Effect of Antihypertensive Therapy on Focal Stroke in Spontaneously Hypertensive Rats

Andrew Slivka, MD

Spontaneously hypertensive rats subjected to focal cerebral ischemia develop larger infarcts than normotensive rat strains. To determine whether antihypertensive therapy decreases infarct volume in hypertensive rats, 60 13-week-old animals were treated with 20 mg/kg hydralazine added daily to the drinking water for 1.5, 6, 10, or 16 weeks and then subjected to focal cerebral ischemia by tandem right common carotid artery and middle cerebral artery occlusion. Blood pressure in the treated groups was substantially lower than that in untreated groups after 1 week of hydralazine therapy and remained lower for the entire treatment period in all four experiments. Mean infarct volume in spontaneously hypertensive rats treated for 10 (p=0.02) or 16 (≥0.005) weeks, but not 1.5 or 6 weeks, was significantly less than that in the untreated controls. The percentage reduction of infarct volume in animals treated for 10 and 16 weeks was similar. This study demonstrates that antihypertensive therapy decreases infarct volume in hypertensive rats subjected to focal cerebral ischemia. This treatment effect appears to be dependent on the duration of therapy, and the magnitude of the treatment effect seems to plateau by 10 weeks of therapy. (Stroke 1991;22:884–888)

Hypertension is a well-documented, independent risk factor for both atherothrombotic cerebral infarction and intracerebral hemorrhage, and treatment of hypertension has been shown to reduce this risk.1–4 Absolute blood pressure measurements are positively correlated with stroke risk in epidemiologic and treatment studies.5–8 Furthermore, in a large cohort of men followed prospectively at 3–5-year intervals for an average of 26 years, blood pressure elevation prior to stroke correlated with increased 30-day mortality following stroke.9

The clinical finding of a relation between blood pressure before stroke and survival after stroke has experimental correlates. The extent of histological infarction in spontaneously hypertensive rats (SHR) subjected to focal cerebral ischemia has been consistently reported to be greater than that in normotensive rat strains.10–12 Fujishima et al13 reported lower lactate and higher adenosine triphosphate levels in SHR treated with antihypertensive therapy and subjected to bilateral common carotid artery (CCA) occlusion compared with untreated controls. In fact, a linear correlation was seen in that study between mean arterial blood pressure and supratentorial brain lactate levels, suggesting that long-term blood pressure reduction prior to CCA occlusion decreases the extent of cerebral ischemia.

The effect of blood pressure reduction on infarct size has not been examined previously either clinically or experimentally. This study was designed to determine whether antihypertensive therapy decreases infarct volume as measured histologically in SHR and whether the duration of antihypertensive treatment influences this effect.

Materials and Methods

Four separate experiments were completed. In each experiment, male SHR averaging 13 weeks of age and weighing 210–300 g were randomly divided into treatment and control groups and housed individually. The animals were fed a regular diet. Treated rats received 20 mg/kg hydralazine mixed with the drinking water. The amount of water consumed per day (30–40 ml) was measured to ensure appropriate hydralazine dosing. Hydralazine solutions were prepared fresh and changed daily. Controls received tap water alone.

Treatment was given for 1.5 weeks in experiment 1 (15 treated and 15 control rats), 6 weeks in experiment 2 (11 treated and 13 control rats), 10 weeks in experiment 3 (18 treated and 17 control rats), and 16 weeks in experiment 4 (16 treated and 16 control rats). Sufficient numbers of animals were used in all four 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 pressure were mea-
sured after the first week of treatment, then at regular 1–3-week intervals thereafter in each animal in all four experiments. Blood pressure was measured by a tail cuff method (Harvard Apparatus, South Natick, Mass.) in an awake restrained rat.

The SHR were fasted for 24 hours prior to 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 a polyethylene catheter (PE-50) to monitor blood pressure and obtain blood samples for assessing physiologic variables. The right CCA was exposed through a midline neck incision and occluded with 4-0 surgical silk. The right middle cerebral artery (MCA) was exposed through a 2-mm burr hole drilled under a continuous normal saline drip 2–3 mm rostral to the fusion of the zygomatic arch with the squamosal bone. Using a micromanipulator (MM 3, Narishige Instruments, Tokyo, Japan), a hook formed of 20-gauge silver wire was positioned under the MCA. The MCA was then lifted 0.5–1 mm above the cortical surface and cauterized. Body temperature was maintained at 37°C throughout the procedure with a heat lamp connected to a rectal thermometer. Immediately after CCA/MCA occlusion, all wounds were sutured closed and the animals were allowed to recover from anesthesia. Sham-operated SHR (n = 3) were subjected to temporalis muscle dissection, craniotomy to expose the MCA, and positioning of the wire hook under the MCA. The hook was subsequently removed, and all wounds were sutured closed. Sham operations were done during the course of three experiments. 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. Blood pressure 4–6 hours after MCA occlusion was strongly correlated with blood pressure 24 hours after surgery (product-moment coefficient of correlation r = 0.88, n = 24). \( \text{PaO}_2 \), \( \text{PaCO}_2 \), arterial pH, glucose concentration, and hematocrit were measured just after tail artery cannulation. Arterial blood gases were measured again prior to MCA occlusion and 4–6 hours after CCA/MCA occlusion. Hematocrit was measured again just prior to decapitation. The concentration of halothane used during the surgical procedure was such that mean arterial blood pressure was always above 90 mm Hg in untreated and 60 mm Hg in treated animals. These blood pressures are the lower limit of cerebral autoregulation in each group. \( \text{PaO}_2 \) was maintained above 80 mm Hg by adjusting the oxygen concentration.

