Effect of Hachimijogan, an Oriental Herbal Medicinal Mixture, on Experimental Amnesia in Mice

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The effect of Hachimijogan on cognitive disturbance was investigated using step-through passive avoidance failure techniques: scopolamine-, cycloheximide- and cerebral ischemia-induced amnesia. Pre-acquisition trial administration of Hachimijogan (0.5 g/kg, p.o.) prolonged the step-through latency reduced by scopolamine and cycloheximide. Hachimijogan (0.5 and 1.0 g/kg, p.o.) also ameliorated the cerebral ischemia-induced amnesia. Physostigmine (0.1 mg/kg, i.p.) ameliorated all three amnesia models. The ameliorating effects of Hachimijogan and physostigmine on cycloheximide-induced amnesia were diminished by the combination with scopolamine. These results suggest that Hachimijogan possesses a wide-ranging pharmacological profile in anti-amnesic actions and that its anti-amnesic activities may be related to the cholinergic neuronal system.

Keywords Hachimijogan; physostigmine; passive avoidance; amnesia; scopolamine; cycloheximide

Memory loss and cognitive disturbance are caused by dysfunction of the neurotransmitter system and a decrease in cerebral metabolism and blood flow. These impairments are frequently observed in patients with cerebrovascular diseases and dementia. Drugs that enhance neuronal functions would be useful in the treatment of these diseases. It is well known that amnesia can be caused by an antagonist like scopolamine, by the administration of a protein synthesis inhibitor like cycloheximide, or by cerebral ischemia. Therefore, these amnesic models have been used for evaluating cognitive-enhancing drugs.

Hachimijogan (HJ), an oriental herbal medicinal mixture, has been used for treating various diseases associated with aging, such as prostatic hypertrophy, oligozoospermia and senile colitis. In our previous paper, we reported that HJ had a phystostigmine-like protective effect on cerebral anoxia. Furthermore, HJ ameliorated the scopolamine-induced cognitive disturbance of the radial maze performance, and increases in acetylcholine content and choline acetyltransferase activity were observed in the frontal cortex of HJ-treated rats. Thus, we speculated that the anti-amnesic effect of HJ was partly related to the central cholinergic system. However, the effect of HJ on other nervous systems or mechanisms is still unknown.

In this paper, we studied the effects of HJ on passive avoidance failures induced by scopolamine, cycloheximide and cerebral ischemia to ascertain whether HJ exerts its anti-amnesic effect in various amnesic models. Spontaneous locomotive activity was also tested to verify the specificity of the effect of HJ on learning.

MATERIALS AND METHODS

Animals Male ddY mice, 8 weeks old, were purchased from SLC Japan (Hamamatsu, Japan). The animals were housed in standard plastic cages in an air-conditioned room (24°C) and given a commercial diet (CE-2, Kureha Co., Tokyo, Japan) and water ad libitum. The animals were kept at least 6 days after their arrival. The animals weighed 32 to 40 g at the time of each experiment.

Reagents Crude drug mixtures of HJ were authenticated and provided by Tsumura Co., Ltd. (Tokyo, Japan). The following drugs were obtained commercially: physostigmine sulfate, cycloheximide (Sigma, St. Louis, U.S.A.) and scopolamine hydrobromide (Nakalai Tesque, Kyoto, Japan). HJ was dissolved in distilled water, and physostigmine sulfate, scopolamine hydrobromide and cycloheximide were dissolved in a 0.9% saline solution. The drugs were administered in a dosage of 0.1 ml per 10 g of body weight.

Preparation of HJ The crude drug composition of HJ is noted in Table I. The mixture of crude drugs was boiled in 12 volumes of water and extracted for 1 h. The decoction was centrifuged, passed through a 1.7-mm mesh sieve and evaporated under 50°C. The concentrate was spray-dried to produce a powdered extract. The yield was 18%.

<table>
<thead>
<tr>
<th>Table I. Crude Drug Composition of HJ a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plant name</td>
</tr>
<tr>
<td>Rehmannia glutinosa LIBOCH. var. purpurea MAKINO (Scrophulariaceae)</td>
</tr>
<tr>
<td>Cornus offficinalis SIEB. et ZUCC. (Cornaceae)</td>
</tr>
<tr>
<td>Dioscorea batatas DENCQ (Dioscoreaceae)</td>
</tr>
<tr>
<td>Alisma orientale JUZEP. (Alimataceae)</td>
</tr>
<tr>
<td>Porta cocos WOLF (A Fungi)</td>
</tr>
<tr>
<td>Paonia suffruticosa ANDR. (Paoniaceae)</td>
</tr>
<tr>
<td>Cinnamomeum cassia BLUMI (Lauraceae)</td>
</tr>
<tr>
<td>Aconitum carmichaeli DEBEX. (Ranunculaceae)</td>
</tr>
</tbody>
</table>

a) As provided by Tumura Co., Ltd. (Tokyo, Japan).

