Efficacy of Retrograde Perfusion of the Cerebral Vein With Verapamil After Focal Ischemia in Rat Brain

T. Hosaka, MD; Y.L. Yamamoto, MD, PhD; and M. Diksic, PhD

Background and Purpose: For treatment of acute stroke, drug therapy administered systemically has been unreliable due to inadequate delivery of drug into ischemic tissue. We have developed a new method to deliver drugs into the ischemic tissue by retrograde perfusion of the cerebral vein.

Methods: We examined in rats the effectiveness of administering verapamil into ischemic tissue by retrograde perfusion through the cerebral vein, starting 3 hours after occlusion of the middle cerebral artery. Twenty-four Fischer-344 rats with occlusion of the middle cerebral artery were divided into four groups of six rats each. Group A rats had no treatment, group B rats received verapamil intravenously, and groups C and D rats received verapamil by transvenous perfusion of the brain with blood and with saline, respectively. We studied local cerebral blood flow using the autoradiographic method with carbon-14-labeled iodoantipyrine and examined cerebral infarct volume with cresyl violet and Luxol fast blue staining.

Results: As compared with group A rats, in groups C and D rats we found a significant and extensive increase of cerebral blood flow in the ischemic cortical and subcortical areas (55–119%, \( p < 0.05 \)) and a significant reduction of cerebral infarct volume (31–39%, \( p < 0.05 \)). We found no significant changes in group B rats.

Conclusions: This study shows that transvenous perfusion of the brain with verapamil starting 3 hours after occlusion of the middle cerebral artery produces a significantly beneficial effect in rats. (Stroke 1991;22:1562–1566)

In recent years, the introduction of various classes of calcium channel blocking agents stimulated numerous experimental and clinical studies, the results of which proved conflicting. In these studies, improvement of local cerebral blood flow (CBF) or reduction of infarct volume in rats with focal ischemia occurred only after either pretreatment or early postischemic treatment (less than 1 hour after infarct).\(^1,2\) The recent clinical studies for treatment of acute stroke sponsored by the National Institutes of Health indicated that it is exceedingly difficult to start treatment within 3 hours of the stroke.\(^3\) We previously reported effective treatment of acute stroke by retrograde perfusion of the cerebral vein with the calcium channel blocker verapamil beginning 1 hour after occlusion of the middle cerebral artery (MCA) in rats.\(^4,5\) Recently, Duverger et al\(^6\) showed that Fischer-344 rats displayed consistent and sizeable infarcted tissue volume following MCA occlusion. Osborne et al\(^7\) also described a method for quantitative assessment in rats of the volume of early cerebral tissue damage by ischemia that was found to be reliable for assessing drug therapy and management strategies in the treatment of cerebral ischemia. We have, therefore, examined the effectiveness of 2-hour transvenous perfusion of the brain with verapamil (0.1 mg/kg/2 hr) and blood or saline, starting 3 hours after MCA occlusion.

Materials and Methods

We used 24 adult male Fischer-344 rats weighing 250–330 g. The rats were in a fasting condition overnight, with water provided ad libitum before the experiment. We produced focal cerebral ischemia by occlusion of the left MCA at the level proximal to the most lateral branch of the lenticulostriate artery, as described by Tamura et al.\(^8\) The rats were divided into four groups of six rats each. Group A (control) rats underwent surgical preparation for MCA occlusion without any treatment. The other three groups...
received verapamil 0.1 mg/kg/2 hr. Group B rats received intravenous administration of verapamil. Group C rats received verapamil by transvenous perfusion of the brain with autologous arterial blood at 150 mm Hg perfusion pressure, and group D rats received verapamil by transvenous perfusion with saline at the same perfusion pressure as group C. All rats were examined by single-tracer autoradiography using carbon-14–labeled iodoantipyrine for the measurement of local CBF, and frozen sections adjacent to those used for autoradiography were used for quantitative volumetric assessment of brain infarction with a modified method of Osborne et al.7 The 14C-iodoantipyrine (specific activity 55 mCi/mmol) was purchased from American Radiolabeled Chemicals, Inc., St. Louis, Mo., and verapamil hydrochloride [Isoptin; 2.8-bis(3,4-dimethoxyphenyl)-6-methyl-6-isopropyl-6-azaocanitrile], 2.5 mg/ml in isotonic aqueous solution, was obtained from Knoll Pharmaceuticals, Markham, Canada.

