Nimodipine Posttreatment Does Not Increase Blood Flow in Rats With Focal Cortical Ischemia

Ulrich Dirnagl, MD, PhD, Michael Jacewicz, MD, and William Pulsinelli, MD, PhD

We used laser-Doppler flowmetry to study the effect of nimodipine administered after the onset of focal cortical ischemia on regional cerebral blood flow in 16 halothane-anesthetized, mechanically ventilated Wistar rats. We selected the Wistar rat strain since it would provide a wide range of ischemia severities to test the vascular response to nimodipine. Laser-Doppler probes continuously recorded regional cerebral blood flow at two or three sites over the parietal cortex (dura intact) while brain temperature was regulated at 37° C. Occlusion of the right middle cerebral and common carotid arteries reduced cerebral blood flow to a mean of 38% (range 13–77%) of baseline. Thirty minutes later, either 2 μg/kg/min nimodipine (n=8) or its vehicle, polyethylene glycol 400 (n=8), was administered by a continuous intravenous infusion. Over 60 minutes of treatment, both the nimodipine-treated and vehicle-treated groups showed a trivial (3%) mean increase in cerebral blood flow. Nimodipine failed to augment cerebral blood flow regardless of whether the cortex was severely, moderately, or mildly ischemic. (Stroke 1990;21:1357–1361)

When administered before the onset of focal cerebral ischemia, nimodipine attenuates the degree and extent of ischemia, tissue acidosis, and infarction.1–3 Started after the onset of ischemia, the efficacy of nimodipine treatment becomes uncertain, with some studies4–7 showing benefit while others8–10 do not. The reasons for the divergent results are unclear. Since different stroke models involve different ischemic insults, one possibility is that nimodipine elicits different cerebral responses when ischemic insults vary in severity and duration, as has been shown for nimodipine binding.11 Because of its role as a potent vasodilator, nimodipine also carries the risk that blood may be shunted from regions that are reversibly injured (i.e., the “ischemic penumbra”) to adjacent nonischemic regions. Alternatively, marginally perfused brain regions may become more ischemic due to nimodipine-induced hypotension as other regions demonstrate improved blood flow.

Materials and Methods

We anesthetized 16 fasted male Wistar rats (240–300 g) with 1–2% halothane in a mixture of 70% N₂ and 30% O₂. The right femoral artery was cannulated for recording systemic arterial blood pressure (BP) and serial sampling of blood for determining PaO₂, PaCO₂, arterial pH, and blood glucose concentration, and the right femoral vein was cannulated for the intravenous administration of drug or vehicle. The rats were tracheotomized and mechanically ventilated, and mean±SD body temperature was controlled at 37.5±0.5° C.

Via a right subtemporal exposure, two burr holes, 2 mm in diameter, were drilled, one 8 mm lateral and 2 mm anterior to the bregma, and the other 8 mm lateral and 8 mm posterior to the bregma, without rupturing the dura. A gentle normal saline drip (25° C) during drilling prevented thermal injury to the underlying cortex. Two or three small-caliber LDF probes (TSI Inc., St. Paul, Minn.) were gently
placed using Narishige micromanipulators (Green-va, N.Y.) under x40 magnification over relatively avascular areas of the parietal cortex. To maintain a clear optical medium through the translucent dura and to keep brain temperature at 37° C, the probe sites were continuously bathed with a gentle stream of warmed (38° C) normal saline. Serial recordings of regional cerebral blood flow (rCBF) using a TSI model P433 monitor began 10 minutes before occlusion of the right MCA and common carotid artery (CCA) and continued throughout the experiment.

The principles and technical details of LDF have been described elsewhere. 13,14 This method permits on-line measurement of changes in rCBF (as percentage of baseline) in approximately 1 mm3 of cortex without disrupting brain tissue (unlike the H2 clearance technique) and without requiring multiple infusions of tracer (unlike the umbelliferone clearance technique). Correlations between LDF and standard rCBF measurement techniques have been excellent. 15-18 In a validation study, 19 we used quantitative 14Ciodoantipyrine autoradiography to demonstrate that the current LDF method accurately measures relative rCBF changes in tissue that is severely, moderately, or mildly ischemic.

