Transvenous Perfusion of the Brain With Verapamil During Focal Cerebral Ischemia in Rats

Takashi Ueda, MD, Y. Lucas Yamamoto, MD, PhD, and Mirko Diksic, PhD

We report on the effect of the calcium channel blocker verapamil administered into the inferior cerebral vein in rats 1 hour after occlusion of the middle cerebral artery. Twenty-four rats were divided into four groups of six rats each. Group A rats received no medication. The other three groups received 0.1 mg verapamil/kg/2 hr. Group B rats received verapamil intravenously. Group C and D rats received verapamil and autologous arterial blood by transvenous perfusion of the brain. Group C rats at 100 mm Hg perfusion pressure and Group D rats at 150 mm Hg perfusion pressure. The administration of verapamil started 1 hour after middle cerebral artery occlusion and lasted for 2 hours. Three hours after occlusion, we used double- or single-tracer autoradiography with 4-\([^{18}F]\)fluoroantipyrine or \([^{14}C]\)iodoantipyrine and \([^{14}C]\)\(-\)aminoisobutyric acid as tracers to study the brains for local cerebral blood flow and blood–brain barrier permeability changes. Group C showed a significant increase of local cerebral blood flow in the parietal cortex (89%, *p*<0.01) and sensorimotor cortex (64%, *p*<0.05) compared with Group A. Group D showed an extensive and striking increase in local cerebral blood flow of the ischemic cortical and subcortical areas (57–100%, *p*<0.05). Group B showed no significant changes but exhibited further reduction of local cerebral blood flow in the ischemic cerebral hemisphere associated with slightly increased local cerebral blood flow in the nonischemic cerebral hemisphere compared with Group A. There was no change of blood–brain barrier permeability in any group. Our findings demonstrate that transvenous retrograde perfusion of the brain can deliver cytoprotective and vasoactive agents selectively and efficiently into ischemic brain tissue. This procedure may have great potential for the treatment of acute cerebral ischemia. (Stroke 1989;20:501–506)

Recent reports suggest that calcium channel blockers may improve ischemic cerebral tissue damage through their vasodilatory effects on cerebral vessels, particularly at the microcirculatory level, and by preventing excessive calcium influx into the cytoplasmic and mitochondrial compartments.1–10 However, most experiments have failed to demonstrate improved local cerebral blood flow (LCBF) in focal cerebral ischemic tissue after systemic administration of calcium channel blockers.11–14 Local application of the calcium channel blocker verapamil over the subarachnoid space does, however, produce a significant vasodilatory effect associated with a marked improvement of LCBF in the ischemic cerebral tissue induced by cerebral vasospasm in vivo.15,16

In acute stroke, the systemic and arterial routes often fail to deliver an adequate amount of promising cytoprotective agents because of advanced atherosclerotic processes in the cerebral arterial system or occlusion of the major supplying artery. Since the cerebral venous system has rich microcollateral channels17 and minimum atherosclerotic changes,18,19 we examined it as a possible therapeutic route for delivering cytoprotective agents into ischemic brain tissue. We recently established that the cerebral venous system of rats with focal cerebral ischemia can tolerate up to 150 mm Hg perfusion pressure without any change of blood–brain barrier (BBB) permeability or LCBF.20 However, we were unable to significantly improve LCBF by administering autologous arterial blood alone by transvenous perfusion of the brain (TVPOB).20 We compare the results of TVPOB with verapamil and those achieved through systemic administration of verapamil in the same ischemic rat model.
Materials and Methods

