Effect of Nimodipine on Ischemia-Induced Brain Edema and Mortality in a Novel Transient Middle Cerebral Artery Occlusion Model

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Abstract—A novel transient middle cerebral artery (MCA) occlusion model in the rat was used to evaluate the effect of nimodipine on brain edema and mortality. Nimodipine (30 μg/kg) administered immediately after 3 hr of transient unilateral MCA occlusion attenuated significantly the post-ischemic increase of tissue water content and partly attenuated ⁴⁵Ca accumulation in the parieto-temporal cortex ipsilateral to the left MCA occlusion 3 hr after reperfusion. Nimodipine decreased the mortality rate at 6 and 9 hr after recirculation, although the survival rate at 24 hr after recirculation was not different from the control group. These results suggest that nimodipine has beneficial effects in the early phase of the reperfusion period.

It has been widely assumed that disruption of calcium homeostasis is one of the detrimental factors leading to cell death after cerebral ischemia (1–4). Beneficial effects of calcium channel blockers after cerebral ischemia in animal and human studies have been reported (5–9). Nimodipine, a Ca²⁺ channel blocker of the dihydropyridine type, has also been shown to improve clinical outcome and has been reported to be protective in animal models of ischemia (5, 10–13). Permanent unilateral MCA occlusion models in the rat have been commonly used to analyze the protective roles of various drugs against ischemic brain damage (14, 15). In human ischemic strokes, however, recanalization after MCA occlusion is not uncommon, particularly in the case of MCA occlusion caused by embolization. Thus, a recirculation model after MCA occlusion may be of great value for simulating focal ischemia in humans. We have developed a transient MCA occlusion model in the rat, which was produced by inserting a piece of silicone-coated nylon thread into the internal carotid artery (16). The aim of the present report was to investigate the effect of nimodipine on post-ischemic brain edema and on animal mortality after reperfusion using a novel transient MCA occlusion model. The effect of hyperosmotics (glycerol) on brain edema was also studied.

Materials and Methods

1. Animals: Male Wistar rats weighing 230–250 g were used. Animals were purchased from Japan S.L.C., Ltd. and allowed free access to food and water.

2. MCA occlusion: Transient focal ischemia was induced in rats by means of left MCA occlusion (16, 17). With the animals under anesthesia (a mixture of 70% N₂O, 30% O₂, and 1% halothane), a piece of nylon thread, one end of which was pre-coated with silicone resin, was inserted into the left common carotid artery in order to occlude the origin of MCA.

3. Brain edema and mortality: Three hours after reperfusion (6 hr after induction of ischemia), the animals were decapitated and tissue samples of both hemispheres were quickly dissected in a humidified chamber. The water content of the samples was determined by the dry-weight method. Brain areas used for analysis are presented in Fig. 1. The water content of animals killed 6 hr after...
ischemia without reperfusion was also examined. The survival of animals treated with nimodipine or vehicle alone was compared at the time points of 3, 6, 9, 24 hr and 7 days after reperfusion following 3-hr ischemia.

4. $^{45}$Ca autoradiography and histological study: $^{45}$Ca autoradiography was performed according to the method of Kato et al. (18). In brief, for the $^{45}$Ca autoradiographic study, the animals were injected intravenously with $^{45}$CaCl$_2$ (Amersham; 300 μCi/animal, dissolved in 0.15 ml physiological saline) when the MCA was reperfused. Three hours after reperfusion following 3-hr ischemia, the brains were removed quickly and frozen in powdered dry-ice. Serial coronal sections, 20-μm thick, were cut in a cryostat at −20°C, and they were exposed to Kodak NMC-1 film for 2 weeks. For histopathology, animals were perfusion-fixed (10% formalin, 10% acetate and 80% methanol) under deep pentobarbital anesthesia 3 hr after reperfusion, and paraffin sections, 5 μm in thickness, were stained with Hematoxylin/Eosin and cresyl violet. Each group consisted of 3 experiments.

5. Test compounds: The doses of test compounds were as follows: Nimodipine (30 μg/kg, 6 ml/kg), nimodipine vehicle (6 ml/kg; it consisted of 200 g/l ethanol, 170 g/l macrogol 400, 2 g/l sodium citrate and 0.3 g/l citrate), 60% glycerol (4 g/kg, 5.3 ml/kg; Wako Co., Ltd., Japan), glycerol vehicle (5.3 ml/kg, physiological saline). Nimodipine and its vehicle were kindly donated by Bayer Co., Ltd. (FR Germany). The intravenous administration of these compounds was started immediately after reperfusion using a pump (Terumo STC-521, Japan) at the rate of 0.2 ml/min for 30 min.

6. Statistics: For statistical analyses, Duncan’s multiple range test, Dunnett’s multiple range test and Fisher’s exact probability test were used.

