The Effect of the Calcium Antagonist Nimodipine on the Gerbil Model of Experimental Cerebral Ischemia

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SUMMARY The gerbil model was used to assess the therapeutic effects of the calcium antagonist nimodipine on cerebral ischemia. Transient cerebral ischemia was produced in each gerbil by bilateral common carotid occlusion of 10-, 15-, or 20-min duration. Nimodipine (0.01 or 0.1 mg/kg) was administered intraperitoneally just before the carotid occlusion or 10–30 min after the removal of the arterial clips. Morbidity of each animal was evaluated using the stroke index, and the sum of stroke indices was calculated for evaluating the overall morbidity during a particular period of reperfusion. Mortality was observed for 24 hours after clip removal. Although, depending on the timing of the drug administration, the low-dose (0.01 mg/kg) nimodipine worsened the morbidity in the gerbils with 10-min ischemia, the high-dose (0.1 mg/kg) of the drug had a clear beneficial effect on the mortality associated with cerebral ischemia. These results are considered worthwhile for further trials to assess the usefulness of nimodipine as a therapeutic agent in the management of the acute ischemic stroke.

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RECENTLY there has been increasing interest in the pathophysiological role of calcium in ischemic brain injury.\(^1\)\(^-\)\(^4\) Calcium has been implicated in the final common pathway not only for contraction of vascular smooth muscle in a variety of pathophysiological conditions,\(^5\) but also for cell death caused by membrane degradation.\(^6\)\(^-\)\(^8\) It seems justified, therefore, to consider whether calcium entry blockers may have therapeutically useful effects on ischemic brain injury.

Amongst several calcium antagonists, nimodipine [BAY e 9736, isopropyl (2-methoxyethyl)-1,4-dihydro-2,6-dimethyl-4(3-nitrophenyl)-3,5-pyridinedicarboxylate] has been shown to be one of the most promising ones with a preferential action on cerebral blood vessels.\(^9\)\(^-\)\(^10\) Kazda et al\(^10\) suggested that nimodipine prevents the posts ischemic impairment of reperfusion following 7-min global cerebral ischemia in cats, and Hoffmeister et al\(^11\) showed in the same model, that the circulatory effects are paralleled by improvement of functional recovery with increased survival rates. Following these studies, several investigations\(^12\)\(^-\)\(^16\) were reported on the pathophysiological effects of this drug on cerebral ischemia.

The mongolian gerbil (Meriones unguiculatus), which lacks a functional connection between the carotid and verteobasilar arterial circulations,\(^17\)\(^-\)\(^18\) has great advantages for the screening of compounds as protective agents against brain ischemia.\(^19\)\(^-\)\(^23\) There have been, however, no reports on the effects of nimodipine on the gerbil model of cerebral ischemia. In the present study, by assessing the changes both in morbidity and mortality following temporary bilateral common carotid occlusion in gerbils with nimodipine or vehicle treatment, we attempted to evaluate the therapeutic potency of the drug in the acute phase of ischemic stroke.

Methods A total of 202 adult Mongolian gerbils of both sexes weighing 60–80 g were used in the present study. The gerbils were anesthetized with ketamine hydrochloride (Ketalar\(^8\), 50 mg/kg i.p.) and placed in the supine position. Bilateral common carotid arteries were then exposed through a ventral midline incision of the neck, and were looped with silk sutures. As the gerbils emerged from the anesthetic, the two common carotid arteries were occluded simultaneously by pulling the sutures around them and thereafter they were occluded by aneurysmal clips for 10, 15 or 20 min. Following these ischemic periods, the clips were removed to restore the blood flow and the patency of the arteries was ascertained by visual inspection. After this, the animals were returned to their cages again for recovery and observation. Morbidity and mortality were evaluated and scored by an evaluator who was blinded as to the duration of cerebral ischemia, the drug or the dosage used. Morbidity of each animal was evaluated every one hour using the McGraw's method.\(^19\) The neurological signs included ptosis, paucity of movements, cocked head, circling behavior, splashed hindlimb, seizure and coma, and were tabulated as the stroke index such that a higher score indicated a more severe neurological deficit. We further calculated the sum of stroke indices in each gerbil for evaluating the morbidity of a particular period of reperfusion. The number of deaths in each category was ascertained 24 hours after clip removal. In order to compare the animals receiving nimodipine with the control animals receiving vehicle, life tables were also constructed by actuarial methods to indicate the cumulative probability of dying as influenced by a specific time following the ischemic insult. In all animals which survived 24-
hour reperfusion after global cerebral ischemia, the existence of forebrain ischemia during bilateral common carotid occlusion was confirmed by the carbon black perfusion method.18

