The therapeutic value of nimodipine in experimental focal cerebral ischemia

Neurological outcome and histopathological findings

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Recent studies suggest that nimodipine, a potent calcium-channel antagonist that causes significant cerebrovascular dilatation, may improve neurological outcome after acute experimental permanent focal cerebral ischemia when given before or immediately after occlusion of the middle cerebral artery (MCA) in various animals. The authors describe the effect of nimodipine on cerebral ischemia in a rat model. At 1, 4, or 6 hours after occlusion of the MCA, rats were treated in a double-blind technique with either nimodipine, placebo, or saline. Neurological and neuropathological evaluation was performed at 24 hours. Neurological outcome was better in rats treated with nimodipine 1, 4, or 6 hours after occlusion (p < 0.001, p < 0.01, p < 0.05, respectively), and the size of areas of infarction was statistically smaller in nimodipine-treated groups (p < 0.01, p < 0.01, p < 0.05, respectively) when compared with control rats treated with saline or placebo. The best neurological outcome and the smallest area of infarction were found in nimodipine-treated rats 1 hour after occlusion. Compared with controls, the size of the periphery of the infarcted area was smaller in nimodipine-treated rats. The results show that nimodipine improves neurological outcome and decreases the size of infarction when administered up to 6 hours after ischemic insult. These results suggest a possible mechanism of action of nimodipine on the "penumbra" of the ischemic area.

KEY WORDS • nimodipine • focal cerebral ischemia • middle cerebral artery • occlusion • rat

There is significant evidence that calcium ions are involved in the final common pathway of cell death after cerebral ischemia. In this process, calcium ions adversely affect mitochondrial function and stimulate breakdown of membrane phospholipids. It has been suggested that the liberation of polyunsaturated fatty acids, predominantly arachidonic acid, contributes to the worsening of cerebral edema. Moreover, it is widely accepted that calcium ions have a role in the contraction of arterial smooth muscle. In particular, the hypoperfusion that generally begins within 30 minutes of the onset of ischemia is thought to be caused by an increase in the influx of calcium ions into potassium-depolarized vascular smooth muscle.

Because of these findings, calcium-channel antagonists are a logical choice for investigation as possible therapeutic agents for the treatment of cerebral ischemia of various etiologies. Nimodipine, a dihydropyridine derivative, is one of the most potent calcium-channel blocking agents that has a selective action on intracranial vessels and minimal metabolic effects. Parenteral infusion of nimodipine before or immediately after induction of experimental focal cerebral ischemia improves neurological outcome and the size of infarction in various animals (JB Bederson, et al., unpublished data). To be of clinical value, however, a mode of treatment must have beneficial effects when initiated at least 1 hour after ischemic insult. In the clinical setting, treatment can rarely be started much earlier than 1 hour after insult, but it is realistic to expect that treatment may be initiated by 6 hours after the onset of ischemia. Therefore, the effects of administration of nimodipine on neurological outcome and
the size of infarction when administered were studied 1, 4, or 6 hours after permanent middle cerebral artery (MCA) occlusion in the rat.

**Materials and Methods**

**Surgical Procedure**

The experimental procedure followed National Institutes of Health guidelines and was approved by the Animal Experimentation Committee of the University of California at San Francisco. Sixty-one adult male Sprague-Dawley rats weighing 350 to 400 gm each were anesthetized with intraperitoneal chloral hydrate in normal saline (40 mg/100 gm body weight). Polyethylene catheters (25 cm long) were introduced into the left femoral artery and vein to monitor mean arterial blood pressure (MABP) and to offer access to the vein. Body temperature was maintained within normal limits with a heating pad.

The left temporo-parietal region was shaved, the rats were placed in the lateral position, and a 2-cm curved vertical incision was made midway between the lateral margin of the left orbit and the external auditory canal. The temporalis muscle was elevated from the skull and the inferotemporal fossa was exposed. With microsurgical technique, a 5-mm craniectomy was made in the inferotemporal fossa by means of a saline-cooled dental drill; care was taken not to damage the zygomatic bone or the mandibular nerve. The dura was opened through a stellate incision and the MCA was exposed. Bipolar coagulation was used to occlude the MCA from its origin at the carotid artery continuously up to 2 mm above the point where it is crossed by the inferior cerebral veins. Brain retraction was not necessary. After occlusion, the MCA was transected to avoid recanalization. The craniectomy site was covered with a small piece of Gelfoam, the soft tissues were allowed to fall back into place, and the skin was sutured. The rats remained on the heating pad while they recovered from anesthesia and were then returned to their cages.