Preparatory surgery and LDF probe placement required approximately 40 minutes to complete. When BP and baseline rCBF readings were stable (after approximately 5 minutes), the right MCA and CCA were occluded using the method described by Brint et al. 12 The concentration of halothane was then reduced to 0.5% to minimize respiratory acidosis and hypotension associated with anesthesia. Ischemia severity was classified as severe (rCBF <25% of baseline), moderate (rCBF 25-50% of baseline), or mild (rCBF 50-77% of baseline). Thirty minutes after occlusion, an intravenous infusion of 2 μg/kg/min nimodipine (n=8) or its vehicle, polyethylene glycol 400 (n=8), was initiated in rats randomly assigned to a treatment group, treatment continued for 60 minutes. We used the Student’s t test to compare physiologic variables between the nimodipine-treated and vehicle-treated groups. We compared changes in rCBF during treatment across the three ischemia severities using analysis of variance. The relation between changes in BP and changes in rCBF during treatment was analyzed using linear correlation.

**Results**

Except for mild hypotension that evolved over 60 minutes in the nimodipine-treated group, physiologic variables did not differ between groups (Table 1).

Occlusion of the right MCA and CCA immediately decreased rCBF, resulting in a wide range of ischemia severities (Table 2). Between occlusion and the start of treatment there was no further change in rCBF (Table 2). Immediately before treatment was started, rCBF ranged from 16% to 59% of baseline over 23 recording sites in the eight vehicle-treated rats and from 13% to 77% over 22 sites in the eight rats randomly selected to receive nimodipine. Nimodipine had little effect on rCBF. Over 60 minutes of treatment, mean±SD rCBF in the nimodipine-treated group rose from 39.5±16.2% to 42.6±17.3% of baseline values, but the vehicle-treated group showed a similar small rise from 37.3±11.9% to 40.6±13.0%.

Even when the rats in each group were classified by ischemia severity, there was no significant change in rCBF in any class (Table 3). The mild hypotension induced by nimodipine did not aggravate the ischemic

### TABLE 1. Physiologic Variables in 16 Wistar Rats Subjected to Focal Cortical Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Baseline</th>
<th>Start of treatment</th>
<th>End of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle</td>
<td>Nimodipine</td>
<td>Vehicle</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>88±4.8</td>
<td>92±10.5</td>
<td>89±7.4</td>
</tr>
<tr>
<td>PacO2 (mm Hg)</td>
<td>35±3.4</td>
<td>36±3.4</td>
<td>35±2.8</td>
</tr>
<tr>
<td>PaCO2 (mm Hg)</td>
<td>87±7.6</td>
<td>96±15.6</td>
<td>87±7.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.38±0.03</td>
<td>7.36±0.03</td>
<td>7.40±0.03</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>42±3.1</td>
<td>43±3.4</td>
<td>...</td>
</tr>
<tr>
<td>Blood glucose (mg/dl)</td>
<td>114±14.1</td>
<td>120±17.5</td>
<td>...</td>
</tr>
</tbody>
</table>

Data are mean±SD; n=8 for each treatment group. *p<0.05 different from vehicle-treated group by Student’s t test.

### TABLE 2. Regional Cerebral Blood Flow in 16 Wistar Rats Subjected to Focal Cortical Ischemia

<table>
<thead>
<tr>
<th>Variable</th>
<th>Vehicle-treated (n=8)</th>
<th>Nimodipine-treated (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD Range</td>
<td>Mean±SD Range</td>
</tr>
<tr>
<td>Recording sites</td>
<td>23 22</td>
<td>22 20</td>
</tr>
<tr>
<td>Baseline</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>After occlusion</td>
<td>38.9±10.6% 14-59%</td>
<td>40.7±15.3% 15-78%</td>
</tr>
<tr>
<td>Before treatment</td>
<td>37.3±11.9% 16-59%</td>
<td>39.5±16.2% 13-77%</td>
</tr>
<tr>
<td>After treatment</td>
<td>40.6±13.0% 16-67%</td>
<td>42.6±17.3% 18-87%</td>
</tr>
<tr>
<td>Change during treatment</td>
<td>3.3±7.28% -8.6-19%</td>
<td>3.1±6.89% -12-14%</td>
</tr>
</tbody>
</table>
insult. There was no significant correlation between individual rCBF changes and BP changes (data not shown) in the three classes (severe ischemia, $r=0.06$; moderate ischemia, $r=0.08$; mild ischemia, $r=-0.43$).

