Age as a Modifying Factor on the Effect of Antihypertensive Therapy in Focal Stroke in Rats

Andrew Slivka, MD

**Background and Purpose:** Antihypertensive treatment with hydralazine for 10 weeks but not 6 weeks reduces infarct size in 13-week-old spontaneously hypertensive rats subjected to focal cerebral ischemia. This study was designed to examine whether the duration of treatment needed to reduce infarct size depends on how long hypertension is present before the initiation of antihypertensive therapy.

**Methods:** Six-week-old spontaneously hypertensive rats were treated for 6 weeks and 10-month-old spontaneously hypertensive rats for 10 weeks with 20 mg/kg hydralazine added daily to the drinking water. The animals were then subjected to focal cerebral ischemia by tandem permanent common carotid and middle cerebral artery occlusion.

**Results:** Blood pressure in the treated groups was lower than that in the untreated groups for the entire treatment period in both experiments. Infarct volume in 10-month-old spontaneously hypertensive rats treated for 10 weeks, but not in 6-week-old spontaneously hypertensive rats treated for 6 weeks, was significantly less than in untreated controls (p=0.02).

**Conclusions:** This study emphasizes the importance of duration of antihypertensive treatment in reducing infarct volume in spontaneously hypertensive rats after focal cerebral ischemia and demonstrates that the effect appears to be independent of the duration of hypertension before the initiation of treatment. (*Stroke* 1993;24:241–244)

**Key Words** • cerebral ischemia • hypertension • hydralazine • rats

Hypertension is a risk factor for myocardial infarction and stroke.1-2 Hypertension also leads to structural cardiovascular changes including left ventricular hypertrophy, decreased left ventricular function, and increased peripheral vascular resistance,3-5 although long-term serial changes of these factors in a population of patients with untreated hypertension has not been well documented. In spontaneously hypertensive rats (SHR), however, intimal and medial lesions involving the aorta and intrarenal arteries progress with age.6 Regression or arrest of this process occurs with antihypertensive therapy although the extent of the treatment effect depends on the age at which the treatment is initiated.7 8 The role that age plays in cerebrovascular changes following antihypertensive therapy has not been clearly elucidated. I have previously demonstrated that antihypertensive therapy decreases infarct size in 13-week-old SHR subjected to focal cerebral ischemia.9 This effect is dependent on the duration of treatment and is seen after 10, but not 6, weeks of treatment. This study was designed as an extension of the prior work, to examine whether the duration of antihypertensive treatment necessary to reduce infarct size in SHR varies depending on the duration of hypertension present before the initiation of treatment.

To determine if a short duration of antihypertensive treatment reduces infarct size when therapy is begun before the onset of hypertension, 6-week-old SHR were treated with hydralazine for 6 weeks. Ten-month-old SHR were treated with hydralazine for 10 weeks to determine whether 10 weeks of antihypertensive therapy reduces infarct volume in SHR with prolonged hypertension as it does in younger SHR.

**Materials and Methods**

Two separate experiments were completed. Animals in both experiments were randomly divided into treatment and control groups and housed individually. Treated SHR received 20 mg/kg/day hydralazine mixed with the drinking water. Control SHR received tap water alone. Hydralazine solutions were prepared fresh and changed daily.

Treatment was given for 6 weeks to 6-week-old male SHR weighing 110–135 g in experiment 1 (15 treated and 15 control rats) and for 10 weeks to 10-month-old male SHR weighing 370–430 g in experiment 2 (14 treated and 15 control rats). Sufficient numbers of animals were used in both experiments to avoid a Type II error for a 25% reduction in infarct volume (β=0.2, α=0.05) in this model.10 Body weight and blood pres-

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See Editorial Comment, page 244
sure were measured after the first week of treatment and then at regular 1–2-week intervals in each animal in both experiments. Blood pressure was measured by the tail cuff method (Harvard Apparatus, South Natick, Mass.) in awake restrained rats.

SHR were fasted for 24 hours before surgery. Halothane (1.5–2.0%) was mixed with oxygen and nitrogen and delivered through a nose cone using a flow regulator. The tail artery was cannulated with polyethylene tubing to monitor blood pressure and to obtain blood samples for measurement of physiological variables. Focal necortical ischemia was produced by tandem right common carotid artery (CCA) and middle cerebral artery (MCA) occlusion as previously described. Body temperature was maintained at 37°C throughout the surgical procedure with a heat lamp connected to a rectal thermistor. Surgery for each experiment was completed by a single investigator over a 2-week period on rats delivered from a single shipment (Harlan Sprague Dawley, Inc., Indianapolis, Ind.). Arterial blood pressure was monitored throughout the surgical procedure and then checked 4–6 hours after surgery when the animals had recovered from anesthesia. PaO₂, PaCO₂, arterial pH, glucose, and hematocrit were measured just after tail artery cannulation. Arterial blood gases were measured again before MCA occlusion and 4–6 hours after CCA/MCA occlusion. Hematocrit was measured again just before decapitation. During the surgical procedure, mean arterial blood pressure was maintained above 90 mm Hg in control and 60 mm Hg in treated animals and PaO₂ was maintained above 80 mm Hg.