The animals 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 \( \mu \)m thick were cut at 500-\( \mu \)m 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 retraced onto a digitizing tablet interfaced to an IBM personal computer (Video Image Analysis System, Ted Pella Inc., Redding, Calif.) that computes infarct areas for each section. To calculate total infarct volume, the infarcted area of sequential sections was summed and multiplied by the thickness between sections. Image analysis for each experiment was done by a technician who was blinded to the treatment groups. Intraborder variability using this method on two separate occasions was excellent (product-moment coefficient of correlation \( r = 0.98, n = 12 \)).

Mean infarct volume and standard deviation were computed for control and treated groups in all four 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

Throughout the treatment phases of all four experiments body weights did not differ significantly between the treated and control groups (data not shown). Serial blood pressure measurements for each experiment are presented in Table 1. Blood pressure was substantially lower in the treated groups after 1 week of hydralazine treatment than in the control groups and remained lower for the entire treatment period in all four experiments.

Physiological data for all four experiments are shown in Table 2. Anesthetized animals in all groups developed mild respiratory acidosis and blood pressure depression before MCA occlusion that normalized by 4–6 hours after surgery. The hematocrit was unchanged over 24 hours in all treated and control groups.

Infarct volumes for each experiment are shown in Table 3. Sham-operated animals exhibited small infarcts, confined to the region of MCA manipulation, averaging 5±4 mm\(^3\). Treatment with hydralazine for 1.5 weeks in experiment 1 did not influence infarct volume (\( t_{21} = 0.25, p = 0.8 \)). Mean infarct volume was 14% smaller in animals treated for 6 weeks in experiment 2 than in the untreated controls, but this difference did not reach statistical significance (\( t_{21} = 1.70, p = 0.10 \)). Rats treated with hydralazine for 10 weeks in experiment 3 exhibited an 18% reduction in infarct volume compared with the untreated controls, which was significant (\( t_{15} = -2.43, p = 0.02 \)); the 95% confidence interval for the difference in infarct volume between the treated and control groups was 6–76 mm\(^3\). An 18% reduction in infarct volume was also noted in animals treated for 16 weeks compared with the untreated controls in experiment 4 (\( t_{21} = -2.99, p = 0.005 \)); the 95% confidence interval for the difference in infarct volume between the treated and control groups was 11–55 mm\(^3\).
TABLE 1. Serial Blood Pressures in Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Baseline</th>
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<tr>
<td>Control</td>
<td>15</td>
<td>207±25</td>
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<tr>
<td>Treated</td>
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<td>231±22</td>
<td>138±11*</td>
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<tr>
<td>Control</td>
<td>13</td>
<td>194±15</td>
<td>181±26</td>
<td>188±29</td>
<td>182±20</td>
<td>180±16</td>
<td>175±18</td>
<td>187±18</td>
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<tr>
<td>Treated</td>
<td>11</td>
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<td>134±36</td>
<td>115±22*</td>
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<tr>
<td>Control</td>
<td>16</td>
<td>192±16</td>
<td>206±14</td>
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<tr>
<td>Treated</td>
<td>16</td>
<td>200±18</td>
<td>121±17</td>
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Values are mean±SD mm Hg.

*Direct mean arterial pressures measured from tail artery cannula 4–6 hours after middle cerebral artery occlusion. All other values are indirect systolic pressure measurements.

Discussion

Most studies suggest that blood pressure is elevated in SHR only after 4 weeks of age, although Gray reported that within 24 hours after birth SHR have mild though significant elevations in mean arterial blood pressure compared with Wistar-Kyoto rats. The SHR used in this study had an average age of 13 weeks, and thus hypertension had been present for at least 9 weeks. The results demonstrated a trend toward infarct volume reduction in hydralazine-treated SHR after 6 weeks and a significant decrease in infarct volume after 10 and 16 weeks of therapy compared with untreated SHR.

Although the influence of antihypertensive therapy on infarct size had not been examined previously, treatment of hypertension in SHR has been demonstrated to affect cerebral vessel morphology and cerebral blood flow (CBF). The medial thickness of cerebral arteries and arterioles has been shown to decrease in 20-week-old SHR that received prior antihypertensive therapy for 10 weeks, although this effect was dependent on vessel size. Harper also reported a decrease in arteriolar diameter and cross-sectional wall area in 18-19-week-old SHR that were treated for 25 days compared with untreated SHR and normotensive Wistar rats.