**Treatment Groups**

Forty-eight rats were treated 1, 4, and 6 hours after occlusion (Groups 1, 2, and 3, respectively; 16 rats in each group). The MCA was occluded in 10 untreated control rats, and three rats underwent a sham operation. In each group, in a double-blind technique, either nimodipine in saline (2 μg/kg/min, eight rats), placebo (four rats), or saline alone (four rats) was administered intravenously over a 10-minute period. Ampules of nimodipine were diluted with saline. Ampules of placebo were diluted with the same volume of saline used to dilute nimodipine.* The total volume given to all rats was approximately 2 cc.

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* Nimodipine (BAY e 9736) Lot No. 861179 and placebo Lot No. 861195 manufactured by Miles Laboratories, Inc., New Haven, Connecticut.

**Neurological Examination**

The neurological status of each rat was carefully evaluated in a double-blind manner 24 hours after MCA occlusion. The following grading scale, described by Bederson, et al., was used: Grade 0, no observable deficit; Grade 1, forelimb flexion; and Grade 2, forelimb flexion and decreased resistance to lateral push.

**Neuropathological Examination**

After neurological evaluation, the rats were anesthetized with chloral hydrate, and a lethal intracardiac perfusion of 2,3,5-triphenyltetrazolium chloride (TTC) was performed (IM Germano, et al., unpublished data). The TTC-stained brains were removed carefully and the surface was examined for any change in vascularity in the area of the stroke, to verify the occlusion and sectioning of the MCA, and to evaluate the size of infarcted areas on the surface of the cerebral cortex. Brains were then fixed in 10% phosphate-buffered formalin for 24 hours, 2 mm-thick coronal sections were cut, and the TTC-stained sections were photographed for measurement of the size of infarction. Histological sections prepared from the same photographed surface of the TTC-stained slice were treated with hematoxylin and eosin (H & E) to confirm the size of the infarcted area. Size was quantified by differential cutting and weighing techniques of traced images and was expressed as a percentage of the coronal section.

**Statistical Analysis**

Intergroup analysis of MABP values was performed by two-way analysis of variance (ANOVA). Intragroup MABP values were analyzed using repeated measures ANOVA (the null hypothesis: there is no difference over time for each treatment) and by one-way ANOVA (the null hypothesis: there is no difference between treatments at each given time). Bonferroni's method was used for the post hoc tests when necessary. Intergroup neurological outcome scores were analyzed by the nonparametric Kruskal-Wallis test corrected for tied ranks. Intragroup values were analyzed by the nonparametric Mann-Whitney U test. Intergroup infarction size values were analyzed by two-way ANOVA; intragroup infarction size values were analyzed by one-way ANOVA. A probability (p) of less than 0.05 was considered significant.

**Results**

All sham-operated rats had a neurological grade of 0, and no infarction was found on neuropathological examination. All MCA-occluded rats, whether treated or untreated, had areas of infarction confirmed by TTC and H & E staining. Neurological outcome, MABP, infarction size, and neuropathological findings were compared within and between the three groups. The MABP values were not statistically different within groups during or at the end of treatment compared to MABP's before treatment (Table 1). However, a statis-
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<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Before Treatment</th>
<th>During Treatment</th>
<th>After Treatment</th>
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<tbody>
<tr>
<td>nimodipine</td>
<td>77 ± 13</td>
<td>71 ± 11</td>
<td>74 ± 12</td>
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<tr>
<td>placebo</td>
<td>78 ± 6</td>
<td>81 ± 17</td>
<td>74 ± 10</td>
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<tr>
<td>saline</td>
<td>81 ± 7</td>
<td>71 ± 11</td>
<td>72 ± 13</td>
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Group 2 (treated 4 hrs after MCA occlusion)

<table>
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<th>After Treatment</th>
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<tr>
<td>nimodipine</td>
<td>97 ± 7</td>
<td>91 ± 8</td>
<td>101 ± 12</td>
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<tr>
<td>placebo</td>
<td>91 ± 24</td>
<td>91 ± 20</td>
<td>93 ± 18</td>
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<tr>
<td>saline</td>
<td>106 ± 8</td>
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<td>105 ± 8</td>
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Group 3 (treated 6 hrs after MCA occlusion)

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<th>During Treatment</th>
<th>After Treatment</th>
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<tr>
<td>nimodipine</td>
<td>94 ± 15</td>
<td>92 ± 16</td>
<td>97 ± 13</td>
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<tr>
<td>placebo</td>
<td>89 ± 3</td>
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<td>90 ± 11</td>
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<td>saline</td>
<td>89 ± 1</td>
<td>89 ± 7</td>
<td>92 ± 4</td>
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* Values are means ± standard deviations (mm Hg). MCA = middle cerebral artery.

