Failure of Nimodipine to Prevent Ischemic Neuronal Damage in Rats

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The efficacy of nimodipine in preventing ischemic brain injury was tested in rats subjected to a 20-minute period of high-grade forebrain ischemia by 4-vessel occlusion. Three minutes after restoration of circulation to the brain, an intravenous bolus of 5 μg/kg nimodipine or an equivalent amount of vehicle or saline was given, followed by continuous intravenous infusion of the respective solution at 1 μg/kg/min for 2 hours. In a second series, a larger bolus (20 μg/kg of nimodipine) and longer infusion period (6 hours) were employed. Histopathology of the brain was evaluated blindly 72 hours later and graded on a conventional 3-point scale. There was no significant effect of treatment in either series. In the 6-hour series, the percent of cerebral hemispheres showing damage of Grades 2 or 3 in zone CA1 of the hippocampus and in the striatum, respectively, was 100 and 40% for the nimodipine-treated rats, 100 and 42% for rats receiving vehicle, and 75 and 25% for animals receiving saline. Thus, this study revealed no beneficial effect of nimodipine when given following a 20-minute period of severe forebrain ischemia. (Stroke 1987;18:210–216)

Materials and Methods

Twenty-seven fasted male Wistar rats weighing 250–350 g were used. The operative procedures were carried out in two stages. In the first stage, anesthesia was induced with 3% halothane and maintained with 1.5% halothane, 70% nitrous oxide, and the balance oxygen. The vertebral arteries were electrocoagulated bilaterally through the alar foramina of the first cervical vertebra. Animals were allowed to recover. The following day, under the same anesthesia, the common carotid arteries were exposed bilaterally, and ligatures made from loops of polyethylene tubing (PE-10) in dual bore Silastic tubing were placed loosely around the arteries. Femoral arteries and veins were cannulated for blood pressure monitoring and arterial blood sampling. Wounds were then infiltrated with 1% lidocaine and closed. The animals were then constrained in a plaster body cast and allowed to recover from the effects of anesthesia for 1 hour prior to induction of ischemia. Arterial blood gases and plasma glucose were sampled at multiple time points. Body temperature was maintained at 37°C with a heating lamp throughout the experiment.

Induction of Cerebral Ischemia

Global cerebral ischemia was induced by tightening the common carotid ligatures bilaterally and was maintained for 20 minutes. Successful ischemia was signaled by drooping of the head, loss of tone in the tail, and diminished responsiveness to tail pinch. Non-comatose rats and animals exhibiting seizures were discarded.

Circulation was restored following ischemia by release of the carotid ligatures. The carotid arteries were inspected to assure patency. Three minutes later, nimodipine was administered. The drug was dissolved in a vehicle of 200 g 96% ethanol, 170 g polyethylene glycol 400, 2 g sodium citrate, and 0.5 g citric acid in 1 liter of water. The drug was prepared fresh each day under sodium light, and syringes and catheters were...
shielded from light with aluminum foil. Two treatment series were employed. In the first series, animals received an initial i.v. bolus of 5 μg/kg of nimodipine followed by a continuous i.v. infusion of 1 μg/kg/min for 2 hours. Vehicle- and saline-treated animals received comparable volumes. In the second series, a larger bolus of nimodipine (20 μg/kg) and a longer duration of infusion (6 hours) were employed. The remainder of the experimental conditions were the same as in the first series.

Following the treatment period, rats were briefly reanesthetized with halothane for removal of body casts and catheters and closing of all incisions. Rats were returned to their cages and given free access to food and water. Seventy-two hours later, brains were fixed by transcardiac perfusion with a mixture of formaldehyde-glacial acetic acid-methanol as previously described. Heads were immersed in the fixative overnight prior to brain removal.