TABLE 2. Physiological Variables for Spontaneously Hypertensive Rats

<table>
<thead>
<tr>
<th>Variable</th>
<th>Before occlusion</th>
<th>After occlusion 4–6 hrs</th>
<th>After occlusion 24 hrs</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control(n=15)</td>
<td>Treated(n=15)</td>
<td>Control(n=17)</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>124±10</td>
<td>99±15</td>
<td>120±10</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.34±0.03</td>
<td>7.35±0.02</td>
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<tr>
<td>PaO2 (mm Hg)</td>
<td>105±16</td>
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<tr>
<td>Paco2 (mm Hg)</td>
<td>44±4</td>
<td>45±2</td>
<td>40±3</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>145±15</td>
<td>148±18</td>
<td>110±10</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>49±1</td>
<td>47±2</td>
<td>47±2</td>
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</table>

Values are mean±SD. MABP, mean arterial blood pressure.
These histological vessel changes, in turn, may explain changes in CBF noted in SHR receiving antihypertensive therapy. The lower limit of cerebral autoregulation is increased in hypertensive rats, mildly but significantly reduced after 4 weeks of blood pressure control, and within the range seen in age-matched normotensive Wistar rats after 8–10 weeks of blood pressure normalization.16,17,24 Fujishima et al25 demonstrated that 20-week-old SHR that received prior antihypertensive therapy for either 8 or 16 weeks had a higher resting CBF than untreated SHR. These workers noted a greater increase in CBF in animals treated for 16 weeks than in those treated for 8 weeks. Furthermore, this increased CBF was correlated with the degree of blood pressure reduction.25

The findings of CBF elevations with antihypertensive therapy discussed above may explain the results in this study. The increase in CBF associated with longer durations of antihypertensive therapy may explain the trend toward infarct volume reduction in SHR treated for 6 weeks and the significant reduction in infarct volume after 10 weeks of treatment. Presumably, this increase in CBF would plateau at some point despite continued treatment, and this may explain why no further reduction in infarct volume was seen in SHR treated for 16 weeks. Supporting evidence for the existence of a plateau effect of antihypertensive therapy is found in a study by Weiss.26 After 5 weeks of antihypertensive treatment, the blood pressure of 8-month-old SHR normalized and hypertrophic vascular changes in hindquarter resistance vessels regressed as reflected by a decrease in the resistance at maximal dilatation and maximal pressor response. An additional 5 weeks of treatment did not further alter these effects.26

Another possible explanation for the treatment effect demonstrated in this study may relate to a direct action of hydralazine, such as cerebral arteriolar vasodilation or platelet inhibition,27–29 rather than its nonspecific antihypertensive effect. Limas et al30 found that some of the changes in SHR heart/body weight and aorta and small intrarenal arterial vessel morphology following treatment with hydrochlorothiazide, hydralazine, or captopril could be explained by selective actions of the agents and that other changes could be explained by a nonspecific antihypertensive effect. If a direct action of hydralazine were responsible for the treatment effect, however, the infarct volume of hydralazine-treated animals in experiment 1 should have been significantly less than that of the untreated controls.

In summary, this study demonstrates that prolonged antihypertensive therapy decreases infarct volume in SHR subjected to focal cerebral ischemia and that infarct volume is significantly correlated with mean arterial blood pressure. This effect appears to be dependent on the duration of treatment, being undetectable after 1.5 weeks, appearing after 6 weeks, and becoming significant after 10 weeks of therapy. Furthermore, the magnitude of the treatment effect seems to plateau by 10 weeks of therapy. While recognizing that translating results from animal studies to the clinical setting should be done cautiously, this study suggests that antihypertensive therapy reduces the extent of infarction when cerebral ischemia occurs in the setting of hypertension. If true, this finding may explain the decreased stroke mortality in patients with lower prestroke blood pressures described by Rabkin et al9 and supports the hypothesis that the improved stroke survival observed during the 1970s is due to the widespread treatment of hypertension, which in turn is postulated to reduce stroke severity.31

Acknowledgments
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References
3. Veterans Administration Cooperative Study Group on Antihypertensive Agents: Effects of treatment on morbidity in

<p>| TABLE 3. Infarct Volume in Spontaneously Hypertensive Rats |
|-------------------|-------------------|-------------------|
| Group             | Control           | Treated           |
|                   | Weeks of treatment | Infarct volume (mm³) | Infarct volume (mm³) |</p>
<table>
<thead>
<tr>
<th>Experiment</th>
<th>n</th>
<th>Infarct volume (mm³)</th>
<th>n</th>
<th>Infarct volume (mm³)</th>
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<td>4</td>
<td>16</td>
<td>16</td>
<td>187±29</td>
<td>16</td>
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</table>

Values are mean±SD.
*p<0.05 different from control by Student's two-tailed t test.
hypertension: Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. *JAMA* 1967;202:116–122

4. Veterans Administration Cooperative Study Group on Antihypertensive Agents: Effects of treatment on morbidity in hypertension: II. Results in patients with diastolic blood pressure averaging 90 through 114 mm Hg. *JAMA* 1970;213:1143–1152


**Key Words** • antihypertensive agents • cerebral ischemia • rats
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