Spatial Features of Focal Infarction After Hydralazine Treatment in Stroke-Prone Spontaneously Hypertensive Rats

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**Background and Purpose:** Rapid occlusion of the middle cerebral artery above the rhinal fissure produces a large ischemic infarct in hypertensive rats, but this occlusion results in a minimal lesion in young normotensive rats. Our purpose was to attenuate rising blood pressure in young stroke-prone spontaneously hypertensive rats with hydralazine before the occlusion to determine if the gross infarct volume is smaller, and if it is, to determine whether length, width, depth, or surface area of the infarct changes, which could suggest a mechanism of protection.

**Methods:** Untreated rats (n = 6) and rats receiving hydralazine for 1.5 (n = 6) or 5 (n = 5) weeks were anesthetized, and the middle cerebral artery was rapidly occluded with a ligature. One day later the rats were killed and the brains were fixed in formalin. Fine-grain-release film that is sensitive to spectral properties of the infarct was used to photograph the tissue. Infarcted areas were traced on paper and then digitized for measurements and computations with a microcomputer.

**Results:** Compared with untreated rats, tail systolic blood pressure (120 ± 3 versus 138 ± 4 mm Hg), infarct volume (61 ± 4 versus 93 ± 6 mm³), infarct surface area (39 ± 1 versus 54 ± 2 mm²), infarct width (3.8 ± 0.1 versus 4.8 ± 0.2 mm), and infarct length (6.0 ± 0.3 versus 8.1 ± 0.3 mm) were less in rats receiving hydralazine for 5 weeks (p < 0.05). No change was detected in infarct depth.

**Conclusions:** Treatment of young stroke-prone spontaneously hypertensive rats with hydralazine for 5 weeks before middle cerebral artery occlusion results in a smaller infarct. The narrower, shorter dimensions indicate increased protection against lateral enlargement of the infarct and the possibility that protection was due to increased collateral blood flow through modified blood vessels. (*Stroke* 1993;24:253–258)

**KEY WORDS** • cerebral arteries • cerebral infarction • hydralazine • rats

Sudden, permanent occlusion of the rat middle cerebral artery (MCA) above the rhinal fissure beyond the lenticulostriate arteries reduces blood flow to the distal MCA cortical territory. The reduced blood flow precedes a large cortical infarct in adult spontaneously hypertensive rats with chronic hypertension. In contrast, 5-week-old normotensive Wistar rats usually escape this lesion, whereas in adult normotensive rats the infarct is variable in frequency and size. In adult normotensive Wistar rats blood flow to the territory of the occluded MCA is reduced shortly after occlusion. After weeks of MCA occlusion arterial anastomoses leading to the MCA territory have wider lumens, blood flow is normal, and blood flow reserve is restored in normotensive Wistar rats protected from infarction. We conjecture that in young spontaneously hypertensive rats the continuous rising blood pressure (BP) produces vascular wall structural changes that reduce the blood flow reserve of collateral anastomoses, thus increasing the risk of infarction after sudden occlusion of the MCA.

Our objective was to study the effect of MCA occlusion on cortical infarction in young stroke-prone spontaneously hypertensive rats (SHRSP) before fully developed vascular wall changes become present during the established stage of hypertension (systolic BP of >150 mm Hg). Because young normotensive Wistar rats usually escape this lesion after MCA occlusion, they were not included in this study. Our first goal was to determine whether gross infarct volume was smaller in young SHRSP after attenuating the rise in BP with hydralazine before MCA occlusion. If volume is smaller, our second goal was to examine infarct length, width, and depth to see where significant change occurs because the place of protection may signify a mechanism of protection.

**Materials and Methods**

Group I rats (n = 6, weight 64 ± 3 g) drank tap water without hydralazine before occlusion of the MCA at 6 weeks of age. Group II rats (n = 6, weight 101 ± 14 g) received hydralazine (50 mg/500 ml tap water, changed daily) in their drinking water ad libitum for 1.5 weeks.
before MCA occlusion at 7 weeks of age. Group III rats \((n=5, \text{ weight } 113\pm18 \text{ g})\) were treated with hydralazine ad libitum (same as group II) for 5 weeks before MCA occlusion at 9 weeks of age. Each group of rats was obtained from two or more inbred litters and fed standard rat chow. Two litters provided animals for both group II and group III. Both sexes were present in all groups.

