Lack of Effect of PN 200-110 on Neuronal Injury and Neurological Outcome in Middle Cerebral Artery-Occluded Rats

M. Marinov and H. Wassmann

We evaluated the influence of a continuous intravenous infusion of 0.24 mg/kg PN 200-110 started 20 minutes before the induction of ischemia and continued for 2 hours on infarct size, histopathology, and neurological outcome in middle cerebral artery-occluded rats treated with PN 200-110 (n=8), placebo (n=7), or saline (n=8). Neurological examination was performed 24 hours after occlusion. We quantified infarct size by 2,3,5-triphenyl-tetrazolium chloride, hematoxylin and eosin, and Nissl staining and by computerized analysis of tracings of the infarcted areas and evaluated neuronal injury at the infarct periphery. The different types of ischemic cell damage were quantified by direct visual counting. We found no differences among saline-, placebo-, and PN 200-110-treated rats regarding infarct size, amount of neuronal alteration, and neurological outcome. Our results indicate the lack of a significant protective effect of this drug in experimental focal ischemia. (Stroke 1991;22:1064–1067)

In a search for effective cerebroprotective agents for use in neurosurgical practice, we became interested in dihydropyridine calcium antagonists. In another experimental study,1 we demonstrated the therapeutic efficacy of nimodipine in lessening the extent of focal ischemia and the severity of peri-infarct neuronal injury. We explained these beneficial effects predominantly by the vasoactive properties of the drug, which increases cerebral blood flow (CBF) in the penumbra zone to above the ischemic threshold. Despite existing controversies regarding the mechanisms of the anti-ischemic action of calcium channel blockers, their most probable role is in restoration of tissue perfusion at the infarct periphery, rather than in protection of the neuronal cell by preventing Ca2+ overload.2–6 On the other hand, some calcium antagonists are considered dangerous since they may worsen postischemic brain edema in experimental conditions by increasing the blood flow threshold for ion homeostasis (impaired membrane protection) and by increasing blood flow.7–10

Recently, some investigators have reported encouraging results in ameliorating cell damage and reducing brain edema by using a new dihydropyridine calcium antagonist, PN 200-110.11,12 In this study we evaluated the cerebroprotective properties of this drug.

Materials and Methods

We permanently occluded the right middle cerebral artery (MCA) in 23 male Fischer-344 rats weighing 250–300 g by means of microsurgical transcra-nial exploration and coagulation.13 The MCA trunk was occluded by microbipolar coagulation from a point proximal to the origin of the lateral striate artery to 1 mm beyond where the vessel crosses the olfactory striae.14 The animals were anesthetized with 50 mg/kg i.m. ketamine HCl and 2 mg/kg i.m. xylazine. The left femoral artery and vein were cannulated with polyethylene catheters to allow continuous blood pressure monitoring, arterial blood sampling, and drug administration. Animals were maintained at normothermia by external heating.

Isradipine (PN 200-110 N) was donated by Sandoz AG, Nureenberg, FRG. Based on practical experience11 and recommendations from Sandoz, PN 200-110 was used at a concentration of 0.24 mg/kg; 1 mg of the substance was dissolved in 1 mg of 96% ethanol plus 1 ml of polyethylene glycol 400 and then diluted with 5% glucose to the desired volume. Starting 20 minutes before MCA occlusion, the solution was administered intravenously to eight rats for 2 hours at a rate of 0.4 ml/hr. The drug, catheters, and syringes were protected from light. The control group (n=8) and the placebo group (n=7) received corresponding volumes of saline and solvent, respectively.
Anesthesia was maintained until the end of drug administration, and after recovery the rats were returned to their cages. A simple neurological examination was performed 24 hours after MCA occlusion using the following scale: 0, no observable deficit; 1, forelimb flexion; 2, decreased resistance to lateral pushing without circling; and 3, the same as 2, but with circling.14

Immediately after neurological examination the rats were killed, and the brains were rapidly removed and sectioned coronally at 5 and 7 mm from the frontal pole. The sections were immersed for 30 minutes at 37°C in a 2% solution of 2',3',5'-triphenyl-2H-tetrazolium chloride. The anterior surface of a 2-mm-thick brain slice at the level of the anterior commissure was photographed, and the brains were then fixed in 10% formalin for further paraffin processing. Histological 7-μm-thick sections stained with hematoxylin and eosin and cresyl violet corresponding to stereotactic plane A 7,470 μm from the atlas of König and Klippel15 were always used. A very good correlation between this plane and the photographed color changes was observed. Tracings of the photographed infarcted areas were quantified by a computerized image analysis system and simultaneously by cutting out and weighing traced sections. Cortex and basal ganglia infarcts were outlined separately, and their sizes were calculated as a percentage of the total cross-sectional area. Neuronal injury in the infarct periphery (a zone 1 mm wide at the dorsal lateral cortical infarct margin) was assessed by a pathologist who was unaware of the experimental conditions employed. Direct visual counting of neurons with different types of ischemic injury was carried out.

The results, presented as mean±SEM, were statistically analyzed using Student's unpaired t test. A probability value less than 0.05 was considered significant.