### Histological Evaluation

Brains were embedded in paraffin, and 6-μm coronal sections of the forebrain were prepared and stained with hematoxylin and eosin. Microscopic sections of each animal were analyzed by 2 separate observers who were blinded to the experimental conditions. At the coronal levels of the anterior commissure (striatum, frontal cortex) and of the dorsal hippocampus (thalamus, parietal cortex), ischemic cell change was graded in multiple structures on a semiquantitative scale: 0 = normal; 1 = few affected neurons; 2 = many affected neurons; 3 = all neurons affected. When different degrees of histological change coexisted within various subregions of a given structure, the assigned grade denoted the most severe degree of injury present. A high degree of concordance of grading was observed between the two examiners, so that their grades were subsequently combined for the presentation below.

### Statistical Analysis

Physiological variables were assessed by analysis of variance. Histopathological scores for the 3 treatment groups were compared by the Mann-Whitney U test.

### Results

The physiological variables are presented in Table 1. Prior to and during ischemia, there were no significant differences in these variables among the treatment groups in either series. Thus, they were combined. There was no significant change in postischemic mean blood pressure (MAP) from the preischemic state in saline-treated rats. In the first series, nimodipine reduced MAP from a preischemic value of 123 ± 6 (mean ± SD) to 102 ± 4 mm Hg at 2 hours — a significant reduction of 18%. Nimodipine also reduced MAP in the second series, by 16% at 6 hours postischemia. Vehicle-treated animals of the second series showed a significant MAP reduction of 12% at 6 hours as well. Nonetheless, MAP at 6 hours in the vehicle-treated rats was significantly higher than in the nimodipine-treated rats. Mean preischemic plasma glucose in the 2 series was 126 ± 31 and 118 ± 18 mg/dl, respectively. Plasma glucose levels rose in all groups during ischemia and declined postischemia, but there were no significant differences among treatment groups.

The histopathological findings in the series are summarized in Tables 2 and 3 and Figure 1. In the 2-hour series, the percent of cerebral hemispheres showing Grade 2 or 3 ischemic damage in zone CA1 of the

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### Table 1. Mean Arterial Pressure (MAP), Arterial Blood Gasses, and Plasma Glucose

<table>
<thead>
<tr>
<th>State</th>
<th>Treatment group</th>
<th>n</th>
<th>MAP (mm Hg)</th>
<th>P&lt;sub&gt;CO2&lt;/sub&gt; (mm Hg)</th>
<th>P&lt;sub&gt;O2&lt;/sub&gt; (mm Hg)</th>
<th>pH</th>
<th>Plasma glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preischemia</td>
<td>Series 1*</td>
<td>12</td>
<td>123 ± 6</td>
<td>93 ± 7</td>
<td>39 ± 2</td>
<td>7.41 ± 0.02</td>
<td>126 ± 31</td>
</tr>
<tr>
<td></td>
<td>Series 2</td>
<td>15</td>
<td>125 ± 9</td>
<td>84 ± 5</td>
<td>36 ± 1</td>
<td>7.40 ± 0.04</td>
<td>118 ± 18</td>
</tr>
<tr>
<td>Ischemia†</td>
<td>Series 1</td>
<td>1</td>
<td>145 ± 17</td>
<td>113 ± 17</td>
<td>21 ± 4</td>
<td>7.49 ± 0.05</td>
<td>178 ± 37</td>
</tr>
<tr>
<td></td>
<td>Series 2</td>
<td>15</td>
<td>142 ± 12</td>
<td>101 ± 9</td>
<td>22 ± 2</td>
<td>7.51 ± 0.04</td>
<td>170 ± 26</td>
</tr>
<tr>
<td>Postischemia‡</td>
<td>Series 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>3</td>
<td>112 ± 3</td>
<td>93 ± 4</td>
<td>40 ± 2</td>
<td>7.39 ± 0.01</td>
<td>148 ± 31</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>3</td>
<td>120 ± 10</td>
<td>97 ± 7</td>
<td>38 ± 4</td>
<td>7.40 ± 0.00</td>
<td>147 ± 49</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>6</td>
<td>102 ± 4</td>
<td>91 ± 7</td>
<td>35 ± 4</td>
<td>7.39 ± 0.01</td>
<td>162 ± 28</td>
</tr>
<tr>
<td></td>
<td>Series 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>4</td>
<td>110 ± 4</td>
<td>91 ± 1</td>
<td>34 ± 0</td>
<td>7.43 ± 0.01</td>
<td>130 ± 28</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>6</td>
<td>115 ± 5</td>
<td>89 ± 2</td>
<td>32 ± 4</td>
<td>7.38 ± 0.04</td>
<td>119 ± 19</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>5</td>
<td>103 ± 7</td>
<td>86 ± 3</td>
<td>34 ± 3</td>
<td>7.41 ± 0.03</td>
<td>97 ± 26</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