We used 24 adult male Sprague-Dawley rats weighing 320-400 g. In all rats, cerebral ischemia was produced by occlusion of the left middle cerebral artery (MCA), and 1 hour later TVPOB commenced. The rats were divided into four groups of six rats each. Group A (control) rats underwent surgical preparations for middle cerebral artery occlusion (MCAO) and cannulation into the cerebral vein, but no TVPOB was performed. The other three groups received 0.1 mg verapamil/kg/2 hr: Group B rats received verapamil intravenously, Group C rats received verapamil by TVPOB with autologous blood at 100 mm Hg perfusion pressure, and Group D rats received verapamil by TVPOB with autologous blood at 150 mm Hg perfusion pressure. All six Group A rats, three Group C rats, and three Group D rats were examined by simultaneous quantitative double-tracer autoradiography using 4-[18F]fluoroantipyrine ([18F]FAP) and [14C]a-aminoisobutyric acid ([14C]AIB) as tracers to measure LCBF and local BBB permeability, respectively. All six Group B rats, three Group C rats, and three Group D rats were examined by single-tracer autoradiography using [14C]iodoantipyrine ([14C]IAP) for the measurement of LCBF.

We prepared [18F]FAP (specific activity approximately 600 mCi/mmole) in the Medical Cyclotron Unit of the Montreal Neurological Institute. [14C]IAP (specific activity 55 mCi/mmole) and [14C]AIB (specific activity 55 mCi/mmole) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, Missouri). Verapamil HCl was obtained from Knoll Pharmaceuticals (Markham, Canada).

Details of the surgical preparation, anesthesia, medication, and monitoring of physiologic changes have been published. Briefly, under general anesthesia with 1.5-2.0% halothane, the femoral artery and veins were catheterized and a tracheostomy was performed. A small cranietomy was made at the inferior and posterior part of the squamosa bone. The inferior cerebral vein, anatomically comparable with Labbé’s vein in humans, collects venous blood from the centroparietotemporal regions. The midpoint of the main trunk of the inferior cerebral vein was cannulated backwards using a PE-10 polyethylene catheter connected to the conduit system, which consisted of four three-way stopcocks. The distal end of the conduit system was then connected to a polyvinyl chloride bottle of isotonic saline containing 5 units/ml heparin (Organon Canada Ltd., West Hill, Canada). To avoid blocking the catheter and conduit system, <0.1 ml of this heparinized saline was infused intermittently at <30 mm Hg perfusion pressure until the beginning of the autologous blood infusion.

Arterial blood (1 ml) was initially drawn from the femoral artery with a heparinized syringe during 3-4 minutes to avoid any changes in systemic blood pressure. This heparinized arterial blood was then injected into the catheter and the proximal portion of the conduit system through the second three-way stopcock. Autologous arterial blood was infused continuously into the inferior cerebral vein during TVPOB at 0.17 ml/min, 100 mm Hg perfusion pressure and 0.2 ml/min, 150 mm Hg perfusion pressure for Groups C and D, respectively. To replace the blood in the conduit system, the heparinized arterial blood was withdrawn 1 ml at a time at rates similar to those of TVPOB from the femoral artery. The infusion pressure was obtained by compressing the polyvinyl chloride bottle filled with saline using a mercurial sphygmomanometer (Tycos, Taylor Instrument Companies, Asheville, North Carolina) connected at the conduit system’s distal end and by gradually increasing the pressure in a stepwise manner from no pressure to the desired pressure. At each step, the pressure rose 20-30 mm Hg; approximately 5 minutes elapsed between steps. During the infusion, the catheter and conduit system were always filled with autologous blood that was replenished periodically according to the infusion schedule described above.

Forty micrograms of verapamil was diluted in 1 ml of 0.9% saline (40 μg/ml, pH 6.76). This diluted verapamil solution was connected to the conduit system’s proximal three-way stopcock and infused at a constant rate of 0.8 μg/kg/min using a Sage infusion pump (model 355, Orion Research, Inc., Boston, Massachusetts). Therefore, the infusion rate of TVPOB for Group D was 0.2 ml arterial blood+8 μl verapamil/min. The infusion pressure was constantly measured through the conduit system’s third-three way stopcock by the transducer mentioned previously. The pressure was maintained within 5 mm Hg using the high-pressure ranges of a blood pressure monitor (Sirecust 322, Siemens Energy and Automation, Cherry Hill, New Jersey) during TVPOB.

