Effect of Antihypertensive Treatment on Focal Cerebral Infarction

Kenichiro Fujii, Barbara L. Weno, Gary L. Baumbach, and Donald D. Heistad

The goal of the current study was to determine whether treatment of hypertension reduces cerebral infarction after occlusion of the middle cerebral artery in stroke-prone spontaneously hypertensive rats (SHRSPs). Three-month-old SHRSPs received untreated drinking water or drinking water containing cilazapril, an angiotensin converting enzyme inhibitor, or hydralazine and hydrochlorothiazide. After 3 months of treatment, the left middle cerebral artery was occluded and neurological deficit was evaluated. Infarct volume was measured 3 days after occlusion using computer imaging techniques from brain slices. Cilazapril and hydralazine with hydrochlorothiazide were equally effective in reducing systolic blood pressure in SHRSPs. One day after occlusion of the middle cerebral artery, neurological deficit was decreased by both cilazapril and hydralazine with hydrochlorothiazide compared with untreated SHRSPs, and the deficit 3 days after occlusion was decreased significantly only by cilazapril. Infarct volume was 178±7 mm³ (mean±SEM) in untreated SHRSPs, and it was significantly reduced to 117±15 mm³ by hydralazine with hydrochlorothiazide and to 101±17 mm³ by cilazapril. Infarct volume in Wistar-Kyoto rats was 27±16 mm³. Thus, reduction in arterial pressure by hydralazine with hydrochlorothiazide or an angiotensin converting enzyme inhibitor is protective against focal cerebral ischemia in SHRSPs.

KEY WORDS • angiotensin converting enzyme inhibitors • hydralazine • cerebral infarction • antihypertensive therapy

Hypertension increases the incidence of stroke and also exaggerates cerebral infarction after arterial occlusion.1-2 Studies in rats have shown that occlusion of the middle cerebral artery (MCA) produces large infarcts in hypertensive rats and no or small infarcts in normotensive rats.3-5 Prevention of development of hypertension may protect against cerebral infarction in stroke-prone spontaneously hypertensive rats (SHRSPs).6 Even after development of chronic hypertension, antihypertensive treatment attenuates the metabolic derangements after bilateral carotid occlusion in spontaneously hypertensive rats (SHRs)6 and decreases infarct volume in SHRses subgrouped to focal cerebral ischemia.7 We have suggested6 that some antihypertensive drugs have somewhat selective effects on cerebral vessels. For example, cilazapril, an angiotensin converting enzyme (ACE) inhibitor, prevented both hypertrophy and "remodeling" of cerebral arterioles in SHRSPs, whereas hydralazine was effective only in attenuating the hypertrophy.8

The first goal of this study was to determine whether treatment of hypertension reduces cerebral infarction or neurological deficits after MCA occlusion in SHRSPs. The second goal was to determine whether different antihypertensive agents (cilazapril and the combination of hydralazine and hydrochlorothiazide) are protective against cerebral ischemia.

Methods

Experiments were conducted on male Wistar-Kyoto (WKY) rats (n=9) and male SHRSPs (n=41). Procedures were in accordance with institutional guidelines. At 3 months of age, SHRSPs were divided into three treatment groups: a control group that received tap water (n=13), a group that received hydralazine (200 mg/l) and hydrochlorothiazide (H&H) (500 mg/l) in the drinking water (n=13), and a group that received cilazapril (500 mg/l) in the drinking water (n=15). Hydrochlorothiazide was added to hydralazine because, in our previous study,8 hydralazine (100 mg/l) alone was not sufficient to decrease blood pressure to a level similar to that in rats treated with cilazapril. Rats in the hydralazine and cilazapril groups drank approximately 40 and 30 ml of water per day, so the dosages of hydralazine and cilazapril were approximately 24 and 45 mg/kg per day, respectively. WKY rats were not treated with either hydralazine or cilazapril, because our previous study had shown that these treatments have no significant effect on cerebral vascular mechanics.8 Animals were allowed free access to food, housed at 25°C, and exposed to a 12-hour light/dark cycle each day. Systolic blood pressure was measured with the tail-cuff method in all rats at 2-week intervals after they reached

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2½ months of age. Experiments were conducted when the animals were approximately 6 months of age.