Details of the surgical preparation and monitoring of physiological parameters have been previously published.5 In brief, in rats under general anesthesia of 1.5–2.0% halothane, we catheterized both the femoral artery and vein and performed a tracheostomy. The general anesthesia was then switched to ketamine (50 mg/kg i.m.) and xylazine (8 mg/kg i.m.)10 and maintained until the end of the experiment. The left MCA was occluded by a small clip (Zen temporary clip, Ohwa Tsusho Ltd., Tokyo, Japan) proximal to the origin of the most lateral lenticonstriate and electrocoagulated just distal to the Zen clip. In groups C and D, another small craniectomy was made at the inferior and posterior part of the squamosal bone for cannulation of the inferior cerebral vein. The inferior cerebral vein was cannulated backward using a PE-10 polyethylene catheter connected to the conduit system, which consisted of four three-way stopcocks. Systemic administration of verapamil through the femoral vein (group B) or by transvenous perfusion of the brain (groups C and D) was started 3 hours after occlusion of the MCA and continued for 2 hours. Verapamil was diluted in 1 ml 0.9% saline solution and infused into the femoral vein or the cerebral vein at a constant rate of 0.8 μg/kg per minute using a Saga infusion pump (model 355, Orion Research, Inc., Boston, Mass.). In group C rats, 1 ml arterial blood was initially drawn from the femoral artery with a heparinized syringe (50 units heparin per 1 ml blood) over 3–4 minutes to avoid any changes in systemic blood pressure. This heparinized arterial blood was then transferred to the catheter and proximal portion of the conduit system through the second three-way stopcock. To replenish arterial blood in the conduit system, heparinized arterial blood was withdrawn 1 ml at a time at rates similar to those of transvenous perfusion from the femoral artery. The perfusion pressure was obtained by compressing the polyvinyl chloride bottle filled with saline using a mercurial sphygmomanometer (Tycos, Taylor Instrument Companies, Asheville, N.C.) connected at the conduit system's distal end and increasing the perfusion pressure in a gradual stepwise fashion up to 150 mm Hg.5 At each step, the pressure rose 20–30 mm Hg, and approximately 5 minutes elapsed between steps. During transvenous perfusion treatment, autologous blood (group C) or saline (group D) was infused continuously into the inferior cerebral vein at the rate of 0.2 or 0.3 ml/min, respectively, at 150 mm Hg perfusion pressure. The verapamil solution was connected to the proximal three-way stopcock of the conduit system and infused at the same rate. In all rats, body temperature was kept at approximately 37°C with a heating lamp positioned over the rats. Systemic blood pressure and blood gases were serially checked and maintained within physiological ranges during the experiments. Hematocrits were determined intermittently in arterial blood samples using an Eppendorf Geraleban Model 5412 centrifuge.

Local CBF was measured by an autoradiographic technique 4 hours and 59 minutes after MCA occlusion. Thirty microcuries 14C-iodoantipyrine was injected into the femoral vein over 1 minute. A 20-μl arterial blood sample was drawn every 5 seconds during injection of the tracer. Local CBF was calculated using the operational equation described by Sakurada et al.9

The tissue–blood partition coefficient of 0.80 was used. The optical density was measured six times in each of three consecutive tissue autoradiograms. The mean values of the tissue 14C radioactivities were obtained for each locus indicated in the rat brain atlas.11 The densitometric measurements were performed with a digital image analyzer (The Image Calculator, McGill University, Montreal, Canada). A 20-μm section of coronal frozen tissue adjacent to those used for autoradiography was dried at room temperature, fixed in 10% formaldehyde for 24 hours, and stained by a method combining cresyl violet and Luxol fast blue to determine the early cerebral infarction.7 The infarcted region was manually outlined and measured on a digitized image of seven equally spaced coronal sections using a digital image analysis system (The Image Calculator). Infarct regions were recognized as poorly stained areas. Any indistinct border of infarction was verified under light microscopic examination for neuronal ischemic damage.7 The total volume of infarction was determined by seven sectional areas of infarction multiplied by the interval thickness (1,280 μm). The amount of infarcted volume was expressed both in absolute value (mm³) and as a percentage of the total volume of the ischemic hemisphere (infarcted volume/ischemic hemispheric volume×100).

