n=16)**
- Candesartan 0.1mg/kg (n=7)
- Candesartan 0.3mg/kg (n=11)
- Candesartan 1mg/kg (n=11)

Bederson score (0-3)

- before reperfusion
- before sacrificing

* indicates statistical significance.
Figure 5A

- Saline (n=8)
- Candesartan 0.1mg/kg (n=7)
- Candesartan 0.3mg/kg (n=11)
- Candesartan 1mg/kg (n=8)

Beam walk (0-3 points)

* denotes statistical significance
Paw grasp (0-3 points)

- Saline (n=8)
- Candesartan 0.1mg/kg (n=7)
- Candesartan 0.3mg/kg (n=11)
- Candesartan 1mg/kg (n=8)

* Figure 5B
Figure 6

Hb (ng/mg of protein)

Saline (n=16)  
Candesartan 0.1mg/kg (n=7)  
Candesartan 0.3mg/kg (n=11)  
Candesartan 1mg/kg (n=11)