The Effect of Tacrine (THA) on Cycloheximide- and Basal Forebrain Lesion-Induced Memory Deficit in Rats

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ABSTRACT—The effects of 9-amino-1,2,3,4-tetrahydroacridine (tacrine), an active acetylcholinesterase inhibitor, on cycloheximide- and basal forebrain (BF) lesion-induced memory deficit in the water maze and passive avoidance task were investigated. While cycloheximide (1.5 mg/kg, s.c.) produced amnesia in the passive avoidance task, chronic administration of tacrine (1, 3 and 10 mg/kg, once a day for 1 week) improved the amnesia. BF lesion produced amnesia in both the water maze and passive avoidance tasks. Chronic tacrine (0.1 3 mg/kg, passive avoidance task, or 0.3 mg/kg, water maze task, once a day for 1 week) improved BF lesion-induced amnesia in the passive avoidance and water maze tasks. These results suggest that tacrine may be useful for senile dementia.

Alzheimer's disease is a slowly progressive neuropsychiatric illness of unknown cause, principally characterized by memory deficits. Some specific neurochemical derangements have been observed in the brains of patients with Alzheimer's disease. One of the major changes is a decrease in activity of the enzyme choline acetyltransferase in the cerebral cortex and hippocampus, leading to a degeneration of cholinergic neurons in the basal forebrain (BF) (1-3). The increase in central cholinergic function by the use of anti-cholinesterase drugs to prevent the breakdown of acetylcholine (ACh) constitutes one pharmacological strategy for alleviating cognitive decline in Alzheimer's disease patients.

Recent studies have reported the beneficial effect of tacrine on memory and learning in experimental animals (4-7). It has been suggested that tacrine acts through AChergic systems, such as inhibition of ACh esterase (AChE) activity (8) and regulation of ACh release (9). Interestingly, some clinical studies have indicated that tacrine is effective in treating Alzheimer's disease (10, 11). In previous animal experiments, the single high dose of tacrine (1-5 mg/kg) was administered intra-peritoneally (i.p.) or subcutaneously (s.c.) (4-7). Therefore, to clarify the efficiency of tacrine, the present study investigated the effect of oral, subacute (1 week) administration of tacrine at a low dose for clinical trial.

MATERIALS AND METHODS

Animals

Male Kbl Wistar rats (Kitayama Laboratories Co., Ltd., Kyoto, Japan) weighing about 300 g were used. They were given food and water ad libitum and were kept in a regulated en-
vironment (23 ± 1°C, 50 ± 5% humidity), with a 12 hr light-dark cycle (8 a.m. to 8 p.m.: light).

Drugs
Tacrine was dissolved in distilled water. Cycloheximide (Sigma, St. Louis, MO) was dissolved in a 0.9% saline solution. Ibotenic acid (Sigma) was dissolved in small volume of 0.36 N sodium hydroxide solution, neutralized by 0.36 N hydrochloric acid solution and diluted to the appropriate concentration by 50 mM phosphate-buffer (pH 7.4). Tacrine and cycloheximide were administered p.o. and s.c., respectively. Ibotenic acid was injected into BF.

Surgery
Animals were given pentobarbital (40 mg/kg, i.p., Abbott, U.S.A.) immediately before surgery. Bilateral neurotoxic lesions of the BF were produced by injections of ibotenic acid using a 0.5-μl microsyringe inserted into the BF (Bregma: A, –1.5 mm; L, 2.6 mm; H, 8.3 mm) according to the stereotaxic atlas of Paxinos and Watson (12). Ibotenic acid was infused in a volume of 0.5 μl over 5 min. Bilateral BF lesions were made by injecting ibotenic acid on each side at an interval of 3 days. The injection needle was left in the place for 5 min more to ensure that the drug had diffused away from the needle tip. Sham-operated rats received 50 mM phosphate-buffer in the same way.

Passive avoidance task
The apparatus consisted of two compartments, one light compartment (25 cm long, 15 cm wide and 15 cm high) and one dark compartment of the same size, connected via a guillotine door (13). On day 1, just before the acquisition trial, each rat was placed in the light compartment and then allowed to enter the dark compartment; the time taken to do so was recorded in seconds. In the acquisition trial, once the rat entered the dark compartment, the guillotine door was closed and an electric shock (2.2 mA for 3 sec) was delivered via the grid floor. The animal was then put back into the home cage until the retention trial. The retention trial was carried out 24 hr after the acquisition trial. At that time, we returned the rat to the light compartment and recorded the time taken to enter the dark compartment (step-through latency, STL). In the screening of tacrine using cycloheximide-induced and BF-lesioned models, maximum cut-off latencies of 600 and 300 sec were used, respectively.