A statistically significant difference in neurological outcome was found within groups between rats treated with nimodipine versus rats treated with saline or placebo. All nimodipine-treated rats had a better neurological outcome than the placebo- and saline-treated rats of the same group (Group 1, p < 0.001; Group 2, p < 0.01; Group 3, p < 0.05). These data are shown as histograms in Fig. 1. Group 1 nimodipine-treated rats had the best outcome, even though no statistically significant difference was found between groups (Fig. 2).

At neuropathological examination, it was found that the MCA had been occluded and transected in all operated rats, but was intact in all sham-operated rats. The size of infarction of the cortical mantle represented approximately five-sixths of the hemisphere in TTC-stained brains of all placebo- and saline-treated rats of each group (Fig. 3A). The size of infarction in nimodipine-treated rats of all groups was more variable, with infarcted areas ranging between one-sixth and four-sixths of the hemispheric cortex (Fig. 3B). The brains were evaluated for increased vascularity in the area of ischemia. The MCA and its branches were barely discernible in the placebo- and saline-treated rats of all groups (Fig. 3A), while the MCA vascular tree was strikingly visible in the majority of nimodipine-treated rats of all groups (Fig. 3B).

The size of infarction on coronal sections of nimodipine-treated rats of each group was significantly smaller than the size in placebo- and saline-treated rats of the same group (Group 1, p < 0.01; Group 2, p < 0.01; Group 3, p < 0.05 by one-way ANOVA). Data are plotted as histograms in Fig. 4 and tabulated in the first column of Table 2. Group 1 rats (nimodipine-treated) had the smallest areas of infarction, but differences between the groups were not statistically significant.

The morphology of infarcted areas was very different for nimodipine-treated rats compared with placebo- or saline-treated rats. The overall size of infarction was smaller in nimodipine-treated rats because of a decrease in the size of the infarction in the peripheral area of the lesion (Fig. 5). No significant differences within and between groups were found in the distribution of infarction size into the cortex and basal ganglia for nimodipine-treated rats compared with rats treated with placebo or saline. These data are reported in Table 2.

A linear correlation was found between the size of the infarcted area and the results of the neurological
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FIG. 3. Whole rat brain showing the size of the infarcted area in the cortex of a placebo-treated rat (A) versus a nimodipine-treated rat (B). The infarcted area is visualized in white, while the TTC-stained normal brain is darker. × 1.5.

FIG. 4. Size of infarction correlated with treatment. Differences were significant for Group 1 (p < 0.01 for nimodipine- vs. placebo- or saline-treated rats). Group 1, 2, and 3 rats were treated 1, 4, and 6 hours, respectively, after middle cerebral artery occlusion. P values were determined by one-way analysis of variance.

FIG. 5. Coronal sections of placebo-treated rat brain (A) and nimodipine-treated rat brain (B). The infarcted area is visualized in white, and the TTC-stained normal brain is dark. Note the peripheral decrease in size in B compared to A. × 1.5.

TABLE 2
Infarction distribution in cortex and basal ganglia*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TI (% coronal section)</th>
<th>Cortex (% TI)</th>
<th>Basal Ganglia (% TI)</th>
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<tr>
<td>Group 1 (treated 1 hr after MCA occlusion)</td>
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<tr>
<td>nimodipine</td>
<td>24 ± 8</td>
<td>76 ± 6</td>
<td>24 ± 5</td>
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<tr>
<td>placebo</td>
<td>31 ± 5</td>
<td>68 ± 2</td>
<td>32 ± 4</td>
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<tr>
<td>saline</td>
<td>39 ± 4</td>
<td>70 ± 5</td>
<td>30 ± 5</td>
</tr>
<tr>
<td>Group 2 (treated 4 hrs after MCA occlusion)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nimodipine</td>
<td>25 ± 8</td>
<td>74 ± 3</td>
<td>26 ± 13</td>
</tr>
<tr>
<td>placebo</td>
<td>37 ± 1</td>
<td>61 ± 4</td>
<td>39 ± 10</td>
</tr>
<tr>
<td>saline</td>
<td>37 ± 2</td>
<td>66 ± 1</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Group 3 (treated 6 hrs after MCA occlusion)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>nimodipine</td>
<td>28 ± 8</td>
<td>69 ± 2</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>placebo</td>
<td>37 ± 4</td>
<td>69 ± 4</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>saline</td>
<td>36 ± 5</td>
<td>65 ± 3</td>
<td>35 ± 7</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations. TI = total infarction; MCA = middle cerebral artery.
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Fig. 6. Infarction size correlated with the results of neurological examination in three rat groups. Group 1, 2, and 3 rats were treated 1, 4, and 6 hours, respectively, after middle cerebral artery occlusion. A linear correlation was found (p < 0.001; r = 0.85; y intercept = 11%).