The treatment consisted of a single intraperitoneal injection of either nimodipine (0.01 or 0.1 mg/kg) or vehicle just prior to bilateral carotid occlusion or 10–30 min after reestablishment of cerebral blood circulation. The volume of injection was the same (0.1 ml) for each gerbil. The vehicle contained, per liter of solution, 200 g 96% ethanol, 170 g polyethylene glycol 400, 2g sodium citrate, and 0.5 g citric acid. Because nimodipine is very sensitive to white light, the drug was always dispensed under sodium light and the syringe for injection was always covered in aluminum foil. Each drug and dose was also administered to 5 gerbils without carotid occlusion to verify the absence of toxicity in the dosage selected.

Results

There were no intraoperative deaths among the gerbils. The morbidity for each treatment group was represented by the sum of stroke indices obtained at 1–6 hours after bilateral common carotid occlusion, and summarized in table 1. In the gerbils with 15- or 20-min bilateral cerebral ischemia, there was no significant difference in the sum of stroke indices between the nimodipine-treated and vehicle-treated animals though the tendency of decrease of stroke indices was noted in the gerbils with nimodipine treatment that survived 24-hour reperfusion. On the other hand, in the gerbils with 10-min ischemia, significant increase of the morbidity scores was observed in the groups treated with the low-dose (0.01 mg/kg) nimodipine and the group with the high-dose (0.1 mg/kg) nimodipine 30 min after reperfusion. Depending on the timing of the administration of the drugs, significant differences were observed in the morbidity even among the gerbils with the same dose of nimodipine.

Mortality rates for each treatment group are summarized in table 2. Although a significant increase of mortality rate was observed in 10-min ischemic gerbils with the low-dose nimodipine just before bilateral common carotid occlusion, no significant differences were noted in mortality rate between the vehicle-treated gerbils and the remaining nimodipine-treated animals with 10-min ischemia, and no significant effects of nimodipine on mortality rate were observed in 15-min ischemic gerbils. On the other hand, significant decrease of mortality rate was observed in the 20-min ischemic gerbils with the high-dose nimodipine, as compared to the vehicle-treated controls. This beneficial effect of the high-dose nimodipine on the mortality rate of the 20-min ischemic gerbils was clearly demonstrated in figure 1. With respect to the 20-min ischemic gerbils, there were no significant differences in the probability of dying among the low-dose nimodipine-treated groups and among the high-dose nimodipine-treated groups before bilateral carotid occlusion. Statistical significance was determined by the unpaired t-test.

Table 1: The Sum of Stroke Indices Obtained at 1–6 Hours after 10, 15 and 20 Min of Bilateral Carotid Arterial Occlusion in Gerbils Treated with Vehicle or Nimodipine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of Bilateral Carotid Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>19.3 ± 3.2</td>
</tr>
<tr>
<td>Nimodipine (0.01 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>before carotid occlusion</td>
<td>56.6 ± 4.0*</td>
</tr>
<tr>
<td>10 min after reperfusion</td>
<td>45.5 ± 3.7*</td>
</tr>
<tr>
<td>30 min after reperfusion</td>
<td>36.0 ± 5.1†</td>
</tr>
<tr>
<td>Nimodipine (0.1 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>before carotid occlusion</td>
<td>14.8 ± 5.1</td>
</tr>
<tr>
<td>10 min after reperfusion</td>
<td>29.3 ± 6.2</td>
</tr>
<tr>
<td>20 min after reperfusion</td>
<td>—</td>
</tr>
<tr>
<td>30 min after reperfusion</td>
<td>46.0 ± 5.2*†</td>
</tr>
</tbody>
</table>

Each value represents mean ± S.E.M. For the number of animals in each group, see table 2 or 3. *p < 0.01; significantly different from the gerbils with vehicle treatment. †p < 0.05; ‡p < 0.01; significantly different from the gerbils with nimodipine treatment before bilateral carotid occlusion. Statistical significance was determined by the unpaired t-test.