All data were expressed as mean±SEM. The statistical analysis of all data was performed using a two-tailed, unpaired Student's t test. A value of p<0.05 was regarded as a significant difference. The critical values for two-tailed t test with the Bonferroni correction are as follows: for p<0.05, t=3.425; for
FIGURE 1. Autoradiographic images of carbon-14-labeled iodoantipyrine in coronal sections of sensorimotor (left panels) and parietal (right panels) regions in group A (control, panels A and B), group B (intravenous administration of verapamil, panels C and D), group C (transvenous perfusion of verapamil with blood, panels E and F), and group D (transvenous perfusion of verapamil with saline, panels G and H). Groups C and D showed extensive increase of local cerebral blood flow in left sensory motor cortex and parietal cortex in ischemic cerebral hemisphere as compared with group A.

Results

Blood pressures, blood gases, and hematocrits of rats in the four groups did not differ significantly after MCA occlusion, regardless of verapamil administration.

In the autoradiographic studies, group B (Figures 1C and 1D and Table 1) showed further reduction of local CBF (−3 to −15%) in the ischemic cerebral cortices with a nonsignificant increase of local CBF in the contralateral normal cortices, as compared with group A control rats. Group C rats (Figures 1E and 1F and Table 1) showed a significant increase of local CBF in the ischemic cortices (from 68% to 74%, p<0.05; Table 1) and in the subcortical areas (from 39% to 72%, p<0.05; Table 1) as compared with the control rats. Group D rats (Figures 1G and 1H, Table 1) showed a significant increase of local CBF in the ischemic cortices (from 55% to 119%, p<0.05; Table 1) and in the subcortical areas (56%, p<0.05; Table 1) as compared with the group A control rats.

In the quantitative volumetric measurement of the brain infarction, group C rats showed significant reduction of the infarcted cerebral volume in the entire ischemic hemisphere (26%, p<0.01; Figure 2), sensorimotor level (33%, p<0.01; Figure 2), and parietal level (31%, p<0.01; Figure 2) as compared with group A control rats. Group D rats also showed significant reduction of the infarcted cerebral volume in the entire ischemic hemisphere (29%, p<0.01; Figure 2), sensorimotor level (27%, p<0.01; Figure 2), and parietal level (39%, p<0.01; Figure 2) as compared with group A control rats. Group B rats showed no significant change of the infarcted cerebral volume in the entire ischemic hemisphere, either the sensorimotor or parietal level (Figure 2).

Discussion

In focal ischemic animal models, postischemic treatment involving systemic administration of cal-
cium channel blocker given more than 1 hour after ischemic insult did not result in increase of local CBF or reduction of infarct volume. 12,13 Beneficial effects of intravenous calcium channel blockers were obtained only when administered before or shortly after onset of cerebral ischemia. 12 However, in clinical situations, therapy normally begins only 3–5 hours after the stroke. 3

Infarct size is often used to assess potential therapeutic regimens in focal cerebral ischemia models and is considered important in studies designed to evaluate the effect of calcium channel blockers. Selecting the proper animal model and method of quantitative assessment of infarction volume is of critical importance for reliable evaluation of therapeutic regimens. Like Duverger and Mackenzie, 6 we observed more reproducible and extensive infarcts in Fischer-344 rats with MCA occlusion than in Sprague-Dawley rats.