The left MCA was occluded proximal to the lateral striate branch, as described by Tamura et al. The general anesthesia was switched to 50 mg/kg i.m. ketamine and 8 mg/kg i.m. xylazine and maintained until the end of the experiment, when the rats were decapitated. The systemic administration of verapamil through the femoral vein (Group B) or by TVPOB (Groups C and D) was started 1 hour after MCAO and ended 2 hours later. Body temperature was kept at approximately 37°C with a heating lamp positioned over the rat. Systemic arterial blood pressure and blood gases were serially checked and maintained within physiologic ranges during the experiment.

A detailed description of the method for measuring LCBF and the local blood–brain transfer constant (Kr) using [18F]FAP and [14C]AIB, respectively, has been published. Briefly, the autoradiographic method is based on the difference in the physical half-lives of the two tracers (T½ for 18F 110 minutes, E½ 240 keV; T½ for 14C 5730 years, E½ 45 keV).
LCBF was measured by the double- or single-tracer technique, using $[^{18}F]FAP$ or $[^{14}C]IAP$,23 2 hours and 59 minutes after MCAO. To measure LCBF, 3–5 mCi of $[^{18}F]FAP$ (for double-tracer autoradiography) or 30 µCi of $[^{14}C]IAP$ (for single-tracer autoradiography) was injected over 1 minute. LCBF was calculated using the operational equation described by Sakurada et al.25 The tissue–blood partition coefficients of 0.89 and 0.80 were used for $[^{18}F]FAP$ and $[^{14}C]IAP$, respectively.

$K_{t}$ was measured using the $[^{14}C]IAP$ autoradiographic method developed by Blasberg et al.23,24 Thirty microcuries of $[^{14}C]IAP$ in 1 ml normal saline was injected intravenously 2 hours and 30 minutes after MCAO. Arterial blood (50 µl) was drawn 0.25, 0.5, 1, 2, 3, 5, 7.5, 10, 15, 20, 25, and 30 minutes after injection of $[^{14}C]IAP$ and was immediately centri-
fuged. Twenty microliters of plasma was then pipetted from each sample into counting vials. Rats were decapitated 30 minutes after $[^{14}C]IAP$ injection. Carbon-14 radioactivity was measured 3 days (39 half-lives of fluorine-18) later for double-tracer autoradiography using a liquid scintillation counter (1219 Rackbeta Liquid Scintillation Counter, Wallac Oy, Turku, Finland). $K_{t}$ for each locus was calculated from the ratio between the tissue carbon-14 radioactivity [Ci(T):nCi/g] at the end of the experiment and the time integral of the arterial plasma carbon-14 radioactivity from the beginning to the end of the experiment as $K_{t}=[Ci(T)]:\int_{0}^{t}C_{a}dt$, where $T$ is the duration of the experiment in minutes.

The optical density was measured six times in each of three consecutive tissue autoradiograms, and the mean value of the tissue fluorine-18 and carbon-

### Table 1. Mean Arterial Blood Pressure and Blood Gases in Rats During Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control (n=6)</th>
<th>Intravenous verapamil (n=6)</th>
<th>TVPOB with verapamil</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg)</td>
<td>117±8</td>
<td>114±10</td>
<td>109±6</td>
</tr>
<tr>
<td>$Paco_2$ (torr)</td>
<td>38.7±2.4</td>
<td>39.5±3.1</td>
<td>40.0±3.3</td>
</tr>
<tr>
<td>$Paco_2$ (torr)</td>
<td>105±8</td>
<td>103±9</td>
<td>106±7</td>
</tr>
<tr>
<td>pH</td>
<td>7.42±0.05</td>
<td>7.40±0.06</td>
<td>7.39±0.05</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>48±2</td>
<td>48±3</td>
<td>47±2</td>
</tr>
</tbody>
</table>

Values are mean±SD. TVPOB, transvenous perfusion of the brain; MABP, mean arterial blood pressure; Hct, hematocrit. 0.1 mg verapamil/kg/2 hr.

