Effect of Cyclooxygenase and Lipoxygenase Inhibitors on Delayed Neuronal Death in the Gerbil Hippocampus

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The purpose of our study was to examine whether cyclooxygenase and lipoxygenase inhibitors ameliorate delayed neuronal death in the hippocampal CA1 sector in Mongolian gerbils after 5 minutes of forebrain ischemia. Gerbils were injected intraperitoneally with cyclooxygenase inhibitors piroxicam and flurbiprofen or with lipoxygenase inhibitors AA-861 and BW-755C. Seven days after ischemic insult, the animals were perfusion-fixed, and the neuronal density in the hippocampal CA1 sector was estimated. The average neuronal density in unoperated normal gerbils was 247±9/mm (mean±SEM). In ischemic gerbils with vehicle administration, the average neuronal densities were 13±2, 14±2, 13±2, and 13±1 for piroxicam, flurbiprofen, AA-861, and BW-755C, respectively. The average neuronal densities in ischemic gerbils treated with 1.5 and 10 mg/kg piroxicam and 1.5 and 10 mg/kg flurbiprofen were 13±2, 194±9, 19±5, and 143±12, respectively. In ischemic gerbils treated with 15 and 100 mg/kg AA-861 and 30 mg/kg BW-755C, the average neuronal densities were 12±1, 13±1, and 14±2, respectively. At their higher doses, both piroxicam and flurbiprofen significantly (p<0.01) ameliorated delayed neuronal death in the hippocampal CA1 sector. Our results suggest that cyclooxygenase products play an important role in the development of delayed neuronal injury after cerebral ischemia. (Stroke 1989;20:925-929)
TABLE 1. Ninety-Eight Mongolian Gerbils Treated With Cyclooxygenase Inhibitor, Lipoxygenase Inhibitor, or Vehicles After 5-Minute Bilateral Artery Occlusion

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No.</th>
<th>Average neuronal density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal, unoperated</td>
<td>10</td>
<td>247±9</td>
</tr>
<tr>
<td>Ischemic, treated with cyclooxygenase inhibitors or vehicles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Piroxicam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (3% sodium bicarbonate)</td>
<td>8</td>
<td>13±2</td>
</tr>
<tr>
<td>1.5 mg/kg</td>
<td>8</td>
<td>13±2</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>8</td>
<td>194±9</td>
</tr>
<tr>
<td>Flurbiprofen</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (physiologic saline)</td>
<td>8</td>
<td>14±2</td>
</tr>
<tr>
<td>1.5 mg/kg</td>
<td>8</td>
<td>19±5</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>8</td>
<td>143±12</td>
</tr>
<tr>
<td>Ischemic, treated with lipoxygenase inhibitors or vehicles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA-861</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (dimethyl sulfoxide)</td>
<td>8</td>
<td>13±2</td>
</tr>
<tr>
<td>15 mg/kg</td>
<td>8</td>
<td>12±1</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>8</td>
<td>13±1</td>
</tr>
<tr>
<td>BW-755C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (physiologic saline)</td>
<td>8</td>
<td>13±1</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>8</td>
<td>14±2</td>
</tr>
<tr>
<td>Total</td>
<td>98</td>
<td></td>
</tr>
</tbody>
</table>

Materials and Methods

We used ninety-eight adult male Mongolian gerbils weighing 60–70 g in our experiment (Table 1). They were anesthetized with 2% halothane in 70% nitrous oxide and 30% oxygen, and the body temperature was maintained at 37°C with a heating blanket. The bilateral carotid arteries at the neck were exposed microsurgically and occluded with aneurysm clips (Sugita temporary clip type 07-940-51, Mizuho Ikakogyo Co. Ltd., Tokyo, Japan) for 5 minutes. Anesthesia was discontinued as soon as the clips were placed. Thirty minutes before the ischemic insult, gerbils were injected intraperitoneally with cyclooxygenase or lipoxygenase inhibitor. As cyclooxygenase inhibitors, 1.5 and 10 mg/kg piroxicam (Taito Pfizer Company, Tokyo, Japan) dissolved with 3% sodium bicarbonate or 1.5 and 10 mg/kg flurbiprofen (Kakenyaku-kako Company, Tokyo, Japan) dissolved with physiologic saline were used. For lipoxygenase inhibitors, 15 and 100 mg/kg AA-861 (Takeda Chemical Company, Osaka, Japan) dissolved with 3% sodium bicarbonate or 1.5 and 10 mg/kg BW-755C dissolved with physiologic saline were used. For lipoxygenase inhibitors, 15 and 100 mg/kg AA-861 (Takeda Chemical Company, Osaka, Japan) dissolved with dimethyl sulfoxide or 30 mg/kg BW-755C dissolved with physiologic saline were used (Wellcome Research Laboratories, Beckenham, Kent, United Kingdom). The inhibitory action of AA-861 is specific for 5-lipoxygenase.18 BW-755C inhibits 5-, 12-, and 15-lipoxygenases as well as cyclooxygenase.19-23

The volume of these administered solutions was fixed at 0.1 ml/10 g body wt except for the AA-861 solution. The AA-861 solution was administered at 0.01 ml/10 g body wt, followed by physiologic saline of 0.09 ml/10 g body wt. Gerbils injected with the vehicle served as controls. In gerbils injected with piroxicam or flurbiprofen, 1 mg/kg of an H2 blocker, famotidine (Yamanouchi Pharmaceutical Co., Ltd., Tokyo, Japan), dissolved with physiologic saline was administered intraperitoneally just after clip removal and was repeated twice daily for 3 days to prevent gastrointestinal bleeding. Ten unoperated normal gerbils served as normal controls.