Five to seven tail systolic BP recordings were obtained for each animal following procedures detailed previously.\(^8\) The last three recordings were averaged to yield one BP value for each rat. BP readings were obtained 0–2 days before and on the day after MCA occlusion.

All rats were anesthetized with 132 mg/kg i.m. ketamine hydrochloride. Details are given elsewhere\(^8\) of surgery necessary to expose the right MCA above the rhinal fissure beyond the origins of the lenticulostrate arteries supplying subcortical structures. A 2-mm-diameter craniectomy was drilled with a \#6 dental burr about 1 mm rostral and 2 mm dorsal to the rostral fusion point of the zgomatic and squamosal bones. Monofilament nylon thread, about 35 \(\mu\)m in diameter, was used to ligate the MCA. The occlusion was about 1,700 \(\mu\)m dorsal to the rhinal fissure and 300–500 \(\mu\)m ventral to the MCA bifurcations distributing to the frontal, parietal, and occipital cortical regions. The rats were not paralyzed, no mechanical ventilation was used, and no respiratory gases were administered. After wound closure with sutures, postsurgical care followed procedures previously outlined.\(^8\)

On the day after MCA occlusion, the rats were anesthetized with ether and then injected with 40–50 mg/kg i.v. papaverine hydrochloride to produce maximal vasodilation and death. Tissue fixation was initiated by perfusion with 50 ml of 10% neutral buffered formalin into the thoracic aorta occluded at the diaphragm with a hemostat. The brains were removed and stored in fixative for 9 months. Photographic differentiation of the infarct was achieved with fine-grain-release film that is highly sensitive to the spectral properties of infarcted tissue (Figure 1).

The brains were placed in a custom-made holder to photograph the hemisphere at a 45\(^\circ\) angle to the mid-sagittal plane. This orientation showed the cortical surface of the infarct in one image (Figure 1A). At \(\times14\) magnification coordinates of the infarct border were digitized, and infarct area at the pial surface was calculated using Sigma-Scan (Jandel Scientific, San Rafael, Calif.). The maximum length of the infarct was in the direction of the long axis of the brain. Length was measured at 0.25-mm intervals parallel to the maximum dimension and averaged to one value for each rat. Width was measured at 0.25-mm intervals perpendicular to the length measurement and averaged to one value for each rat.

All brains were cut in the coronal plane using an Activational Systems, Inc. model RBM-4000C holder (Warren, Mich.) to control section thickness at 1 mm. Sections were photographed, and infarct borders were traced from negatives projected at \(\times14\) magnification. Tracings were made without observer knowledge of the treatment group. Infarct borders were digitized and areas computed. Infarct volume for each coronal section was obtained by averaging the surface area of panne-

crotic tissue on each side of the section (Figure 1B) multiplied by the section thickness. Brain infarct volume was obtained by summing the section volumes. Medial–lateral width of the infarct was measured at the pial surface (superficial) and at the interface with the white matter (deep) and averaged. Cortical infarct depth was measured from a pial surface point to the white matter. Three to 10 depth measurements were made for each side of a section and averaged. The two side values were averaged to give a section value, and for each brain average depth was obtained from all section values.

Data for the three groups were compared using one-way analysis of variance. Bonferroni’s correction was applied to multiple posttest comparisons. Intranimal comparisons were made with a paired \(t\) test. An \(\alpha\) error below 0.05 (i.e., \(p<0.05\)) was considered significant. All values are expressed as mean\(\pm\)SEM.

### Results

Tail systolic BP was lower in group III (120\(\pm\)2.9 mm Hg) than in group I (138\(\pm\)3.8 mm Hg, \(p<0.05\)). In group II BP was 132\(\pm\)4.9 mm Hg, not significantly different from that in groups I or III (\(p>0.05\)). One day after MCA occlusion, BP was not significantly different from the preocclusion value for any group (\(p>0.05\)).