Occlusion of Middle Cerebral Artery

Animals were anesthetized with sodium pentobarbital (50 mg/kg body wt i.p.). Details of the surgical procedures have been reported previously. In brief, a craniotomy (1–2 mm in diameter) was made, dura mater was incised, and the left MCA was ligated. The occlusion point was distal to striate branches but ventral to MCA bifurcations distributing to frontal, parietal, and occipital cortical regions. In three rats in each group, a ligature was placed under the MCA but then removed (sham occlusion). During surgery, body temperature was maintained at 37.0°C. The skin was closed with sutures. The animals were allowed to awaken and then returned to their cages. Treatment with drugs continued during the 3 days after MCA occlusion.

Neurological Evaluation

Neurological function was evaluated 1 and 3 days after MCA occlusion by one person who was blinded to the experimental condition of the animal. The animals were scored on a scale of 0–3 based on a neurological scoring system. Scores were 0, no observable deficit; 1, forelimb flexion; 2, decreased resistance to lateral push without circling; and 3, same behavior as 2, with circling.

Quantification of Infarct Volume

After 3 days, animals were anesthetized with sodium pentobarbital (50 mg/kg body wt i.p.). Arterial pressure and blood gases were measured. The animals were decapitated, and their brains were rapidly removed, weighed, and chilled in a freezer. After chilling and with the use of a brain dissection block (Activational Systems, Inc., Warren, Mich.), the entire forebrains were cut into 2-mm-thick coronal sections. The slices were immersed for 30 minutes in 2% buffered triphenyltetrazolium chloride (Sigma Chemical Co., St. Louis, Mo.) and kept at 37°C. This water-soluble compound is enzymatically converted in intact mitochondria to a fat-soluble formazan compound that stains viable tissue crimson red, while infarcted tissue fails to stain and appears white. The slices were stored in 7% Formalin.

The posterior surface of each section was imaged and digitized (Mac II computer, Raster Ops 364 color video board, and Digital Microvax II computer). The digitized image was displayed on a video terminal. The infarct border was outlined by one person (who was blinded to treatment groups) using a cursor, and infarct area was computed. For each rat, infarct volume was computed.
Con

FIGURE 3. Computer images of inferior (top panels) and posterior (bottom panels) views of typical infarcts reconstructed in three dimensions in an untreated stroke-prone spontaneously hypertensive rat (SHRSP) (Con), a SHRSP treated with hydralazine and hydrochlorothiazide (H&H), and a SHRSP treated with cilazapril (Cil). Treatment produced generalized reduction in cerebral infarct size.

as the sum of infarct area times the 2-mm thickness of each section over the rostral-caudal extent of the forebrain.

Statistical Analysis

Values are expressed as mean±SEM. One-way analysis of variance followed by Scheffe's F test for significance for within-group comparisons was used to compare physiological variables and infarct volume. Fisher's exact test (two-tailed) was used to compare neurological grades. A probability value of less than 0.05 was considered significant.

Results

Systolic arterial pressure in SHRSPs was reduced effectively by H&H or cilazapril to the level of WKY rats (Figure 1). Treatment had no significant effect on body weight, but the ventricular weight and ratio of ventricular weight to body weight were smaller in treated than in untreated SHRSPs. Arterial blood gases, pH, and hematocrit 3 days after MCA occlusion were within the physiological range and were similar among all groups.

Treatment with H&H decreased neurological deficit 1 day after MCA occlusion (p<0.05), but the difference was not statistically significant 3 days after occlusion (Figure 2). Cilazapril significantly reduced neurological deficit both 1 and 3 days after MCA occlusion compared with untreated SHRSPs (p<0.05). Neurological deficit 1 and 3 days after MCA occlusion was not significantly different in SHRSPs treated with cilazapril and H&H. WKY rats had the lowest neurological score.

