Effects of a New Thyrotropin-Releasing Hormone Derivative on Behavioral Changes After Focal Cerebral Ischemia in Rats

Minoru Yamamoto, PhD, Akira Tamura, MD, Takaaki Kirino, MD, Masao Shimizu, MS, and Keiji Sano, MD

We observed the effects of a new thyrotropin-releasing hormone derivative, YM-14673 (N\(^{-}\)-[(S)-4-oxo-2-azetidinyl]carbonyl-L-histidyl-L-prolinamide dihydrate), on behavioral changes in rats for 3 weeks after focal cerebral ischemia. Under halothane anesthesia, the left middle cerebral artery was occluded via a transretro-orbital approach. YM-14673 was administered just after the operation and once a day for 3 weeks. Neurologic deficits, including hemiplegia and abnormal posture, and disturbance of passive avoidance learning were present in solvent-treated control rats for the entire 3 weeks. YM-14673 at 0.1 or 0.3 mg/kg i.p. or 1 mg/kg p.o. significantly accelerated the recovery of neurologic deficits and ameliorated cognitive disturbance compared with the solvent-treated controls although the drug at 0.1 and 0.3 mg/kg i.p. did not influence the size of the ischemic infarct. YM-14673 mitigated the behavioral disturbances in this model of chronic focal cerebral ischemia. We also discuss the suitability of this model for the evaluation of drugs. (Stroke 1989;20:362-366)

Pathologic and functional changes during the acute phase of focal cerebral ischemia have been well investigated.\(^1\)-\(^5\) These changes can be modulated by treatment with various drugs, such as cerebral metabolism enhancers,\(^6\) cerebral vasodilators,\(^6\)-\(^9\) opioid antagonists,\(^10\) and central nervous system depressants.\(^10\)-\(^12\) On the other hand, there are only a few reports concerning pathologic and functional changes during the chronic phase of focal cerebral ischemia.\(^11\) Therefore, we monitored behavioral changes for 16 weeks after occlusion of the middle cerebral artery (MCA) in rats to investigate functional changes during the chronic phase of this model.\(^13\),\(^14\) In previous studies we observed neurologic deficits for 4 weeks after MCA occlusion, and learning behavior in a passive avoidance task was disturbed for 8 weeks when the rats were trained 3 days after MCA occlusion.\(^13\),\(^14\)

Yasuhara and Naito\(^15\) reported that thyrotropin-releasing hormone (TRH) decreased the threshold for evoked muscular discharge induced by electrical stimulation of the brain reticular formation in rabbits. Yamazaki et al\(^16\)-\(^19\) reported that shortening of the latency of a passive avoidance task in rodents subjected to anoxia and treated with cycloheximide or scopolamine was prolonged by administration of TRH. These observations indicate that TRH possesses facilitatory effects on motor function and learned behavior. Recently, Latham et al\(^19\) reported that TRH analogues (RX77368 and CG3509) with long lives prevented the loss of cortical somatosensory evoked potentials in MCA-occluded rats. Therefore, long-acting TRH analogues may show protective action against cerebral ischemia.

Since TRH is known to be rapidly metabolized in the body,\(^20\),\(^21\) we have searched for TRH analogues that are more potent and last longer than TRH. A new TRH derivative, YM-14673 (N\(^{-}\)-[(S)-4-oxo-2-azetidinyl]carbonyl-L-histidyl-L-prolinamide dihydrate) (Figure 1), was found to possess analeptic actions such as antagonizing the effects of pentobarbital-induced sleeping time in mice and ameliorating the effects of conscious disturbance in mice with concussions.\(^22\) YM-14673 is approximately 10–100 times more potent than TRH in the context of central nervous facilitatory activities.\(^22\) The half-life of YM-14673 is approximately five times that of TRH in animals and humans (H. Imazaki, personal communication), and the analeptic activities of YM-14673 last approximately 8–36 times longer than those of TRH.\(^22\) Our aim in this study was to

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describe the effects of YM-14673 on behavioral changes, including neurologic deficits and disturbance of learned behavior, during 3 weeks following focal cerebral ischemia in rats.

Materials and Methods

We used 67 male Wistar rats, each weighing approximately 300 g, housed in group cages under 12-hour light:dark conditions and given free access to laboratory chow and water. The rats were anesthetized with 2% halothane, and the proximal portion of the left MCA was permanently occluded using a microsurgical technique modified from our original method (that developed by Tamura et al) for the purpose of chronic experiments. The stem of the MCA was electrocauterized just medial to the olfactory tract and was cut to ensure the completeness of the vascular occlusion.

YM-14673 was prepared in our laboratories and dissolved in saline or distilled water. The drug administrations were begun just after the operation and were repeated once a day for 3 weeks. YM-14673 (0.03, 0.1, or 0.3 mg/kg) or saline in a volume of 0.1 ml/100 g was administered intraperitoneally in 36 rats, and 31 rats received the drug (0.1 or 1 mg/kg) or distilled water in a volume of 0.1 ml/100 g orally. Neurologic deficits and learning behavior were observed randomly 30 or 60 minutes after intraperitoneal or oral administration, respectively, by two persons who did not know to which group the rat had been assigned.