After occlusion of the MCA, mean cortical infarct volume was 34% less in group III than in group I (\(p<0.05\)) and 35% less than in group II (\(p<0.05\), Table 1, Figure 2). Hydralazine treatment for 1.5 weeks before MCA occlusion had virtually no effect on mean infarct volume (\(p>0.05\), Table 1).

In group III surface area of the infarct was 28% smaller than in group I (\(p<0.05\)) and 19% smaller than in group II (\(p<0.05\), Table 1, Figure 2). In group II mean surface area was not significantly different from that in group I (\(p>0.05\), Table 1).

Average width of the infarct was 21% shorter in group III than in group I (\(p<0.05\)) and 17% shorter than in group II (\(p<0.05\), Table 1, Figure 2). Average width of the infarct did not differ significantly in groups I and II (\(p>0.05\), Table 1). Width of the infarct was greater superficially than deep in all groups (\(p<0.05\), Table 1), and the difference made the infarct wedge shaped. Deep width of the infarct was less in group III than in groups I or II (\(p<0.05\), Table 1).

Average length of the infarct was 26% shorter in group III than in group I (\(p<0.05\), Table 1, Figure 2). Average length of the infarct was not significantly different in groups I and II (\(p>0.05\), Table 1).

Infarct length was significantly greater than infarct width in each group, which indicates that the lesion has characteristic (not amorphous) shape and polarity (\(p<0.05\), Table 1). Mean depth of the infarct was similar in all groups (\(p>0.05\), Table 1). Infarct depth was appreciably less than infarct width and infarct length in all groups (\(p<0.05\)). There was no significant linear correlation of BP with infarct volume or infarct surface area, length, width, or depth in any group (\(p>0.05\)).

### Discussion

This study provides three insights. First, in young SHRSP treated for rising BP the smaller infarct volume suggests that hydralazine treatment for 5 weeks provided some protection from infarction after MCA oc-
FIGURE 1. Photomicrographs. Panel A: View of stroke-prone spontaneously hypertensive rat (SHRSP) brain rotated 45° to midsagittal plane. Arrow indicates site where middle cerebral artery (MCA) was occluded. Brain from rat receiving hydralazine for 5 weeks before MCA occlusion and fixation in formalin. Magnification same as in Panel B. Panel B: View of coronal section of untreated SHRSP brain 1 day after MCA occlusion and fixation in formalin.

clusion. Second, the narrower, shorter dimensions of small infarcts reveal protection against lateral (side and end) enlargement of the infarct that may result from blood flow from lateral sources. Third, the similar depths of small and large infarcts is evidence that minimal, if any, protection occurred on the underside of the cortical infarct, which lacks a deeper source of collateral blood supply.

Others have demonstrated smaller infarcts after MCA occlusion in adult SHRSP following reductions in BP with hydralazine and hydrochlorothiazide or with cilazapril for 3 months,7 and after hydralazine treatment for 10 weeks in spontaneously hypertensive rats (SHR) infarcts were smaller.6 We found that 1.5 weeks of hydralazine treatment in combination with ketamine anesthesia was no more protective than ketamine alone, which is evidence that the interaction of the two drugs produced minimal, if any, tissue protection. With hydralazine treatment for 5 weeks, the smaller infarct confirms the finding that hydralazine requires a temporal component to produce some tissue protection. That tissue protection requiring a temporal component was probably independent of an effect of ketamine used in this study or of halothane used in the earlier study.6