Discussion

Two major factors influence the sequelae of cerebral ischemic injury: the delayed hypoperfusion that occurs after focal ischemia and membrane failure that leads to irreversible cell injury. Calcium ions play a major role in both pathophysiological mechanisms. For this reason, nimodipine, one of the most potent calcium-channel blockers that causes prominent cerebral vasodilation, has potential value for the treatment of ischemia.

The effects of nimodipine on postischemic blood pressure are minimal, but a significant decrease in MABP has been reported. Steen, et al., suggested that nimodipine causes no significant changes in MABP in treated dogs compared with untreated control animals. In the studies discussed above, nimodipine was administered either before or immediately after ischemic insult. We found, in agreement with Steen, et al., that nimodipine had no significant effect on the MABP of treated rats. The fact that all Group 1 rats had lower MABP's than Group 2 and 3 rats can be explained by pathophysiological adjustments that occur after cerebral ischemia. Systemic blood pressure decreases in the first several days after ischemic insult in humans and in the first few hours after insult in other mammals, after which values return to normal levels.

The neurological outcome in rats pretreated or treated immediately after insult with nimodipine is better than that of placebo-treated rats with focal cerebral ischemia (JB Bederson, et al., unpublished data).

Other investigators have reported that outcome was improved in dogs treated with nimodipine before ischemic insult only, which raised the question of the potential value of nimodipine administered after injury. Our results show a statistically significantly improved neurological outcome in all nimodipine-treated rats in all groups studied, which indicates that nimodipine may be of clinical value if administered up to 6 hours after ischemic insult in this model. Improved neurological outcome was supported by the neuropathological findings. The size of infarction was statistically smaller in all nimodipine-treated rats compared with placebo- or saline-treated rats. The linear relationship between infarction size and neurological outcome corroborates the findings of Bederson, et al.

The mechanism of action of nimodipine is not fully understood. Pharmacologically, nimodipine could influence cerebral postischemic changes by affecting cerebral vasoconstriction, platelet aggregation, and neuronal membrane and mitochondrial function. There is evidence that nimodipine acts as a vasodilator with or without osmotic disruption of the blood-brain barrier. Our finding that the vascular tree is more visible in nimodipine-treated rats compared with either placebo- or saline-treated rats supports the evidence that nimodipine acts on cerebral vessels as a vasodilator. Aragno and Doni and Hossmann, et al., reported an increase in platelet aggregation in cerebral vessels after ischemic injury. While the importance of calcium entry blockers on this phenomenon is not clearly established, it was found that nifedipine caused a reduction in platelet aggregation factor released from human leukocytes. A direct protective effect of calcium blockers on neurons has not been established, but evidence that in vitro nimodipine affects the calcium channels of neurons has been published. Results of in vivo studies, however, suggest that pretreatment with nimodipine in a primate cerebral ischemia model does not prevent calcium from entering ischemic cells.

Surgical and pharmacological intervention in focal cerebral ischemia is effective only during the period in which ischemic tissue may recover before irreversible damage occurs. Recently, attention has been directed to the "ischemic penumbra," the zone of nonfunctioning but structurally preserved brain tissue at the periphery of the infarcted area. The intrinsic resistance of neurons to ischemia for relatively long periods of complete or partial oxygen deprivation is supported by studies of Ames and Gurian and Hossmann and Kleihues. The viability of neurons in the penumbra of the ischemic lesion seems to be even longer. A decrease in the intracellular concentration of Ca++ after reperfusion in cerebral ischemia has been described. Restoration of blood flow and/or the block of calcium ions entering the cell may therefore prolong viability and restore activity. In this study, the decrease in the size of infarction in nimodipine-treated rats involved both the basal ganglia and the cortex. The reduction in size uniformly affected the periphery of the ischemic area,
a zone that appears to correlate anatomically to the penumbra.

Thus, nimodipine administered up to 6 hours after ischemic insult improves neurological outcome in rats with focal cerebral ischemia. Moreover, neuropathological evidence suggests that nimodipine may act at the penumbra of the ischemic area, preventing cell death by a direct action on neurons and/or by improving blood flow.

Acknowledgments

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