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Apparatus  The passive avoidance apparatus consisted of two compartments, one illuminated (10 × 10 × 15 cm, light [60 W], at a height of 30 cm from the top of the chamber) and one dark (18 × 18 × 15 cm). The floor consisted of a parallel stainless steel grid (6 mm in diameter), and the two compartments were separated with a guillotine door (3 × 4 cm). The intensity of electric foot-shock was 60 V (1 Hz, duration 0.5 s) for 3 s.

Passive Avoidance Response  Before the acquisition trial, the mouse was given a single pre-training trial by the following procedure. The mouse was placed in an illuminated compartment and a few minutes later, the guillotine door was raised. The mouse entered the dark compartment and was allowed to remain there for about 1 min. In the acquisition trial, the mouse was placed in an illuminated compartment. The mouse could enter into the dark compartment through the door. Once the mouse’s four paws were in the dark compartment, the door was shut and an electric foot-shock was delivered to the floor grid. Thereafter, the mouse was returned to its home cage. In the retention trial given 24 h after the acquisition trial, the mouse was again placed in the illuminated compartment, and the response latency in entering the dark compartment was measured. The latency of mice which did not move into the dark compartment during the observation period was 600 s.

Effects of HJ and Physostigmine on Scopolamine-Induced Amnesia  Scopolamine in a dose of 0.75 mg/kg was administered intraperitoneally to mice. The acquisition trial was performed 30 min after scopolamine injection. HJ was orally administered 60 min before or immediately after the acquisition trial or 60 min before the retention trial. Physostigmine was injected subcutaneously 20 min before the acquisition trial.

Effects of HJ and Physostigmine on Cycloheximide-Induced Amnesia  Mice were given cycloheximide subcutaneously in a dose of 150 mg/kg 30 min before the acquisition trial. HJ was administered orally 60 min before the acquisition trial, and physostigmine was injected subcutaneously 20 min before the acquisition trial.

Effects of HJ and Physostigmine in Combination with Scopolamine on Cycloheximide-Induced Amnesia  Mice were given cycloheximide (150 mg/kg, s.c.) 30 min before the acquisition trial. HJ was administered orally 60 min before the acquisition trial and physostigmine was injected subcutaneously 20 min before the acquisition trial. Scopolamine (0.5 mg/kg, i.p.) was injected immediately after the acquisition trial.

Effects of HJ and Physostigmine on Cerebral Ischemia-Induced  After the retention trial, mice were immediately anesthetized with 1% halothane, and both the bilateral common carotid arteries were carefully exposed and occluded for 15 min by clips. The time from electric foot-shock delivery to occlusion was within 20 min. HJ was orally administered 60 min before the acquisition trial, and physostigmine was injected subcutaneously 20 min before the acquisition trial.

Effects of HJ on the Passive Avoidance Response in Normal Mice  HJ was orally administered 60 min before or immediately after acquisition or 60 min before retention trial.

**Effects of HJ on Spontaneous Movement in Mice**  The spontaneous movement of three mice was measured using an Animex III counter (Shimadzu Co., Japan) every 30 min after administration of HJ for 120 min.

**Statistical Treatment**  All data were analyzed by using the Kruskal-Wallis non-parametric one-way analysis of variance, followed by the two-tailed Mann-Whitney U-test for paired comparisons. In all statistical evaluations, p < 0.05 was used as the criterion for statistical significance.

**RESULTS**

**Effects of HJ and Physostigmine on Scopolamine-Induced Amnesia**  Scopolamine in a dose of 0.75 mg/kg significantly shortened the latency of the step-through response in mice (Table II). When HJ (0.5 g/kg, p.o.) was administered 60 min before the acquisition trial, the latency significantly increased. When HJ was administered immediately after the acquisition trial and 60 min before the retention trial, each of the latencies were the same as those in the control group. With the administration 60 min before the acquisition trial, HJ (0.5 g/kg, p.o.) significantly prolonged the shortened latency, and the dose–response curve of HJ was bell-shaped (Fig. 1). Physostigmine (0.1 and 0.2 mg/kg, i.p.) significantly prolonged the shortened latency in scopolamine-treated mice.