*Series 1 = Rats given 5 μg/kg i.v. bolus of nimodipine or equivalent amount of vehicle or saline followed by 1 μg/kg/min infusion for 2 hours. Series 2 = Rats given 20 μg/kg i.v. bolus followed by 1 μg/kg/min infusion for 6 hours.
†Parameters at 15 minutes during ischemia.
‡Parameters at 2 hours postischemia in Series 1 and at 6 hours in Series 2.
hippocampus and in the striatum, respectively, was 67 and 50% for nimodipine-treated rats, 100 and 33% for vehicle-treated rats, and 84 and 50% for saline-treated rats. The respective figures in the 6-hour series were 100 and 40%, 100 and 42%, and 75 and 25%. There was no beneficial effect of nimodipine treatment in saline- or vehicle-treated animals; this change was statistically demonstrable with therapeutic effects occurring. 

**Table 2. Histological Findings in the 2-Hour Infusion Series**

<table>
<thead>
<tr>
<th>Region</th>
<th>Treatment group</th>
<th>Grade of damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>Saline</td>
<td>17 50 33 0</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>67 33 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>50 17 17 17</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>Saline</td>
<td>0 33 67 0</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>67 33 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>42 8 17 33</td>
</tr>
<tr>
<td>Striatum</td>
<td>Saline</td>
<td>17 33 17 33</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>67 0 33 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>33 17 17 33</td>
</tr>
<tr>
<td>CA1</td>
<td>Saline</td>
<td>0 17 17 67</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Vehicle</td>
<td>0 0 67 33</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>25 8 0 67</td>
</tr>
<tr>
<td>CA3,4</td>
<td>Saline</td>
<td>33 33 33 0</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Vehicle</td>
<td>100 0 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>50 50 0 0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Saline</td>
<td>33 67 0 0</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>100 0 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>83 0 17 0</td>
</tr>
</tbody>
</table>

*Data are given as % of hemispheres with Grades 0–3 damage.*

**Discussion**

The results of this study indicate that nimodipine, administered postischemia, fails to prevent ischemic neuronal damage. We chose the 4-vessel occlusion model of global ischemia in rats for its predictable ischemic neuronal changes in various forebrain regions and the advantage of induction of ischemia in awake animals. Previous blood flow studies during 4-vessel occlusion in awake, nonhypotensive Wistar rats by ourselves and others have documented high-grade flow reductions within the neocortex, striatum, and hippocampus (below 3% of control, and 1.3–6.5% of control); these findings have been confirmed in recent autoradiographic studies from our laboratory (M. Globus et al, unpublished observations). Our control animals demonstrated a pattern of neuronal damage following 20 minutes of ischemia similar to that reported by Pulmonelli and colleagues. We chose a 20-minute period of ischemia, which produces a consistently detectable degree of ischemic neuronal injury in all animals while avoiding the more severe changes associated with longer (viz., 30-minute) ischemic periods. Histological injury produced by ischemic periods shorter than 20 minutes is highly variable, making statistical demonstration of therapeutic effects difficult.