Most barbiturates have shown varying protective effect in experimental models of cerebral ischemia. 14 We chose ketamine, a known iV-methyl-D-aspartate receptor antagonist, 15 as the general anesthetic agent in all four groups because histological studies have shown that it ameliorates cerebral infarct volume 16 in a dose-dependent manner. For the original method of Osborne et al, 7 the rats were initially perfused with a combination of 40% formaldehyde, glacial acetic acid, and methanol, then their brains embedded in paraffin. We did not fix the rat brains in this way. Instead, we made frozen sections that were fixed in 10% formaldehyde for over 24 hours to allow measurement simultaneously, in the same rat, of both cerebral infarct volume and local CBF. Osborne’s group observed that sections stained by combining cresyl violet and Luxol fast blue showed a well-demarcated contour of infarct region. 7

### TABLE 1. Local Cerebral Blood Flow in Rats (ml/100 g/min) 3 Hours After Left MCA Occlusion and 2 Hours After Treatment

<table>
<thead>
<tr>
<th>Structures</th>
<th>Control (n=6)</th>
<th>Intravenous verapamil (n=6)</th>
<th>Transvenous perfusion of brain with verapamil</th>
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<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
<td>With blood (n=6)</td>
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<tr>
<td>Frontal cortex</td>
<td>33±4</td>
<td>150±2</td>
<td>28±4</td>
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<tr>
<td>Sensorimotor cortex</td>
<td>33±3</td>
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<td>37±4</td>
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<td>94±5</td>
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<td>Hippocampus</td>
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<td>6±2</td>
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<tr>
<td>Caudate, medial</td>
<td>51±11</td>
<td>142±7</td>
<td>52±5</td>
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<td>Dentate</td>
<td>101±6</td>
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</table>

Values are mean±SEM.  
*p<0.05, **p<0.01 for significant difference from control group by two-tailed unpaired t test.  
†p<0.05, ‡p<0.01 after Bonferroni correction.

### FIGURE 2. Infarcted area at seven coronal levels (1.28-mm interval). Nontreated group A (broken lines) is compared with treated groups B, C, and D (unbroken lines). Data are mean±SEM. Infarct sizes in group B are not different from those in group A. In groups C and D, infarct sizes are significantly reduced in coronal levels of ischemic core as compared with group A. TVBOB, transvenous perfusion of the brain.  
*p<0.05, **p<0.01 by two-tailed unpaired t test.
and we could easily outline the contour of the poorly stained infarct region with a digital image analyzer.

Verapamil has a vasodilating effect, particularly on small, resistant vessels.17 Local application of verapamil produces vasodilation in previously constricted artery both in vitro18,19 and in vivo.20–22 The inferior cerebral vein, anatomically comparable to Labbé’s vein in humans, collects venous blood from the centroparietotemporal regions.23 Our previous study indicated that the inferior cerebral vein can tolerate up to 150 mm Hg of retrograde perfusion pressure without any changes in blood–brain barrier permeability or in local CBF in rats with occlusion of the MCA.5 In another earlier study,6 we found that in focal ischemia, transvenous perfusion with verapamil into the ipsilateral inferior cerebral vein at 150 mm Hg perfusion pressure, with treatment started 1 hour after occlusion of the MCA, resulted in a significant increase of local CBF in the cortexes and subcortical areas of the ischemic cerebral hemisphere, producing potent vasodilation in resistant vessels in the territory of the inferior cerebral vein and resulting in an increase of local CBF in the cortical and subcortical areas of the territory of the inferior cerebral vein. Our present studies, with treatment starting 3 hours after occlusion of the MCA, confirm that transvenous perfusion of the brain with verapamil at 150 mm Hg perfusion pressure results in a significant increase of local CBF in the ischemic cerebral cortexes and deep subcortical structures and results in a reduction of cerebral infarction volume. As carrier solution to transvenous perfusion with verapamil, there was no statistical difference between autologous arterial blood or physiological saline solution. Our results, therefore, indicate that transvenous perfusion of the brain with verapamil provides beneficial therapeutic effects on both cerebral cortical and subcortical ischemic tissue, even 3 hours after occlusion of the MCA in rats.

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References

Key Words • autoradiography • calcium channel blockers • perfusion, venous • rats
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