### Table 2. Effect of TVPOB With 0.1 mg Verapamil/kg/2 hr on Local Cerebral Blood Flow in Rats During Left Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Structures</th>
<th>Control (n=6)</th>
<th>Intravenous verapamil (n=6)</th>
<th>TVPOB with verapamil*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>34±21</td>
<td>137±29</td>
<td>45±14</td>
</tr>
<tr>
<td>Sensorimotor cortex</td>
<td>28±11</td>
<td>139±31</td>
<td>46±16*</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>27±12</td>
<td>148±15</td>
<td>51±12†</td>
</tr>
<tr>
<td>Auditory cortex</td>
<td>25±6</td>
<td>155±32</td>
<td>40±26</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>49±23</td>
<td>141±41</td>
<td>63±20</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>79±16</td>
<td>92±10</td>
<td>84±21</td>
</tr>
<tr>
<td>Amygdala</td>
<td>69±18</td>
<td>94±17</td>
<td>73±21</td>
</tr>
<tr>
<td>Caudate</td>
<td>21±4</td>
<td>139±27</td>
<td>20±9</td>
</tr>
<tr>
<td>Lateral</td>
<td>64±32</td>
<td>134±24</td>
<td>79±25</td>
</tr>
<tr>
<td>Medial</td>
<td>75±22</td>
<td>128±28</td>
<td>105±32</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>73±20</td>
<td>80±19</td>
<td>77±11</td>
</tr>
<tr>
<td>Thalamus</td>
<td>104±26</td>
<td>146±16</td>
<td>96±24</td>
</tr>
<tr>
<td>Lateral</td>
<td>106±18</td>
<td>149±22</td>
<td>103±17</td>
</tr>
<tr>
<td>Medial</td>
<td>79±16</td>
<td>97±21</td>
<td>76±12</td>
</tr>
<tr>
<td>Hypothalamus</td>
<td>76±27</td>
<td>92±10</td>
<td>96±19</td>
</tr>
</tbody>
</table>

Values are mean±SD; ml/100 g/min. TVPOB, transvenous perfusion of the brain.

*tp<0.05, 0.01, respectively, different from control group by two-tailed unpaired t test.
FIGURE 1. Autoradiograms of [14C]fluoroantipyrine (or [14C]iodoantipyrine) from coronal section of frontal region (A, D, and G), sensorimotor cortex (B, E, and H), and thalamus/parietal cortex regions (C, F, and I) showing decrease of local cerebral blood flow (LCBF) in left insular and perirhinal cortices subjacent to operative site in all rats. Group B (intravenous administration of verapamil, D–F) showed no significant changes of LCBF compared with Group A (control, A–C). Group D (transvenous perfusion of the brain with verapamil at 150 mm Hg, G–I) showed extensive increase of LCBF in ipsilateral frontal, sensorimotor, and parietal cortices.

Results

Blood pressure, blood gases, and hematocrit of rats in the four groups did not differ during MCAO regardless of verapamil administration (Table 1). LCBF in the four groups are summarized in Table 2 and depicted in Figure 1. In all rats, LCBF was reduced in the left insular and perirhinal cortices over the surgical site. Compared with Group A, Group C showed a significant increase of LCBF in the parietal (89%, \( p<0.01 \)) and sensorimotor cortices (64%, \( p<0.05 \)) (Table 2; Figure 1, A–C; Figure 2). Group D showed extensive and significant increases of LCBF in the ischemic cortical areas (sensorimotor cortex: 86%, \( p<0.05 \); parietal cortex: 106%, \( p<0.05 \); auditory cortex: 68%, \( p<0.05 \)) as well as in the subcortical area (posterior lateral portion of the caudoputamen: 57%, \( p<0.01 \), Table 2; Figure 1, G–I; Figure 2). In Groups C and D, we observed no significant LCBF increases in the regions supplied by the deep branches of the internal carotid artery or by the anterior or posterior cerebral arteries, such as the occipital cortex, hippocampus, amygdala, thalamus, hypothalamus, and dentate (Table 2). LCBF also did not change significantly in the contralateral nonischemic cerebral regions in Groups C and D. However, Group B showed a slight, nonsignificant, further LCBF reduction in the ischemic cerebral cortex associated with a slight LCBF increase in the contralateral cerebral hemisphere compared with Group A (Table 2; Figure 1, D–F; Figure 2).