Results

1. Brain edema: Effect of nimodipine and glycerol on brain edema after transient focal ischemia is summarized in Table 1. The present ischemic model caused a marked increase in the water content in the areas perfused by MCA. In the brain areas ipsilateral to the left MCA occlusion, the water content in the striatum, parieto-temporal cortex, and frontal cortex (nimodipine vehicle group) of rats killed 3 hr after reperfusion (6 hr after induction of ischemia) increased by 6.3, 6.4 and 2.2%, respectively. By contrast, the increases of water content in the striatum, parieto-temporal cortex, and frontal cortex ipsilateral to the left MCA occlusion in animals killed 6 hr after left MCA occlusion without reperfusion were 4.2, 3.8 and 1.2%, respectively. Water content of the areas contralateral to the left MCA occlusion was unaltered both in the reperfusion group and in the group with permanent left MCA occlusion. Brain edema during the 3-hr reperfusion that occurred after a 3-hr ischemia induced in our rat model was observed to be more severe than that which followed a 6-hr ischemia.

Nimodipine significantly reduced the post-ischemic increase of water content in the frontal and parieto-temporal cortex ipsilateral to the left MCA occlusion 3 hr after reperfusion (6 hr after left MCA occlusion). The increase in water content of the nimodipine group in the parieto-temporal cortex and frontal cortex ipsilateral to the left MCA occlusion was reduced to 3.9 and 1%, respectively. Glycerol significantly reduced the increase of water content in the striatum, frontal cortex, and parieto-temporal cortex ipsilateral to the left MCA occlusion 3 hr after reperfusion. Interestingly, glycerol induced a reduction of water content in the striatum and parieto-temporal cortex contralateral to left MCA occlusion.
Table 1. Effect of nimodipine and glycerol on brain edema after left MCA occlusion (3 hr) in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Ipsilateral to MCA occlusion</th>
<th>Contralateral to MCA occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>parietal c.</td>
<td>frontal c.</td>
</tr>
<tr>
<td>Sham</td>
<td>10</td>
<td>79.62±0.16**</td>
<td>79.28±0.26**</td>
</tr>
<tr>
<td>Vehicle¹</td>
<td>12</td>
<td>84.69±0.36</td>
<td>81.01±0.17</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>12</td>
<td>82.77±0.78*</td>
<td>80.11±0.30*</td>
</tr>
<tr>
<td>Vehicle²</td>
<td>8</td>
<td>85.05±0.27</td>
<td>81.20±0.20</td>
</tr>
<tr>
<td>Glycerol</td>
<td>10</td>
<td>82.15±0.30**</td>
<td>79.29±0.25**</td>
</tr>
</tbody>
</table>

Each value represents a mean±S.E.M. Nimodipine vehicle¹ (composition is given in the text), nimodipine (30 µg/kg), glycerol (4 g/kg) and glycerol vehicle² (physiological saline) were administered intravenously immediately after recirculation. Animals were killed 3 hr after reperfusion (6 hr after induction ischemia). parietal c., parieto-temporal cortex; frontal c., frontal cortex. *: P<0.05, **P<0.01 vs. vehicle, Dunnett’s multiple range test.
Table 2. Effect of nimodipine on mortality after left MCA occlusion (3 hr) and reperfusion in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sham (n=5)</th>
<th>Vehicle (n=15)</th>
<th>Nimodipine (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Reperfusion</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 hr</td>
<td>0</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>47</td>
<td>0*</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>87</td>
<td>27**</td>
</tr>
<tr>
<td>24</td>
<td>0</td>
<td>100</td>
<td>82</td>
</tr>
<tr>
<td>7 days</td>
<td>0</td>
<td>100</td>
<td>82</td>
</tr>
</tbody>
</table>

*P<0.05. **P<0.01 vs. Vehicle. Fisher’s exact probability test.

2. Mortality: Effect of nimodipine on animal mortality after transient focal ischemia is summarized in Table 2. At 3, 6, 9, 24 hr and 7 days after reperfusion, the mortality of the nimodipine vehicle group was 7, 47, 87, 100 and 100%, respectively. A time-dependent increase in mortality was observed following reperfusion. On the other hand, the mortality of the nimodipine group was 0, 0, 27, 82 and 82% at 3, 6, 9, 24 hr and 7 days after reperfusion, respectively, with statistical significance at 6 and 9 hr after reperfusion.