Water maze task
A circular water tank (140 cm in diameter, 45 cm in high) was used. A transparent platform (10 cm in diameter, 28 cm in high) was located in a constant position in the middle of one quadrant inside the tank, equidistant from the center and edge of the pool. The tank was filled with water at approximately 23°C (the platform’s top surface being 2 cm below the surface of the water). The pool was located in a large test room, in which there were many cues external to the maze, which were visible from within the pool, and could be used by the rat for spatial orientation. Positions of the cues were kept unchanged throughout the period of training. For each training session, the rat was placed in the water so that it faced the wall of the pool. Each rat started at one of five starting positions, but the sequence of the positions was selected randomly. In each training session, the goal latency to escape onto the hidden platform was recorded. If the rat was unable to find the platform within 200 sec, the training session was terminated and a maximum score of 200 sec was assigned. The behavioral trace and the distance of swimming in the water maze were monitored by TV camera and analyzed by computer system (BTA-2A, Muromachi Co., Ltd., Tokyo, Japan).

Experimental schedule
Cycloheximide (1.5 mg/kg, s.c.) was administered immediately after training. The dose of cycloheximide used was selected according to previous experiments (13). Briefly, cycloheximide failed to produce amnesia at the dose of
1.0 mg/kg and produced more severe side effects at the dose of 3.0 mg/kg. Tacrine (0.3, 1, 3 and 10 mg/kg) was administered once a day for 1 week before training and once before the retention trial of the passive avoidance task. The water maze task was started 2 weeks after the BF lesion, and the administration of tacrine was commenced 1 week before the water maze task. The water maze task was carried out one trial a day for 6 days. The rats received tacrine at the doses of 0.1, 0.3, 0.5 and 3 mg/kg, p.o. 30 min before every training session of the water maze task. Sham-operated animals received distilled water 30 min before every training. The passive avoidance task was carried out after the water maze task in the BF-lesioned rats. The rats received tacrine (0.1, 0.3, 0.5 and 3 mg/kg, p.o.) 30 min before the acquisition and retention trial of the passive avoidance task.

Assay for choline acetyltransferase (ChAT) activity

ChAT activities in the sham-operated and BF-lesioned groups were assayed. Rats were decapitated 5 days after the last drug administration, and the brain was removed rapidly and dissected into the following regions: the prefrontal cortex, the fronto-parietal cortex and the hippocampus (14). The tissue was stored at -80°C for assay at a later time. ChAT activity was measured by the method of Fonnum (15). The tissue was homogenized (4% w/v) in cold 50 mM phosphate-buffer (pH 7.4). Triton X-100 (0.5% v/v) was added to the homogenates to ensure release of enzyme (enzyme solution). A 125-μl aliquot of substrate mixture (0.35 mM [3H]-acetyl-Coenzyme A, 4.0 mCi/mmol), 300 mM NaCl, 50 mM phosphate-buffer (pH 7.4), 8 mM choline chloride, 20 mM EDTA, and 0.1 mM physostigmine was added to 75 μl of enzyme solution in a scintillation vial and incubated at 37°C for 30 min. After the incubation, 1 ml of cold 50 mM phosphate-buffer, 0.5 ml of acetonitrile containing 2.5 mg of tetraphenylboron, and 1.5 ml of toluene were added to the scintillation vial. The vials were shaken lightly and allowed to sit overnight before counting. Protein was measured by the method of Lowry et al. (16).

Statistical analyses

Data for the water maze task were analyzed by a repeated type of 2-way-analysis of variance (ANOVA), followed by the two-tailed Mann-Whitney U-test. Data for the passive avoidance task were expressed in terms of medians and interquartile ranges and analyzed using the Kruskal-Wallis test, followed by Mann-Whitney’s U-test.

RESULTS

The BF lesion produced aphagia and ataxia for 3 to 5 days after the surgery. These behavioral changes were ameliorated by providing water and small amounts of food inside the home cage and did not persist beyond 7 days after surgery. No drug treatment changed the step-through latency in the training of the passive avoidance task (Table 1).

Effect of subacute tacrine administration on cycloheximide-induced amnesia in the passive avoidance task (Experiment 1)

As shown in Fig. 1, the STL of the cycloheximide (1.5 mg/kg, s.c.-treated group shortened significantly compared to that of the control group. The shortened STL induced by cycloheximide was significantly reversed by subacute administration of tacrine (1–10 mg/kg) for 1 week ($\chi^2 = 18.13$, $P < 0.01$).