**Results**

The values of PaCO₂, PaO₂, arterial pH, glucose level, hemoglobin, and hematocrit remained within the physiological ranges during the experiment (data not shown). In all groups mean arterial blood pressure was reduced following MCA occlusion (Figure 1), but not to <85 mm Hg. Thus, the values were stable above the threshold for maintenance of normal cortical blood flow.8 There was no significant difference in neurological outcome among groups (data not shown). Table 1 demonstrates the infarct sizes expressed as a percentage of the total cross-sectional area at the level photographed. The size and distribution of the whole infarct, as well as of its cortical and striatal components, showed no significant differences among groups.

In most histological sections we observed a border of markedly vacuolated neuropil in the cortical dorsolateral region (peri-infarct zone). This swollen tissue contained both injured and normal-appearing neurons (selective neuronal injury according to DeGirolami et al16 and Nedergaard17). At 1 mm from the cortical infarct rim (containing according to Nedergaard18 the majority of selectively injured neurons), we found damaged neurons in all cortical layers, but predominately the upper one. The ischemic neuronal changes were subdivided into type I, cells with triangular and dark nuclei with peripherally located Nissl substance; type II, pale, swollen cells with peripheral tigrolysis; type III, shrunken cells with dark, dense cytoplasm and pyknotic nuclei; and type IV, severely injured neurons, which were diffusely darkened without identifiable nuclei and had vacuolated cytoplasm. Because of their rarity, types I and II are not given separately

**Table 1. Relative Infarct Size 24 Hours After Middle Cerebral Artery Occlusion in Rats**

<table>
<thead>
<tr>
<th>Region</th>
<th>Control (n=8)</th>
<th>Placebo (n=7)</th>
<th>PN 200-110 (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortex</td>
<td>9.7±1.2</td>
<td>9.49±1.75</td>
<td>8.08±1.7</td>
</tr>
<tr>
<td>Basal ganglia</td>
<td>11.1±0.7</td>
<td>10.22±0.31</td>
<td>10.49±0.58</td>
</tr>
<tr>
<td>Total</td>
<td>20.8±1.4</td>
<td>19.7±1.75</td>
<td>18.57±2.19</td>
</tr>
</tbody>
</table>

Values are mean±SEM percentage of cross-sectional area.
in Table 2. Neuronal damage in the immediate peri-
infarct zone did not differ significantly among groups.
It was not possible with our preparation technique to
hypothesize whether type II and III neurons could be
considered to indicate definitive neuronal death since
they are not acidophilic.

Discussion
PN 200-110 is a derivative of 1,4-dihydropyridine
with a potent calcium blocking action on cerebral
arteries in vitro. In vivo studies with the drug, using
chronic rat models with MCA occlusion, have been published. The former study demonstrates
reduction of the extent of the infarction and improvement of the neurological outcome, as well as of the histological and biochemical data. Only relative CBF was estimated, and the authors state that relative CBF was affected less than the parameters reflecting edema. On the other hand, the results of Abe et al. indicate inhibition of brain edema formation by PN 200-110 that the authors explain by the drug’s prevention of a massive influx of calcium into brain cells. However, CBF was not measured.

The dosage and route of drug administration differed significantly between those studies and ours. We chose pretreatment and posttreatment by continuous intravenous infusion for several reasons. First, this mode of administration is closer to the presumed intraoperative situation in which intentional or urgent interruption of the blood flow in major cerebral vessels is necessary. Second, it is known that the preischemic administration of calcium antagonists allows more effective neuroprotection than postisch-
emic treatment. Considering also the evidence from the literature that calcium antagonists act predominantly by improving postischemic collateral per-
fusion (penumbra “rescue”), we continued postischemic infusion to support tissue perfusion, achieving drug saturation of the periphery of the infarct. We found this mode of administration to be effective in ameliorating ischemic injury when using nimodipine, which supports the above speculations.

In our study continuous preischemic and postisch-
emic treatment with PN 200-110 was not associated with any significant reduction of infarct size or peri-
infarct neuronal alterations or with improved neurological outcome. The lack of a protective action of the drug on the penumbra of the lesion could be explained by insufficient vasoactive properties, but direct CBF studies are needed to support this hypothesis.

Table 2. Quantitative Assessment of Neuronal Injury Adjacent to Infarct in Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Types I-IV</th>
<th>Type III</th>
<th>Type IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>82.9 ± 2.19</td>
<td>72.9 ± 3.4</td>
<td>8.3 ± 1.3</td>
</tr>
<tr>
<td>Placebo</td>
<td>7</td>
<td>85.24 ± 3.84</td>
<td>74.9 ± 3.74</td>
<td>9.38 ± 1.35</td>
</tr>
<tr>
<td>PN 200-110</td>
<td>8</td>
<td>84.15 ± 3.64</td>
<td>72.46 ± 4.68</td>
<td>10.01 ± 1.87</td>
</tr>
</tbody>
</table>

Values are mean±SEM percentage of total neurons.

References
12. Sauter A, Rudin M: Calcium antagonists reduce the extent of infarction in rat middle cerebral artery occlusion model as determined by quantitative magnetic resonance imaging. Stroke 1986;17:1228–1234

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**KEY WORDS** • calcium channel blockers • cerebral ischemia • neuroprotection • rats
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