Nimodipine was given in an effort to retard ischemia-induced entry of calcium ions into neurons and thereby to reduce the extent of ischemic neuronal injury. Postischemic administration was chosen to make this study relevant to the human post-cardiac-arrest setting. In contrast, in most previous studies, the agent was administered prior to ischemia. Improved

**Table 3. Histological Findings in the 6-Hour Infusion Series**

<table>
<thead>
<tr>
<th>Region</th>
<th>Treatment group</th>
<th>Grade of damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frontal cortex</td>
<td>Saline</td>
<td>25 50 0 25</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>33 67 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>20 50 30 0</td>
</tr>
<tr>
<td>Parietal cortex</td>
<td>Saline</td>
<td>25 50 0 25</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>8 83 8 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>0 50 50 0</td>
</tr>
<tr>
<td>Striatum</td>
<td>Saline</td>
<td>25 50 0 25</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>0 58 42 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>30 30 20 20</td>
</tr>
<tr>
<td>CA1</td>
<td>Saline</td>
<td>0 25 0 75</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Vehicle</td>
<td>0 0 0 100</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>0 0 0 100</td>
</tr>
<tr>
<td>CA3,4</td>
<td>Saline</td>
<td>50 50 25 0</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>Vehicle</td>
<td>50 50 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>60 40 0 0</td>
</tr>
<tr>
<td>Thalamus</td>
<td>Saline</td>
<td>25 50 0 25</td>
</tr>
<tr>
<td></td>
<td>Vehicle</td>
<td>83 17 0 0</td>
</tr>
<tr>
<td></td>
<td>Nimodipine</td>
<td>50 20 20 10</td>
</tr>
</tbody>
</table>

*Data are given as % of hemispheres with Grades 0–3 damage.*

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EEG and increased survival were noted in cats after a 7-minute period of total ischemia induced by tourniquet occlusion of neck vessels. Nimodipine also improved functional outcome in dogs when given prior to a 10-minute period of temporary ligation of the aorta and vena cava. In a study of middle cerebral artery occlusion in baboons, however, nimodipine appeared to increase the susceptibility of tissue to ischemic damage. In rats pretreated with nimodipine (100 μg/kg) by i.v. infusion 15 minutes before the induction of a 15-minute period of forebrain ischemia by carotid occlusion and hemorrhagic hypotension, postischemic hypoperfusion was ameliorated but recovery of sensory evoked response and EEG activity was not enhanced. Nimodipine administered before or after ischemia at 1 μg/kg/min also failed to improve other histopathological changes of the spinal cord or functional deficit caused by temporary aortic occlusion in unanesthetized rabbits. Steen et al found that in dogs subjected to 10 minutes of complete cerebral ischemia by temporary ligation of the aorta and vena cava, nimodipine given postischemia at 1 μg/kg/min for 2 hours increased CBF twofold but did not unequivocally improve neurologic outcome.

Other experimental studies have employed different calcium antagonists, e.g., nifedipine and flunarizine, in several animal models with varying results. In one histopathologic study, flunarizine was given orally to rats before a combined hypoxic–ischemic insult (Levine preparation); the extent of ischemic cell change in the cortex was significantly reduced at 24 hours. In the normal state, intracellular calcium is maintained at 10⁻⁷ M despite extracellular calcium concentrations of 10⁻³ M by two major pump mechanisms: the Na⁺-Ca⁺⁺ exchange system energized by the Na⁺, K⁺-ATPase pump, and the Ca⁺⁺-ATPase system. Intracellular calcium is sequestered by both mitochondria and smooth endoplasmic reticulum. In ischemia, ATP depletion leads to depolarization of membranes and to influx of both Ca⁺⁺ and Na⁺. Cessation of mitochondrial Ca⁺⁺ sequestration, in addition, leads to cytosolic Ca⁺⁺ accumulation and subsequent activation of phospholipases, resulting in accumulation of free fatty acids. Recirculation may in turn lead to deleterious free radical reactions and lipid peroxidation. Selective hippocampal vulnerability to ischemia has been postulated by some investigators to be related to calcium-sensitive dendritic regions of vulnerable neurons, which undergo burst-firing, resulting in massive Ca⁺⁺ entry. In one study of 30 minutes of forebrain ischemia in rats induced by bilateral carotid occlusion and hemorrhagic hypotension, calcium deposits were demonstrated by the oxalate/pyroantimonate procedure in pyramidal neurons of the hippocampus and in lamina 3 of the cortex. Thus, on theoretical grounds, it is conceivable that calcium entry blockers might retard the cascade that leads to cell death.