Table 2: Mortality Rates at 24 Hours after 10, 15 and 20 Min of Bilateral Carotid Arterial Occlusion in Gerbils Treated with Vehicle or Nimodipine

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration of Bilateral Carotid Occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Vehicle</td>
<td>13% (15)</td>
</tr>
<tr>
<td>Nimodipine (0.01 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>before carotid occlusion</td>
<td>73%* (11)</td>
</tr>
<tr>
<td>10 min after reperfusion</td>
<td>50% (4)</td>
</tr>
<tr>
<td>30 min after reperfusion</td>
<td>17% (12)</td>
</tr>
<tr>
<td>Nimodipine (0.1 mg/kg)</td>
<td></td>
</tr>
<tr>
<td>before carotid occlusion</td>
<td>10% (10)</td>
</tr>
<tr>
<td>10 min after reperfusion</td>
<td>0% (10)</td>
</tr>
<tr>
<td>20 min after reperfusion</td>
<td>—</td>
</tr>
<tr>
<td>30 min after reperfusion</td>
<td>0% (6)</td>
</tr>
</tbody>
</table>

Each value represents percent mortality at 24 hours after bilateral cerebral ischemia. The number of animals in each group is given in parentheses. *p < 0.05; significantly different from the gerbils with vehicle treatment. Statistical significance was determined by the chi-square test.
Duration of Ischemia (min)

FIGURE 1. Comparison of mortality curves among the vehicle-treated gerbils (●) and the gerbils with 0.01 mg/kg (△) and 0.1 mg/kg (▲) nimodipine treatment at 30 min after clip removal. Significant difference (χ² = 5.46, p < 0.05) from the vehicle-treated control animals was indicated by the asterisk (★). Statistical significance was determined by the chi-square test. No significant difference was observed in mortality rate between the vehicle-treated and 0.01 mg/kg nimodipine-treated animals.

treated ones. Therefore, these groups were combined and analyzed as a single group. Figure 2 presents the life tables for the three treatment groups with 20-min bilateral cerebral ischemia. No significant improvement was observed in the gerbils with the low-dose nimodipine but the gerbils treated with the high-dose nimodipine proved to have a significantly lower probability of dying than the vehicle-treated gerbils at 7–24 hours after reperfusion.

The gerbils with cerebral ischemia often showed seizures, which in turn could affect survival. We therefore checked the incidence of seizures in each group of gerbils and summarized the results in table 3. The gerbils with longer duration ischemia showed higher incidence of seizures. There were no significant differences in incidence of seizures between the vehicle-treated and nimodipine-treated gerbils, except for 10-min ischemic gerbils with the low-dose nimodipine treatment that showed an increased incidence of seizures during the early recovery period.

Discussion

In recent years, the model of transient bilateral cerebral ischemia in the gerbil has been intensively used for testing therapeutic agents in the management of the acute brain ischemia. Bilateral common carotid artery occlusion produces a fairly uniform ischemia in the telencephalon without ambiguity and the surgical procedure is so simple that large number of animals can be assessed statistically. The transient ischemic model can also allow vascular access of administered drugs or improved blood flow to affect the ischemic areas of the brain. In the previous experiments, we characterized the morbidity and established a mortality curve during the duration of ischemia as the dependent variable in this model of transient global cerebral ischemia. Bilateral common carotid artery occlusion resulted in 100% mortality during a 24-hour period in the gerbils subjected to 20-min occlusion, whereas negligible mortality was associated with the bilateral carotid occlusion of 10-min duration. In the present study, we introduced a single administration of nimodipine (0.01 or 0.1 mg/kg) or vehicle as independent variables to determine possible changes in the morbidity and the mortality curve following the identical periods of bilateral common carotid occlusion.

There is no doubt that the outcome of cerebral ischemia can be influenced by the calcium antagonist nimodipine as indicated by the present results. This study also indicates that the effectiveness depended not only on the dose of nimodipine but also on the timing of the drug administration. As a matter of fact, the detrimental effects of the low-dose nimodipine were unexpectedly observed in the gerbils with 10-min
ischemia, especially when the drug was injected just before the carotid occlusion (tables 1, 2 and 3). On the other hand, clear beneficial effects of the high-dose nimodipine were observed in the gerbils with 20-min ischemia, irrespective of the time of administration — whether given prior to or after termination of the ischemic episode (table 2, figs. 1 and 2).