**Discussion**

Nimodipine administered 30 minutes after the onset of focal cortical ischemia did not alter the brain microcirculation in severely, moderately, or mildly ischemic cortex during 60 minutes of treatment. After the onset of focal ischemia, rCBF showed only minor fluctuations without evidence of either local shunting of blood flow or hypoperfusion aggravated by nimodipine’s hypotensive effect. These results suggest that, while deleterious cerebrovascular effects are not observed, posttreatment with nimodipine provides no significant hemodynamic benefit to the ischemic brain of halothane-anesthetized rats.

The reasons for a lack of a cerebrovascular effect with nimodipine posttreatment are not clear. One possible explanation is that nimodipine did not reach the cortex in concentrations adequate to effect a change. The nimodipine dosage we used, however, was twice that found to be maximally effective in halothane-anesthetized rabbits, which suggests that nimodipine does not provide adequate concentrations of drug. Another possible explanation is that the ischemic insult was too severe for any therapy to be effective. However, pretreatment with the same dosage of nimodipine in spontaneously hypertensive and Wistar rats subjected to MCA and CCA occlusion has been shown to improve rCBF to the ischemic vascular bed, to reduce edema, and to decrease the volume of cortical infarction.

Anesthesia can substantially modify the effects of therapeutic drugs (e.g., MK-801) on BP, rCBF, and cerebral metabolism, and halothane, a known vasodilator, may have masked the vasodilatory action of nimodipine. Because it can accentuate the hypotensive effect of nimodipine, halothane has the potential to further reduce cerebral perfusion in ischemic regions that have lost autoregulation and thereby offset any rCBF increase due to nimodipine. Although the latter possibility cannot be excluded, it seems unlikely because 0.5% halothane in a 70%-30% N₂O-O₂ mixture does not prevent cortical blood flow increases after treatment with 1-4 μg/kg/min nimodipine in normal rats or in rats pretreated with 1 μg/kg/min nimodipine and subjected to MCA occlusion.

The 60-minute treatment period may have been too short for us to observe an increase in rCBF. However, Meyer et al. reported a nimodipine-mediated increase in rCBF within 30 minutes of therapy started 20 minutes after MCA branch occlusion in anesthetized rabbits. Mohamed et al. also found increased rCBF when 1 μg/kg/min nimodipine was administered for 1 hour beginning 30 minutes before MCA occlusion in anesthetized rats. In contrast, with longer treatment intervals (90 minutes, 4 hours), administration of 0.5 μg/kg/min nimodipine beginning 15 minutes after the onset of ischemia did not increase rCBF in rats recovering from halothane anesthesia.

Other studies involving nimodipine posttreatment have yielded conflicting data. Nimodipine increased rCBF in the ischemic cortex when given 20 minutes after MCA branch occlusion in anesthetized rabbits, but had no effect on rCBF when administered 5 minutes after MCA occlusion in anesthetized rats or 15 minutes after MCA occlusion in rats recovering from anesthesia. These discrepancies are not easily explained, but they could involve species and strain differences in vascular responsiveness to...
low doses of nimodipine,29 variations in the degree and duration of ischemia, the type and depth of anesthesia,30 the mode of drug delivery, associated hypotension,28 an open-versus a closed-skull preparation,25 use of low-molecular-weight dextran,32 and other factors unique to each paradigm.

The absence of improved rCBF with nimodipine posttreatment does not preclude a therapeutic effect by other mechanisms. Nimodipine can directly antagonize calcium entry into neurons via voltage-sensitive channels23,24 and can act as an anticonvulsant.35,36 In support of this view, Berger and Hakim28 have found that nimodipine treatment can ameliorate cerebral lactic acidosis without increasing rCBF after MCA occlusion in rats. Whether this results in long-term histologic protection, however, is less clear. Germano et al6 have reported reduced infarct size in their posttreated rats, but other studies8–10 have not found such benefit. The question of the efficacy of nimodipine treatment started after the onset of ischemia remains unresolved.

References


KEY WORDS • cerebral blood flow • cerebral ischemia • nimodipine • rats
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