Several possibilities need to be considered as to why nimodipine was ineffective in this study. First, during severe ischemia with consequent tissue energy depletion, the derangement of mitochondrial sequestration of calcium is expected to lead to massive, possibly irreversible, increases in cytosolic calcium; these intracellular translocations of calcium might not be susceptible to calcium blockers. Second, although radioligand binding studies show that there are specific binding sites for nimodipine in human cerebral cortex and rat brain membranes, it is possible that cerebral Ca⁺⁺ binding sites were not saturated to achieve full effect, even though the dose administered (1 μg/kg/min) was sufficient to decrease systemic blood pressure significantly below control levels. Other calcium antagonists exhibiting greater potency and enhanced blood–brain barrier penetration might prove efficacious. Third, the 6-hour postischemic nimodipine infusion in our study might not have been long enough to cause a sustained improvement in the delayed hypo-
perfusion that occurs in this and other ischemia models and may contribute to development of ischemic neuronal injury.

As noted earlier, several studies have shown that nimodipine increases cerebral blood flow (CBF) in both the normal state and following transient ischemia. In a recent study, nimodipine infusion in awake rats at 1 μg/kg/min was shown to increase CBF by 39–84% without altering the rate of glucose utilization in various brain regions.8 These observations are consistent with a primary site of action of nimodipine on cerebral blood vessels rather than on the cerebral parenchyma itself. In the 4-vessel occlusion model, 30 minutes of ischemia is followed by an initial postischemic hyperemia then by hypoperfusion, which lasts between 3 and 24 hours, depending on the region.18 A prolonged infusion for 24–48 hours, e.g., by intraperitoneal infusion, might therefore be of possible benefit.

In a recent study, Steen et al29 produced complete cerebral ischemia by neck cuff inflation in monkeys and administered nimodipine as a 10 μg/kg bolus 5 minutes after a 17-minute period of ischemia, followed by i.v. infusion at 1 μg/kg/min for 10 hours. The neurologic outcome and histopathology of treated animals at 96 hours were statistically improved over that of untreated controls. Differences in animal species, ischemia model, durations of ischemia and drug administration, and completeness of ischemia may have accounted for the differences observed. Of note in that study was the occurrence of histopathologic findings in the distal arterial perfusion zones of the cerebral cortex, suggesting a possible beneficial effect of nimodipine therapy on cerebral perfusion in zones of borderline hypoperfusion.

In another recently reported study, pretreatment with nimodipine in rats undergoing middle cerebral
artery (MCA) occlusion ameliorated ipsilateral cortical hypo-perfusion and reduced the volume and extent of cellular damage at the periphery but not in the core of the infarct lesion. These results are supported by a recent report, in abstract form, that nimodipine (20 μg/kg) given at 1, 4, or 6 hours after MCA occlusion in rats decreased infarct size and improved neurologic recovery. These studies, taken together, suggest that nimodipine might be of benefit in acute focal cerebral infarction, primarily because of improved blood flow at the infarct periphery. In contrast, in reversible cerebral ischemia resulting in selective neuronal necrosis, such as that of the present study, the efficacy of nimodipine remains to be convincingly substantiated.

The seemingly beneficial effect of the ethanol-polyethylene glycol vehicle in some regions of the ischemic forebrain in our study (Tables 2 and 3, Figure 1) is not readily explained. Polyethylene glycol prevents acute ischemic renal failure in dogs by enhancing transglomerular hydrostatic pressure and thereby preventing secondary tubular obstruction.

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3. Stone PH, Antman EM, Muller JE, Braunwald E: Calcium channel antagonist nimodipine in cerebral ischemia resulting in selective neuronal necrosis, infarction, acute focal cerebral ischemic forebrain in our study (Tables 2 and 3, Figure 1). Stroke 1984;15:527-530

**KEY WORDS** • nimodipine • ischemic neuronal damage • rats • four-vessel occlusion
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