3. 45Ca autoradiography and histological study: Figure 2 shows typical 45Ca autoradiograms of each experimental group. After 3 hours of ischemia followed by 3-hr reperfusion, 45Ca accumulation and neuronal cell damage were high in the dorsolateral striatum, moderate in the MCA cortex, and slight in the anterior cerebral artery (ACA) cortex (Fig. 2, a, b, data of histological findings not shown). The contralateral areas and sham-operated rats showed no abnormal calcium accumulation and neuronal cell damage (Fig. 2, g, h). The abnormal 45Ca accumulation distribution was in close accordance with the neuronal cell damage area in the histological findings. Nimodipine partly attenuated the abnormal 45Ca accumulation in the MCA and ACA cortex, not in the striatum, in two of three animals (Fig. 2, c, d). On the other hand, glycerol attenuated the abnormal 45Ca accumulation in all areas (Fig. 2, e, f).

Discussion

The present results showed that the increase in tissue water content was accentuated by reperfusion following ischemia, correlating well with previous reports (19–21). Calcium antagonists have been reported to be beneficial for the treatment of permanent MCA occlusion in rats (6, 14). In these models, the beneficial effects of drugs were obtained by influencing only the peripheral areas surrounding the ischemic core, where drugs could not reach. We observed that nimodipine administered immediately after recirculation attenuated the formation of brain edema in the areas perfused not only by MCA (parieto-temporal cortex) but also by ACA (frontal cortex), although water content in the striatum was not reduced by nimodipine treatment. Furthermore, nimodipine partly attenuated the 45Ca accumulation in the MCA and ACA areas. In contrast, glycerol, a hyperosmotic agent, was potent enough to ameliorate brain edema and water content in the striatum ipsilateral to the MCA occlusion.

One reason why nimodipine failed to save the striatum seems to be that the damage to the striatum was greater than that to the cortex. Abe et al. (22) has reported that recovery from brain edema and of protein synthesis following a 1-hr ischemia in the same animal model used in our study differed between the cerebral cortex and the striatum, and that the inhibition of protein synthesis and the formation of brain edema began sooner in the striatum than in the cortex. Recently, it has been generally accepted that an excitotoxic mechanism is involved in the neuronal damage that occurs after ischemia.
Marked calcium accumulation is noted in the dorsolateral striatum, the MCA cortex and the anterior cerebral artery (ACA) cortex. Abnormal calcium accumulation was attenuated partly in the MCA and ACA cortex. Abnormal calcium accumulation was attenuated almost completely in the MCA cortex, ACA cortex and striatum. No abnormal calcium accumulation is noted.

The dorsolateral striatum has been observed to be rich in receptors of excitatory neurotransmitters such as glutamate (24) and dopamine (25). Receptors of inhibitory neurotransmitters such as GABA and benzodiazepine have been found to exist in smaller amounts in the striatum than in the cortex (26). The release of glutamate and dopamine have been seen to greatly increase during ischemia and/or the early period of reperfusion (27, 28). Therefore, the damage to the striatum may be more severe than that to the cortex.
cortex because of the imbalance of excitatory and inhibitory innervation.

Although the mechanisms of such beneficial effects of nimodipine seen in the present study on MCA occlusion-induced brain edema in rats are by no means definitely established, there seem to be at least two possible explanations. The first one is an improvement of postischemic hypoperfusion. It has been suggested that the neurologic damage that occurs subsequent to ischemia is due at least in part to a delayed hypoperfusion state following reperfusion (29, 30). Steen et al. has reported previously that infusion of the nimodipine, administered before or after ischemia (7, 8), improved both the postischemic cerebral blood flow and the neurologic outcome. Similar results with nimodipine have been reported by Hofmeister et al. (31) and Kazda et al. (32).

The second one is a reduction of Ca2+ overload in the neurons. Recently, it has been reported that a massive influx of calcium occurs during the reperfusion period and may result in further damage to brain cells. This influx may occur as the result of other reperfusion injuries, such as lipid peroxidation and acidosis (2, 33). Furthermore, Uematsu et al. (34) has reported that nimodipine prevents an increase in cytosolic free calcium concentrations following cerebral ischemia in vivo. Therefore, nimodipine may prevent partly the formation of brain edema by a direct action (blocking the entry of calcium ions into the cell) on neurons.

The effect of calcium antagonists on the histopathological outcome after cerebral ischemia is still unclear. Interestingly, studies that reported the beneficial effect of calcium antagonists analyzed the drug effects at earlier periods after ischemia (6, 14). The beneficial effect of nimodipine on animal mortality after transient left MCA occlusion was shown only at 6 and 9 hr after reperfusion but not at 24 hr and 7 days. Taken together, later administration of nimodipine may be less effective for the prevention of ischemic brain damage. However, since we administered nimodipine only once, repeated infusion of the drug might ameliorate animal survival.

In conclusion, nimodipine treatment is beneficial in early phases of the recirculation period. The present reversible MCA occlusion model provides a good system for estimating drug effects against ischemic brain damage and is of value for simulating human stroke.

References
Brain Edema and Nimodipine

336 (1984)