**Effects of HJ and Physostigmine on Cycloheximide-Induced Amnesia**  The latency in the cycloheximide-treated group was shortened considerably (Fig. 2). HJ (0.5 g/kg, p.o.) and physostigmine (0.1 mg/kg, i.p.) significantly prolonged the shortened latency with a bell-shaped response curve.

**Effects of HJ and Physostigmine in Combination with Scopolamine on Cycloheximide-Induced Amnesia**  HJ (0.5 g/kg, p.o.) and physostigmine (0.1 mg/kg, i.p.) significantly prolonged the shortened latency by cycloheximide (Fig. 3). Additional treatment with scopolamine (0.5 mg/kg, i.p.) did not significantly prolong the latency of the [HJ + scopolamine]-treated group compared with that of the cycloheximide-treated group. The latency of the (HJ + scopolamine)-treated group was shorter than that of HJ-treated group but not significantly so. The latency of the [physostigmine + scopolamine]-treated group was not prolonged any more than that of the cycloheximide-treated group, but a significant difference

**TABLE II.** Effect of HJ Administered Pre-, Post-acquisition and Pre-retention Trial on Scopolamine-Induced Amnesia in Mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Scopolamine 0.75 mg/kg, i.p.</th>
<th>No. of mice</th>
<th>Latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>−</td>
<td>8</td>
<td>502.5 ± 57.9b</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>7</td>
<td>52.1 ± 21.8</td>
</tr>
<tr>
<td>HJ 0.5 g/kg, p.o.</td>
<td>+</td>
<td>10</td>
<td>199.2 ± 53.3a</td>
</tr>
<tr>
<td>Pre-acquisition trial</td>
<td>+</td>
<td>9</td>
<td>90.2 ± 42.3</td>
</tr>
<tr>
<td>Post-acquisition trial</td>
<td>+</td>
<td>11</td>
<td>115.4 ± 37.3</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. Pre-acquisition trial: 60 min before acquisition trial administration, post-acquisition trial: immediately after acquisition administration, pre-retention trial: 60 min before retention trial administration.  a) p < 0.05, b) p < 0.01 vs. control.
Fig. 1. Effect of HJ and Physostigmine on the Cognitive Disturbance of Scopolamine-Treated Mice in the Passive Avoidance Response
Each column indicates the mean ± S.E.M. of 8–11 mice. Significance: a) p<0.05, b) p<0.01 vs. control.

Fig. 2. Effect of HJ and Physostigmine on the Cognitive Disturbance of Cycloheximide-Treated Mice in the Passive Avoidance Response
Each column indicates the mean ± S.E.M. of 10–11 mice. Significance: a) p<0.05, b) p<0.01 vs. control.

Fig. 3. Effects of HJ and Physostigmine (PHY) in Combination with Scopolamine on Cycloheximide-Induced Amnesia
Each column indicates the mean ± S.E.M. Significance: a) p<0.05, b) p<0.01 vs. control. c) <0.05 vs. (PHY+cycloheximide) group.

was exhibited in comparison with the physostigmine-treated group.

**Effects of HJ and Physostigmine on Cerebral Ischemia-Induced Amnesia** The latency of the 15 min ischemic group immediately after acquisition trial was shortened significantly compared with that of the control group (Fig. 4). HJ (0.5 and 1.0 g/kg, p.o.) and physostigmine (0.1 and 0.2 mg/kg, i.p.) significantly prolonged the shortened
Fig. 4. Effect of HJ and Physostigmine on Cerebral Ischemia-Induced Cognitive Disturbance in Mice in the Passive Avoidance Response
Each column indicates the mean ± S.E.M. of 11–18 mice. Significance: a) p < 0.05, b) p < 0.01 vs. control.

<table>
<thead>
<tr>
<th>Drug and treatment time</th>
<th>No. of mice</th>
<th>Latency (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8</td>
<td>505.6 ± 57.5</td>
</tr>
<tr>
<td>HJ 0.5 g/kg, p.o.</td>
<td>8</td>
<td>507.3 ± 51.9</td>
</tr>
<tr>
<td>Pre-acquisition trial</td>
<td>8</td>
<td>519.6 ± 55.2</td>
</tr>
<tr>
<td>Post-acquisition trial</td>
<td>8</td>
<td>501.3 ± 73.1</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. Pre-acquisition trial: 60 min before acquisition trial administration; post-acquisition trial: immediately after acquisition administration; pre-retention trial: 60 min before retention trial administration.

latency.