In each SHRSP, ligation of the MCA produced a single cerebral infarct. Treatment with H&H or cilazapril produced a generalized reduction in the size of cerebral infarcts. Figure 3 shows typical infarcts (which are close to the mean size of infarction for the groups) reconstructed in three dimensions in an untreated SHRSP, a SHRSP treated with cilazapril, and a SHRSP treated with H&H. Infarct volume was 178±7 mm³ in untreated SHRSPs, 117±15 mm³ in SHRSPs treated with H&H, 101±17 mm³ in SHRSPs treated with cilazapril, and 27±16 mm³ in WKY rats. Infarct volume in SHRSPs treated with H&H and cilazapril was significantly lower than in untreated SHRSPs (p<0.05) but was larger than in WKY rats (p<0.05). Infarct volume did not differ between the H&H and cilazapril groups. There was no detectable infarction in sham-occluded rats. We attempted to minimize effects of edema on infarct volume as in a previous report, but differences between groups were virtually unaltered (corrected infarct volume: untreated, 158±6 mm³; H&H, 101±13 mm³; cilazapril, 87±14 mm³; WKY, 24±14 mm³).

Discussion

There are three new findings in the present study. First, antihypertensive treatment, after hypertension has developed, reduces the extent of infarction after MCA occlusion in SHRSPs. Second, H&H and cilazapril were both effective in reducing infarct volume. Third, H&H and cilazapril were also effective in reducing neurological deficit after MCA occlusion. In addition, the results suggest (but do not prove) that the antihypertensive agents protect against cerebral infarction principally by their antihypertensive effects.

In SHRs, antihypertensive treatment with hydralazine reduces the extent of infarction after unilateral occlusion of the common carotid and middle cerebral arteries. Our study has demonstrated that chronic treatment of hypertension can also reduce infarct size following MCA occlusion after hypertension has developed in SHRSPs. In SHRs, some structural alterations in cerebral vessels, including medial hypertrophy, can be reversed by antihypertensive treatment. Endothelial function in cerebral vessels and the aorta also recovers after treatment. We speculate that these morphological and functional changes during antihypertensive treatment may restore dilator capacity of cerebral arteries and thus improve the cerebral blood flow reserve and collateral vascular capacity.
It is interesting that infarct size is larger in treated SHRSPs than in WKY rats even though arterial pressure is the same. It is possible that alterations in cerebral vessels that contribute to the increase in infarct size have already occurred by 3 months of age. Treatment of hypertension for 3 months may not reverse completely these vascular changes.

Some unique effects of ACE inhibitors on blood vessels have been reported. For example, a study in SHR has shown that treatment with cilazapril but not hydralazine restores normal endothelial function and morphological feature in the aorta. In SHR, cilazapril can normalize the cerebral blood flow reserve, with reversal of the medial thickening that occurs in the cerebral vessels during hypertension. We have suggested that, in SHRSPs, treatment with cilazapril but not hydralazine prevents remodeling of pial arterioles, whereas both cilazapril and hydralazine prevent hypertrophy of pial arterioles. Encroachment on the lumen of cerebral arterioles in SHRSPs is primarily due to reductions in external diameter rather than hypertrophy. Thus, the study implies that an ACE inhibitor may be more effective than hydralazine in restoring dilator reserve of cerebral arterioles. Very recently, it has been shown that acute administration of an ACE inhibitor before incomplete cerebral ischemia improves neurological outcome, suggesting that an ACE inhibitor may have a protective effect against ischemia that is independent of its effect on blood pressure. The present study indicates that H&H and cilazapril, an ACE inhibitor, are similarly effective in reducing neurological deficit and infarct volume after MCA occlusion in SHRSPs.

In summary, antihypertensive treatment reduces infarct volume in SHRSPs subjected to focal cerebral ischemia. This effect appears to be mainly due to reduction in blood pressure.

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