After surgery, gerbils were returned to their cages and permitted free access to food and water. One week after ischemia, these gerbils were perfusion-fixed. Under deep pentobarbital anesthesia, fixation was performed by transcardiac perfusion at a pressure of 120 cm H2O using 3.5% formaldehyde in 0.1 M phosphate buffer (pH=7.3). Fixed gerbils were refrigerated overnight, and the brains were dissected out the following day. Two-millimeter-thick coronal sections were cut, dehydrated through graded series of ethanol, soaked in xylene, and embedded in paraffin. Six-micrometer-thick sections containing the dorsal hippocampus were prepared and stained with hematoxylin and eosin or Luxol fast blue and cresyl violet.

These sections were examined in a blind fashion as reported by Kirino et al.24 One section from each gerbil was used for counting the number of intact neurons in the CA1 sector, since similar neuronal change is seen throughout the rostral-caudal extent of the dorsal hippocampus. Photographs of left and right dorsal hippocampi of each specimen were
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Figure 1. Effect of piroxicam and flurbiprofen on neuronal cell density per 1 mm linear length of hippocampal CA1 sector. Piroxicam (10 mg/kg) and flurbiprofen (10 mg/kg) ameliorated delayed neuronal death of CA1 sector. Values are mean ± SEM. **p<0.01 (vs. vehicle).

In the 10 unoperated normal gerbils, the average neuronal cell density of the CA1 sector was 247±9/mm (mean±SEM) (Figure 1). In gerbils injected with the vehicles 3% sodium bicarbonate for piroxicam or physiologic saline for flurbiprofen, extensive neuronal damage was observed. Average neuronal densities in the CA1 sector were 13±2/mm (n=8) for piroxicam and 14±2/mm (n=8) for flurbiprofen. There was no significant difference in the neuronal densities between these two groups (Figure 1). The CA1 pyramidal neurons were well preserved by treatment with a higher dose of piroxicam or flurbiprofen. At a lower dose of each drug, however, extensive neuronal damage was observed. Average neuronal densities in ischemic gerbils injected with 1.5 and 10 mg/kg piroxicam and 1.5 and 10 mg/kg flurbiprofen were 13±2/mm (n=8), 19±4/mm (n=8), 19±5/mm (n=8), and 14±12/mm (n=8), respectively (Figure 1). Neuronal density in gerbils injected with higher doses of each cyclooxygenase inhibitor was significantly higher than in gerbils injected with a respective vehicle (p<0.01).

In gerbils injected with the vehicles (physiologic saline for BW-755C or dimethyl sulfoxide for AA-861), extensive neuronal damage was observed in the CA1 sector. The average densities of intact neurons were 13±1/mm (n=8) and 13±1/mm (n=8), respectively (Figure 2). The CA1 pyramidal neurons were not preserved by treatment with either lipooxygenase inhibitor AA-861 or BW-755C (Figure 2). Average neuronal densities in ischemic gerbils injected with 15 and 100 mg/kg AA-861 and 30 mg/kg BW-755C were 12±1/mm (n=8), 13±1/mm (n=8), and 14±2/mm (n=8), respectively (Figure 2). No significant difference was noted in the neuronal densities between gerbils treated with each lipooxygenase inhibitor and those injected with a respective vehicle.

Discussion

In our experiment, both cyclooxygenase inhibitors piroxicam and flurbiprofen, at their higher doses, ameliorated delayed neuronal death in the hippocampal CA1 sector after forebrain ischemia. Our results are nearly consistent with those of our previous experiment in which indomethacin at doses of 1, 2, 5, and 10 mg/kg ameliorated delayed neuronal death in gerbils, suggesting that cyclooxygenase metabolites of arachidonic acid are involved in the development of delayed neuronal death. A small discrepancy seems to arise, however, between our results with piroxicam and flurbiprofen and...
FIGURE 2. Effect of AA-861 and BW-755C on neuronal cell density per 1 mm linear length of hippocampal CA1 sector. Neither AA-861 nor BW-755C inhibited delayed neuronal death of CA1 sector. Values are mean ± SEM.

In conclusion, our present experiments demonstrate that cyclooxygenase metabolites of arachidonic acid rather than 5- or 12-lipoxygenase metabolites may play an important role in the development of delayed neuronal death of the hippocampal CA1 sector in the ischemic gerbil brain.

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