The rats were anesthetized with halothane and decapitated 24 hours after CCA/MCA occlusion. The brains were rapidly removed from the cranium and frozen in Freon over dry ice. Coronal sections 20 μm thick were cut at 500-μm intervals, fixed in 90% ethanol, and stained with hematoxylin and eosin. Each brain section was magnified using a photographic lens, and the infarcted area was traced onto paper. Each drawing was then retracted onto a digitizing tablet interfaced to an IBM personal computer (Video Image Analysis System, Ted Pella Inc., Redding, Calif.) that computes infarcted area for each section. To calculate total infarct volume, the infarcted areas of sequential sections were summed and multiplied by the thickness between sections. Areas of the right and left hemispheres of each brain section were also obtained and volumes calculated as described above. Hemispheric volumes due to edema were obtained by subtracting volume of the ischemic right hemisphere from volume of the nonischemic left hemisphere. Infarct volumes adjusted for the presence of edema were calculated by multiplying infarct volume by the ratio of left hemispheric volume to right hemispheric volume. Image analysis for each experiment was done by a technician who was blinded to the treatment groups.

Infarct volumes and hemispheric edema volumes were expressed as mean±SD for control and treated groups in both experiments. Results were analyzed using a two-tailed Student’s t test. The relation between blood pressure and infarct volume was examined for each experiment using the Pearson product moment correlation technique.

## Results

Body weights did not differ significantly between the treated and control groups during the treatment phases of either experiment (data not shown). Serial blood pressure measurements for each experiment are presented in Table 1. The blood pressures of treated and control rats in experiment 1 were not elevated at baseline or at week 1. However, beginning at week 2 blood pressures rose in control animals and remained in the normotensive range in treated SHR. In experiment 2, blood pressure was substantially lower in the treated group than in the control group after 1 week of hydralazine treatment and remained lower for the entire treatment period.

Physiological data for both experiments are shown in Table 2. Anesthetized rats in both groups developed mild respiratory acidosis and blood pressure depression before MCA occlusion that normalized by 4–6 hours after surgery.

Infarct volumes for experiments 1 and 2 are shown in Table 3. Treatment with hydralazine for 6 weeks in 6-week-old SHR did not influence infarct volume (t(28)=0.05, p>0.05). However, 10-month-old SHR treated with hydralazine for 10 weeks exhibited a significant 17% reduction in infarct volume compared with control rats (t(27)=2.45, p=0.02); the 95% confidence interval for the difference in infarct volume between the treated and control groups was −1 to 59 mm³. These results were the same when infarct volumes corrected for the presence of edema were analyzed. No significant difference in corrected infarct volume between the treated and control groups was seen in experiment 1 (t(28)=0.27, p>0.05), but treatment significantly reduced corrected infarct volume by 16% in experiment 2 (t(27)=2.55, p=0.02). Mean arterial blood pressure measured directly 4–6 hours after CCA/MCA occlusion correlated with infarct volume for both treated and

### Table 1. Blood Pressures in Treated and Control Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Baseline</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 4</th>
<th>Week 5</th>
<th>Week 6</th>
<th>Week 8</th>
<th>Week 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n=15)</td>
<td>100±22</td>
<td>140±37</td>
<td>167±42</td>
<td>205±19</td>
<td>184±14</td>
<td>176±11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treated (n=15)</td>
<td>106±31</td>
<td>131±43</td>
<td>111±32</td>
<td>156±22</td>
<td>133±13</td>
<td>139±9*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (n=15)</td>
<td>234±22</td>
<td>245±23</td>
<td></td>
<td>215±27</td>
<td></td>
<td>213±38</td>
<td>238±31</td>
<td>193±10</td>
</tr>
<tr>
<td>Treated (n=14)</td>
<td>239±21</td>
<td>150±33</td>
<td>134±19</td>
<td>142±43</td>
<td></td>
<td>133±26</td>
<td>141±17</td>
<td>149±18*</td>
</tr>
</tbody>
</table>

Values are mean±SD mm Hg.

*Mean arterial blood pressures measured directly from tail artery cannula 4–6 hours after middle cerebral artery occlusion. All other values are indirect systolic pressure measurements.
TABLE 2. Physiological Data for Treated and Control Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Experiment 1</th>
<th></th>
<th>Experiment 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (n=15)</td>
<td>Treated (n=15)</td>
<td>Control (n=15)</td>
<td>Treated (n=14)</td>
</tr>
<tr>
<td>Before occlusion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>124±11</td>
<td>96±7</td>
<td>137±17</td>
<td>113±14</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.32±0.02</td>
<td>7.33±0.03</td>
<td>7.32±0.04</td>
<td>7.32±0.06</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>108±11</td>
<td>112±16</td>
<td>134±20</td>
<td>150±25</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>42±3</td>
<td>41±4</td>
<td>42±4</td>
<td>41±6</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>137±15</td>
<td>139±8</td>
<td>168±20</td>
<td>170±21</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48±1</td>
<td>46±2</td>
<td>46±2</td>
<td>44±2</td>
</tr>
<tr>
<td>After occlusion 4–6 hrs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>176±11</td>
<td>139±9</td>
<td>193±10</td>
<td>149±18</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38±0.04</td>
<td>7.40±0.06</td>
<td>7.36±0.04</td>
<td>7.42±0.04</td>
</tr>
<tr>
<td>PaO2 (mm Hg)</td>
<td>93±12</td>
<td>95±9</td>
<td>100±25</td>
<td>108±15</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>38±5</td>
<td>36±6</td>
<td>38±4</td>
<td>34±5</td>
</tr>
<tr>
<td>24 hrs Hematocrit (%)</td>
<td>46±2</td>
<td>45±1</td>
<td>45±2</td>
<td>44±3</td>
</tr>
</tbody>
</table>