Concerning effects of anesthesia used during MCA occlusion, ketamine hydrochloride, an N-methyl-D-aspartate (NMDA) antagonist, produced no more tissue protection than halothane in adult SHR.19 In adult SHRSP anesthetized with sodium pentobarbital,7 infarct volume was not significantly different from that in adult SHR anesthetized with halothane6 (p > 0.05 for reported means ± SEM). The data suggest that differences in tissue protection produced by acute administration of halothane, sodium pentobarbital, or ketamine...
are minimal in spontaneously hypertensive rat strains with MCA occlusion. Furthermore, a single dose (5 mg/kg) of MK-801, a noncompetitive NMDA antagonist, provided no significant tissue protection after MCA occlusion in adult SHR anesthetized with halothane. A single dose (1 mg/kg) of MK-801 before MCA occlusion had a small but significant protective effect in 8-week-old SHR anesthetized with ketamine, which suggests that a single dose of ketamine was appreciably less effective on NMDA-mediated protection or that MK-801 protected through a mechanism different from that of ketamine. While the possibility was not ruled out that ketamine produced some protection in both control and experimental groups, the protection was probably minimal.

As early as 15 days after birth, BP is elevated in SHR compared with normotensive rats, and medial hypertrophy is evident in cerebral vascular beds at this very early age. Protection against infarction could depend upon reversal or attenuation of vascular structural changes that are produced secondary to increased systolic BP, elevated pulse pressure, or even changes unrelated to high BP. Reversal of medial hypertrophy, recovery from endothelial dysfunction, and prevention or reversal of “remodeling” of vessels into smaller vessels all require time, and complete normalization may never occur after BP reduction. Furthermore, the hypertensive action of hydralazine could reduce perfusion downstream to fully dilated but structurally narrowed anastomoses, thereby making flow pressure dependent in collateral-dependent tissue. Thus, the amount and location of tissue protected by hydralazine after MCA occlusion in SHRSP is probably determined more by flow reserve in the anastomoses and downstream driving pressure within cortical penetrating arterioles than by NMDA receptor blockade from ketamine.

Narrower, shorter, wedge-shaped gross infarcts were indicative of increased tissue protection in a circumferential region surrounding the infarct. This region of increased protection was located closer to pial surface MCA anastomoses with branches of the anterior and posterior cerebral arteries than to the infarct center. Others have demonstrated a gradient of blood flow that increases with distance from the site of MCA occlusion, which suggests that increased tissue protection was obtained from increased blood flow in cortical penetrating arterioles that frequently course parallel to the edge of the wedge-shaped infarct (Figure 1B).

In contrast to the narrower, shorter dimensions of smaller cortical infarcts, we found cortical infarct depth to be similar in large and small lesions. Protection was not observed on the underside of the lesion, possibly because no deep collateral blood supply exists there. However, others have demonstrated that infarcted cortex is thicker due to edema and swelling, which is likely to alter perfusion hemodynamics and obscure normal from injured or infarcted tissue. Thus, we do not

![Figure 2](https://example.com/figure2.png)
exclude the possibility that any real difference within the core of the infarct may have been obscured.

In summary, antihypertensive treatment of young SHRSP with hydralazine for 5 weeks before rapid occlusion of the MCA results in a smaller core infarct than in untreated SHRSP. Spatial features of the smaller, focal infarct indicate that protection is increased laterally and possibly that protection is due to greater blood flow through modified anastomotic blood vessels.

References

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Editorial Comment

Occlusion of the middle cerebral artery produces a marked decrease in blood flow and a large infarct in experimental models of chronic hypertension. In contrast, similar arterial occlusion in normotensive control animals produces little or no infarction.

After chronic hypertension has been established, antihypertensive therapy decreases infarct volume in spontaneously hypertensive rats in response to focal cerebral ischemia. The magnitude of this protective effect is dependent on the duration of treatment. The present study by Coyle and Feng suggest that early treatment of stroke-prone spontaneously hypertensive rats with hydralazine, which essentially prevents the development of hypertension, also protects against brain damage following occlusion of the middle cerebral artery. Protection against infarction may be due to increased blood flow through collateral vessels.

The mechanism by which antihypertensive treatment protects cerebral blood vessels is not fully defined but may include prevention of or reversal of structural changes in cerebral arterioles, maintenance or restoration of normal endothelium-dependent responses, and improvement in cerebral blood flow reserve (minimal vascular resistance) and collateral vascular capacity.

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Stroke 1993;24:253-257
doi: 10.1161/01.STR.24.2.253

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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