One, two, and three weeks after MCA occlusion, the effects of YM-14673 were evaluated by the degree of hemiplegia when the rats' right legs were lifted by a bar and by the degree of abnormal posture when the rats were lifted by their tails. Each sign was scored using the following criteria: 0, no abnormality; 1, mild abnormality; and 2, severe abnormality. Neurologic deficits were assessed by combining the hemiplegia and posture scores. Therefore, a score of 0 is the lowest and a score of 4 is the highest possible.

The rats were trained according to the step-through procedure described by Jarvik and Kopp. Three days after MCA occlusion, each rat was placed in an illuminated safe compartment (20×15×25 cm), which had a grid on the floor. Once the rat's four paws were on the grid a scrambled foot shock (60 V, 50 Hz) was delivered to the grid, and the rat could escape the shock only by stepping back into the illuminated safe compartment. In the test trials given 4 days and 2 and 3 weeks after the operation, the rat was again placed in the safe compartment and the response latency (time to enter the dark compartment) was measured. Results were recorded as the average of the response latency for each group of rats. The response latency of rats that did not move into the dark compartment during the observation period was 600 seconds.

Three weeks after the operation, 31 rats receiving saline (n=8) or 0.03 (n=7), 0.1 (n=8), or 0.3 (n=8) mg/kg i.p. YM-14673 were perfusion-fixed with a 35% formaldehyde solution. After perfusion, the rats were decapitated and the brain was removed. The hindbrain was detached by cutting through the midbrain, and the cerebral hemispheres were cut into five coronal slices, each 2 mm thick. Sections 7–8 μm thick were stained using hemalum and eosin and by a method combining cresyl violet and Luxol fast blue. Each section was photographed through the light microscope, and infarct size was measured using an Avionics Japan TVIP-2000 image analyzer by one of us without knowledge of the rat's history.

Significant differences between groups and their respective saline- or distilled water-treated controls were determined using analysis of variance followed by the Mann-Whitney U test.

Results

Death rates during the 3 weeks after MCA occlusion are given in Table 1. As previously reported, body weight in MCA-occluded rats decreased significantly compared with unoccluded rats approximately 1 week after the operation. In this study, a decrease in body weight was observed in both solvent- and drug-treated rats. Therefore, the decrease in body weight may be attributed to the operation and not to the administration of solvent or drug. Similar death rates were observed during 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th>n</th>
<th>Dying rats</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intraperitoneal administration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td>9</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>YM-14673 (mg/kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.03</td>
<td>9</td>
<td>2</td>
<td>22</td>
</tr>
<tr>
<td>0.1</td>
<td>9</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>0.3</td>
<td>9</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td><strong>Oral administration</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>12</td>
<td>2</td>
<td>17</td>
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<tr>
<td>YM-14673 (mg/kg)</td>
<td></td>
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</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>2</td>
<td>20</td>
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<tr>
<td>1</td>
<td>9</td>
<td>2</td>
<td>22</td>
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</table>
weeks after MCA occlusion in each group; therefore, results corresponding to dying rats were excluded from subsequent analysis.

Maximal neurologic deficits were observed 1 week after MCA occlusion and then gradually recovered, as shown in Figure 2. These changes agree with those we described previously.1314 YM-14673 at 0.1 and 0.3 mg/kg i.p. (Figure 2, left) and 1 mg/kg p.o. (Figure 2, right) significantly accelerated the recovery of neurologic deficits 2 and 3 weeks after MCA occlusion compared with the respective solvent-treated controls.

Mean±SEM response latency in the passive avoidance task in the saline-treated group was 55±14, 26±6, and 36±7 seconds in eight rats 4 days and 2 and 3 weeks after the operation, respectively (Figure 3, top). As previously reported by Yamamoto et al.,14 mean±SEM response latency was 170±43 (n=8) and 53±18 (n=6) seconds after 2 weeks in unoccluded and MCA-occluded saline-treated rats, respectively. Thus, response latency in the passive avoidance task in the current MCA-occluded group was significantly shorter than that in the previously reported nonoccluded group, in agreement with the reports of Tamura et al.13 and Yamamoto et al.14 YM-14673 at 0.1 or 0.3 mg/kg i.p. (Figure 3, top) and 1 mg/kg p.o. (Figure 3, bottom) significantly prolonged the shortened response latency 4 days and 2 and 3 weeks after MCA occlusion compared with the respective solvent-treated controls.

In a preliminary study using normal rats, YM-14673 in doses not affecting spontaneous movement did not affect the response latency.