Values are mean±SD. MABP, mean arterial blood pressure.

control groups in experiment 2 (r=0.38, p<0.05) but not in experiment 1 (r=-0.05, p>0.05). As seen in Table 3, hydralazine treatment did not significantly reduce hemispheric edema in either experiment (t(20)=0.81, p>0.05 in experiment 1; t(15)=0.54, p>0.05 in experiment 2).

Discussion

The results of this study and prior experiments demonstrate a consistent treatment effect on infarct size when SHR are treated with hydralazine for 10 but not 6 weeks. Although hydralazine therapy for 6 weeks decreased infarct volume in 13-week-old SHR by 14% compared with untreated SHR, the result was not statistically significant. In this study, 6 weeks of hydralazine therapy did not affect infarct volume in 6-week-old SHR. However, infarct volume in 13-week-old SHR that were treated for 10 weeks with hydralazine was 18% less than that in untreated SHR (p=0.02). In this study, 10 weeks of hydralazine therapy reduced infarct volume in 10-month-old SHR by 17% compared with control SHR (p=0.02) even when infarct volumes were corrected for the presence of edema. This decrease in infarct volume was not the result of a decrease in edema because hemispheric edema volume did not differ between the treated and control groups. These results suggest that the duration of antihypertensive therapy necessary to decrease infarct volume is not influenced by the duration of hypertension before initiation of treatment.

Antihypertensive therapy of SHR increases cerebral blood flow. This may explain why such treatment is associated with a reduction in infarct volume after focal ischemia. Resting cerebral blood flow is greater in SHR receiving prior antihypertensive therapy than in untreated SHR. The increase in cerebral blood flow seen in 4-week-old SHR treated for 16 weeks was greater than that seen in 12-week-old SHR treated for 8 weeks, although whether this was because treatment was started earlier in 4-week-old SHR or because 4-week-old SHR received a longer duration of treatment is unclear. The results of this study suggest that the duration of treatment is a more critical factor than age at the initiation of therapy. Also, prolonged antihypertensive treatment of 3- and 10-week-old SHR produces similar vascular changes in hindquarter vessels as reflected by decreases in the resistance at maximal dilation, the steepness of the resistance curve, and the maximal pressor response.

Unlike in 4- or 12-week-old SHR, antihypertensive therapy in 24-month-old SHR does not increase resting cerebral blood flow, although treatment does exert some influence on the cerebral vasculature. In 24-month-old SHR receiving antihypertensive treatment the lower limit of autoregulation is reduced to levels seen in normotensive Wistar rats, as has been reported in 6-month-old SHR after 9 weeks of blood pressure normalization. The fact that resting cerebral blood flow does not change after antihypertensive therapy does not exclude the possibility that such treatment will improve cerebral blood flow after CCA/MCA occlusion. Resting cerebral blood flow of 5- to 9-month-old as well as 7- to 12-month-old SHR and Wistar rats is similar, but after bilateral CCA occlusion cortical and thalamic cerebral blood flows are less in SHR than in normotensive Wistar rats. Thus, increased cerebral blood flow after CCA/MCA occlusion is a possible explanation for the treatment effect seen in older SHR.

In summary, this study along with prior work emphasizes the importance of the duration of antihypertensive treatment in reducing infarct volume in SHR subjected to focal cerebral ischemia and demonstrates that this effect appears to be independent of the age at which antihypertensive therapy is initiated. Clinically, blood pressure elevation before stroke correlates with increased 30-day mortality after stroke. Assuming that at least some of the lower stroke mortality in patients with lower blood pressures reflects the presence of smaller infarcts, this study suggests that antihypertensive therapy will be effective in reducing infarct size even when initiated after prolonged periods of untreated hypertension.

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References

In this study, the author reports the effects of antihypertensive treatment with hydralazine at different durations on infarct volume in young (6-week-old) and old (10-month-old) spontaneously hypertensive rats (SHR) following focal cerebral ischemia. The author concludes that the duration of the antihypertensive treatment is important in reducing infarct volume in SHR after focal cerebral ischemia. The mode of the drug action appears unrelated to its effect on brain edema, one of the major factors that could influence the infarct volume measurement. This highly focused and well-planned study, in conjunction with the previous study also conducted by Dr. Slivka, provides evidence that hypertensive therapy is still effective in reducing infarct size even when initiated after prolonged periods of untreated hypertension.

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