The only increase in \( K_t \) was confined to the small focal area around the left rhinal fissure over the surgical site in Groups A, C, and D due to the surgical artifact. However, there were no changes in \( K_t \) in the distribution of the occluded MCA, in the ipsilateral normal cortex, or in the contralateral hemisphere. This was also the case in Group B.

Discussion

Verapamil, a so-called slow-channel Ca\(^{2+}\) blocking agent, has a vasodilating effect, particularly on small, resistant vessels. \(^{29}\) Verapamil is rapidly metabolized in vivo; labeling it with carbon-14 has facili-
hated the study of its metabolic decomposition in human\textsuperscript{30,31} and animals.\textsuperscript{32} The half-life of verapamil in plasma is $<$20 minutes. Its binding to plasma proteins, approximately 90\%, would reduce substantially its pharmacologic activity.\textsuperscript{31,32} Therefore, verapamil has been considered to be a first-pass metabolic agent, the unique pharmacologic property of which makes it a most suitable drug for TVPOB. In locally perfused tissue, verapamil has minimum systemic side effects. Most investigators\textsuperscript{11,12,14} have failed to increase cerebral blood flow after an ischemic insult by the systemic administration of verapamil in spite of long-term continuous infusion. However, local application of verapamil produced vasodilatation in previously constricted artery in vitro\textsuperscript{13,33} and in vivo.\textsuperscript{4,15,16}

We first confirmed that TVPOB with 0.1 mg verapamil/kg at perfusion pressures of 100 and 150 mm Hg did not alter BBB permeability as measured by quantitative double-tracer autoradiography. We then studied the effects of verapamil administered by TVPOB at perfusion pressures of 100 and 150 mm Hg on LCBF. We compared results of this procedure with results following systemic administration of verapamil in the same ischemic rat model. Intravenous systemic administration of 0.1 mg verapamil/kg/2 hr resulted in a slight further reduction of LCBF in the ischemic cerebral hemisphere, associated with slightly increased LCBF in the nonischemic contralateral cerebral hemisphere compared with the control group (Table 2). Roy et al\textsuperscript{12} made similar observations in cats and reported that continuous intravenous systemic administration of 0.1 $\mu$g verapamil/kg/min for 3–24 hours was associated with worsening of LCBF in the ischemic regions and inappropriate increases in regional cerebral blood flow in the nonischemic regions in the MCAO model. Even high doses of verapamil (0.7 mg/kg/30 min) given by intravenous infusion for prolonged periods did not improve functional neurologic recovery in pigs and baboons with focal cerebral ischemia.\textsuperscript{34}

In the nonischemic regions, a relatively low dose of verapamil (0.1 mg/kg/2 hr) did not produce any significant changes in systemic blood pressure or LCBF. Although we administered a relatively low dose of verapamil and autologous arterial blood by TVPOB, a perfusion pressure of 100 mm Hg caused a significant increase of LCBF in the ipsilateral, pial, and sensorimotor cortices. Furthermore, the administration of verapamil and autologous arterial blood by TVPOB at a perfusion pressure of 150 mm Hg caused an extensive and significant increase of LCBF not only in the ischemic cortical areas, but also in the subcortical area in the territory of the MCA. LCBF did not change significantly in the nonischemic contralateral cerebral hemisphere.

TVPOB has made it possible to deliver vasoactive and cytoprotective agents more selectively and efficiently into focally ischemic cerebral tissues with minimum systemic side effects. We are investigating this new therapeutic approach for treating acute focal cerebral ischemia further.

**Acknowledgments**

The authors would like to thank the members of the Medical Cyclotron Unit for preparation of $[^{18}F]$FAP, Mrs. Janet Arts for technical assistance, and Miss F. Lumia for secretarial help.

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Key Words • autoradiography • calcium channel blockers • cerebral blood flow • rats
Transvenous perfusion of the brain with verapamil during focal cerebral ischemia in rats.
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Stroke. 1989;20:501-506
doi: 10.1161/01.STR.20.4.501
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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