As described by Tamura et al.,2325 there was a remarkably constant pattern of ischemic brain damage in saline-treated MCA-occluded rats. Ischemic damage was commonly observed in the cerebral cortex of the frontal, sensorimotor, and auditory areas and in the lateral segment of the caudate nucleus. The average percent infarcted area of both
hemispheres in five coronal sections in saline- and YM-14673-treated groups is given in Table 2. There was no significant difference in infarcted area between groups. In one rat treated with 0.1 mg/kg i.p. YM-14673, the infarcted area was extremely large because of intracerebral hematoma due to operative failure. The infarct size in the saline-treated rats was relatively smaller than that observed by Tamura et al.25 Our neuropathologic study was done 3 weeks after MCA occlusion, however, so some of the differences in infarct size may be due to atrophy during 3 weeks after the occlusion.

**Discussion**

We have reported behavioral changes, including neurologic deficits and learning disturbance, during the chronic phase after focal cerebral ischemia in rats.13,14 There is little precedent for evaluating drug action in this model. This study was designed to examine the effects of YM-14673 on neurologic deficits and disturbance of learned behavior observed during 3 weeks following MCA occlusion. Interestingly, YM-14673 significantly accelerated the recovery of neurologic deficits, including hemiplegia and postural abnormality. In addition, the shortened latency of step-through caused by MCA occlusion in the passive avoidance task was prolonged by administration of YM-14673, indicating that this drug ameliorated the ischemia-induced amnesia. However, the size of ischemic cerebral infarcts 3 weeks after MCA occlusion was not reduced by treatment with the new TRH analogue.

With respect to evaluation of the cerebral antischismic properties of various drugs (such as vasodilators and cerebral metabolic enhancers), their effects on physiologic and functional disturbances have been observed only during the acute phase after focal cerebral ischemia.6-10,12,19,26 However, it is of great importance to perform animal experiments using a chronic cerebral ischemia model to develop drug therapies for the chronic phase of patients with cerebrovascular diseases. YM-14673 ameliorated the behavioral disturbances during the chronic phase of this focal cerebral ischemia model and may be useful as a therapeutic agent in patients during the chronic phase of cerebrovascular diseases and other organic brain syndromes.

We propose a mechanism for the ameliorating effects of YM-14673 on hemiplegia and disturbed learned behavior. In this chronic cerebral ischemia model in rats, hemiplegia may be induced mainly by a disturbance of pyramidal motor function. Therefore, agents with facilitatory effects on the pyramidal motor system may be useful for the treatment of hemiplegia in cerebral vascular disease. TRH seems to enhance the function of the pyramidal motor system.15 Thus, the ameliorating effects of YM-14673 on hemiplegia may be due to its facilitatory effects on the pyramidal system. Involvement of central monoaminergic27 and cholinergic systems29 is well known in regulating learned behavior. Indeed, YM-14673 as well as TRH possess facilitatory effects on central monoaminergic29 and cholinergic30 systems in rodents. Interestingly, there are many reports concerning the antiannemic activity of TRH in mice.16-18 Therefore, ameliorating effects of YM-14673 and TRH on the disturbance of learned behavior may be attributable to their facilitatory effects on the central nervous system such as the monoaminergic and cholinergic systems. In addition, Schmidt-Achetz et al30 reported that TRH exerts a trophic influence on long-term cultures of fetal rat motor neurons. Therefore, synaptic plasticity in the face of damage observed in MCA-occluded rats may be promoted by the TRH analogue.

Latham et al19 reported that long-lived TRH analogues (RX77368 and CG3509), unlike TRH, prevented the loss of cortical somatosensory evoked potentials in rats subjected to MCA occlusion; therefore, long-acting TRH analogues may show protective action against cerebral ischemia. We have already reported that YM-14673 acts longer than TRH in facilitating central nervous system activity.22 Therefore, the ameliorating effects of YM-14673 against hemiplegia and disturbed learned behavior in MCA-occluded rats may be attributable to its TRH-like properties.

In conclusion, behavioral changes during the chronic phase of MCA-occluded rats were ameliorated by YM-14673 without any effect on ischemic brain damage. Furthermore, the rat model of MCA occlusion may be useful for investigating the pathogenesis of, and drug therapy for, the chronic phase of focal cerebral ischemia.

**Acknowledgments**

We are indebted to Mr. H. Okamiya for his making of the brain preparations in the neuropathologic study and to Misses N. Tomukai and T. Iwasawa for their excellent technical assistance.

**Table 2. Effects of YM-14673 on Ischemic Brain Damage After Middle Cerebral Artery Occlusion in Rats**

<table>
<thead>
<tr>
<th>Rat</th>
<th>Ischemic brain damage (% of total area)</th>
<th>YM-14673 (mg/kg i.p.)</th>
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<tr>
<td></td>
<td></td>
<td>Saline (n=8)</td>
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<tr>
<td>1</td>
<td></td>
<td>14.1</td>
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<td>2</td>
<td></td>
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<td>3</td>
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<td>4</td>
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<tr>
<td>5</td>
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<td>10.9</td>
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<tr>
<td>6</td>
<td></td>
<td>6.3</td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>8.2</td>
</tr>
<tr>
<td>8</td>
<td></td>
<td>7.0</td>
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</tbody>
</table>

Data are averages from five coronal sections.
References


KEY WORDS • behavior • cerebral ischemia • rats
American Heart Association

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