Although it is very difficult to explain the exact mechanism of these effects of nimodipine, the effects of this drug on the postischemic reperfusion of the ischemic brain had seemed to be one of the major factors which determine the outcome of the ischemic gerbils. In our preliminary experiment, a single intraperitoneal injection of 0.01 or 0.1 mg/kg nimodipine reduced mean arterial pressure, which was continuously monitored with an intra-aortic catheter, by 3–7% for about 15 min or by 7–20% for more than 30 min, respectively. In this model of cerebral ischemia, significant regional or systemic hemodynamic changes were reported both during and after bilateral cerebral ischemia. 

An initial fall of mean blood pressure, which may cause secondary impairment of cerebral blood flow, was observed soon after the release of the aneurysmal clips. Therefore, it seems to be most probable that when administered just before the carotid occlusion in 10-min ischemic gerbils, the low-dose nimodipine produced only the potentiation of the initial fall of blood pressure after clip removal because of the shorter duration of action and the weaker effects on cerebral blood flow, and showed the detrimental effects on morbidity and mortality through the impairment of the postischemic reperfusion. Even if this dose of nimodipine still has a selective action on brain vessels through which drugs can affect the vessels in the ischemic brain regions, and immediately after the recirculation strong postischemic dilatation of these vessels can be seen without vasodilatory drugs. A different time schedule experiment was then added to clarify this point. A significant difference, detected in morbidity and mortality between the gerbils with the low-dose nimodipine treatment before carotid occlusion and at 30 min after reperfusion, supports the abovementioned suggestion. However it may be the ischemia-produced seizures, and not the lowered blood pressure per se, which more directly accounts for reduced survival in the lower dose regime. As shown in tables 2 and 3, a clear association between seizures and outcome was observed and the ineffective and sometimes harmful dose schedule of nimodipine was indeed accompanied by increased seizures in the early recovery period which might be caused by the fall in blood pressure. But other mechanisms such as the metabolic effects of nimodipine cannot be wholly excluded and, in the intramural model of focal cerebral ischemia, it was suggested that nimodipine increases the susceptibility of tissue to ischemic damage in the areas where blood flow is critically reduced.

On the other hand, the high-dose nimodipine has a greater modifying effect of longer duration on regional cerebral blood flow than the low-dose; this may be a major factor responsible for the observed differences in the survival of the gerbils (table 2, figs. 1 and 2). Our results obtained in the gerbils with the high-dose nimodipine were in agreement with the previous reports. A consistent finding in previous experiments and in ours has been that treatment with nimodipine improves survival after ischemic cerebral damage. In order to understand how nimodipine may benefit the gerbils with predicted 100% mortality, we should know the pathophysiological cause of death. As mentioned above, the lethality of prolonged cerebral ischemia may be, in part, related to the metabolic harm from the seizures triggered by the cerebral ischemia. But the benefits of the higher dose regime cannot be explained by control of seizures, because no significant difference was detected in the incidence of seizures between the gerbils with vehicle treatment and those with high-dose nimodipine treatment (table 3). Avery et al recently reported that in this model of transient global cerebral ischemia, mortality correlated well with the evolution of cerebral edema. In our investigation, we also could detect significant differences in specific gravity between the ischemic telencephalic brain regions from the dying animals and the others. This lethal edema process could resolve with the evolution of cerebral ischemia, and at 30 min after reperfusion, supports the above-mentioned suggestion. However it may be the ischemia-produced seizures, and not the lowered blood pressure per se, which more directly accounts for reduced survival in the lower dose regime. As shown in tables 2 and 3, a clear association between seizures and outcome was observed and the ineffective and sometimes harmful dose schedule of nimodipine was indeed accompanied by increased seizures in the early recovery period which might be caused by the fall in blood pressure. But other mechanisms such as the metabolic effects of nimodipine cannot be wholly excluded and, in the intramural model of focal cerebral ischemia, it was suggested that nimodipine increases the susceptibility of tissue to ischemic damage in the areas where blood flow is critically reduced.

We conclude that nimodipine has beneficial effects in the gerbil model of cerebral ischemia when administered in an adequate dosage and with proper timing. This drug should be considered seriously for further therapeutic trials of acute ischemic stroke.
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