**Effects of HJ on the Passive Avoidance Response in Normal Mice** The latency was not influenced by the oral administration of HJ 60 min before or immediately after the acquisition trial, or 60 min before the retention trial (Table III).

**Effects of HJ on Spontaneous Movement in Mice** HJ (0.1–1.0 g/kg, p.o.) had little effect on spontaneous movement for 120 min after the oral administration of HJ.

**DISCUSSION**

It is well known that the central cholinergic system plays an important role in learning and memory, and that anti-cholinergic drugs, such as scopolamine, induce amnesia in animals and humans. A major neurochemical change in Alzheimer-type senile dementia involves the deterioration of muscarinic and nicotinic presynaptic cholinergic indices in the cerebral cortex and hippocampus. Our previous experiment demonstrated that HJ showed anti-anoxic action, and this effect was diminished by treatment with atropine and potentiated by treatment with physostigmine. Moreover, HJ reduced the scopolamine-induced cognitive disturbance in the radial maze performance, and increases of acetylcholine content and choline acetyltransferase activity were observed in the frontal cortex. These results showed the possibility that the anti-amnesic effect of HJ is related to its facilitation of the central cholinergic system. To clarify the cognitive enhancing effect of HJ, we demonstrated the effect of HJ on scopolamine-induced, cycloheximide-induced and cerebral ischemia-induced passive avoidance response failure in mice.

In these studies, HJ attenuated the scopolamine-induced impairment of memory for pre-acquisition trial administration, but no prolongation of the latency was observed when HJ was administered immediately after the acquisition trial or before the retention trial. In the same schedule of pre-acquisition trial administration of HJ, amnesia induced by cycloheximide and cerebral ischemia was ameliorated. These results indicate that HJ reduces memory impairment by acting on the acquisition and consolidation of cognitive behavior. Moreover, the latency of the HJ-treated normal mice was similar to that of the control group, and HJ had little effect on spontaneous movement. Our previous experiments demonstrated that HJ did not have any influence on the pain thresholds in the Randall-Selitto method and acetic acid-writhing method (unpublished data). Therefore, these results suggest that the effect of HJ on cognitive disturbances may not be a direct effect on movement activity but the facilitation of cognitive function.

Scopolamine and other anti-muscarinic drugs induce cognitive disturbance by blocking postsynaptic muscarinic receptors. However, there are several reports that psychotropic agents influence the central cholinergic system directly or indirectly via the modulation of the dopaminergic or serotonergic system. Some investigators have suggested that cycloheximide-induced amnesia may be related not only to the inhibition of protein synthesis, but also to dysfunction of monoamineergic and cholinergic activity. Our present study demonstrated that HJ and physostigmine reduced the cognitive disturbance in scopolamine- and cycloheximide-induced amnesia. In the cycloheximide-induced amnesia, the ameliorating effects of HJ and physostigmine were diminished by treatment with scopolamine. More importantly, the reduction of latency of the (HJ + scopolamine)-treated group was smaller than that of the (physostigmine + scopolamine)-treated group. These results may indicate that the cognitive enhancing effect of HJ is due...
not only to the improvement of the cholinergic system but also that of the monoaminergic system.

Neurons in the central nervous system are vulnerable to ischemia, and neurological dysfunction is accompanied by deterioration of learning and memory. Our previous experiments demonstrated that HJ exhibited an antianoxic effect on the decapitation model and decreased the cumulative mortality rate after occlusion of the bilateral carotid artery in mice (unpublished data). Therefore, we attempted to investigate whether HJ protects against ischemia-induced memory impairment, and the treatment with HJ or physostigmine ameliorated the ischemia-induced shortened latency in our present experiment. Kakihara et al. have reported that ischemia caused cholinergic dysfunction, such as a decrease in acetylcholine content and an increase in choline content. As described above, HJ increased acetylcholine content in the frontal cortex, so these results suggest that the anti-amnesic effect of HJ in this ischemia-induced model may be related to an improvement of the cerebral cholinergic system, and the direct or indirect action of HJ on the central cholinergic system may play an important role in this memory impairment.

In conclusion, it is suggested that HJ possesses a pharmacological profile in anti-amnesic actions and its anti-amnesic activities may be related mainly to the cholinergic neuronal system. It is still unclear how HJ stimulates the cholinergic neuronal system, although HJ increased acetylcholine content and choline acetyltransferase activity in the rat frontal cortex. We are now investigating the effects of HJ on other central neuronal systems (dopaminergic, serotonergic and so on) to clarify the mechanism by which HJ performs anti-amnesic actions.

REFERENCES