Effect of subacute tacrine administration on the BF lesion-induced amnesia in the passive avoidance task (Experiment 2)

As shown in Fig. 2, ibotenic acid (4–20 μg/rat) significantly shortened STL as compared with the sham-operated group ($\chi^2 = 27.06$, $P < 0.01$).

As shown in Fig. 3, the shortened STL of the BF lesion (ibotenic acid 12 μg/rat-group) was prolonged by subacute administration of tacrine (0.1, 0.3, 0.5 and 3 mg/kg) for 3 weeks ($\chi^2 = 23.69$, $P < 0.01$). The dose-response
Table 1. Step-through latency on the training of passive avoidance task in drug-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>N</th>
<th>Step-through latency (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>18.00 ± 3.54</td>
</tr>
<tr>
<td>Cycloheximide (1.5 mg/kg) + Vehicle</td>
<td>12</td>
<td>10.67 ± 2.66</td>
</tr>
<tr>
<td>Cycloheximide (1.5 mg/kg) + Tacrine (mg/kg)</td>
<td>10</td>
<td>13.20 ± 2.87</td>
</tr>
<tr>
<td>Cycloheximide (1.5 mg/kg) + Tacrine (mg/kg)</td>
<td>11</td>
<td>12.55 ± 2.76</td>
</tr>
<tr>
<td>Cycloheximide (1.5 mg/kg) + Tacrine (mg/kg)</td>
<td>11</td>
<td>11.45 ± 2.66</td>
</tr>
<tr>
<td>Cycloheximide (1.5 mg/kg) + Tacrine (mg/kg)</td>
<td>11</td>
<td>11.36 ± 2.25</td>
</tr>
</tbody>
</table>

[Experiment 2]
Vehicle | 10  | 11.50 ± 3.48              |
Ibotenic acid (μg/rat) | 4.0 | 9.20 ± 1.77               |
Ibotenic acid (μg/rat) | 8.0 | 8.80 ± 1.37               |
Ibotenic acid (μg/rat) | 12.0| 15.00 ± 4.16              |
Ibotenic acid (μg/rat) | 16.0| 10.10 ± 1.93              |
Ibotenic acid (μg/rat) | 20.0| 9.20 ± 2.43               |
Control | 14  | 15.21 ± 3.32              |
Ibotenic acid (12 μg/rat) + Vehicle | 16  | 13.13 ± 2.65              |
Ibotenic acid (12 μg/rat) + Tacrine (mg/kg) | 0.1 | 13.00 ± 2.29              |
Ibotenic acid (12 μg/rat) + Tacrine (mg/kg) | 0.3 | 9.69 ± 2.79               |
Ibotenic acid (12 μg/rat) + Tacrine (mg/kg) | 0.5 | 11.00 ± 4.97              |
Ibotenic acid (12 μg/rat) + Tacrine (mg/kg) | 3.0 | 8.88 ± 2.03               |

Fig. 1. Effect of subacute (1 week) administration of tacrine on the cycloheximide-induced amnesia in the rats in the step-through passive avoidance task. The columns and the numbers in parentheses show the median and interquartile range, respectively. Overall $\chi^2 = 18.13, P < 0.01$ (Kruskal-Wallis test). **$P < 0.01$ vs. vehicle-treated group; *$P < 0.05$, **$P < 0.01$ vs. cycloheximide-treated group (Mann-Whitney's U-test). N = 10 - 12.
Fig. 2. Effect of ibotenic acid on acquisition of the step-through passive avoidance task in rats. The columns and the numbers in parentheses show the median and interquartile range, respectively. Overall $\chi^2 = 27.06, P < 0.01$ (Kruskal-Wallis test). **$P < 0.01$ vs. vehicle-treated group (Mann-Whitney's $U$-test). $N = 10$.

Fig. 3. Effect of subacute (3 weeks) administration of tacrine on basal forebrain lesion-induced amnesia in rats in the step-through passive avoidance task. The columns and the numbers in parentheses show the median and interquartile range, respectively. Overall $\chi^2 = 23.69, P < 0.01$ (Kruskal-Wallis test). **$P < 0.01$ vs. vehicle-treated group; $# P < 0.05$, **$P < 0.01$ vs. ibotenic acid-treated group (Mann-Whitney's $U$-test). $N = 5-16$. 

Tacrine and Amnesia
curve for tacrine was bell-shaped.

Effect of subacute tacrine administration on the BF lesion-induced amnesia in the water maze task (Experiment 3)

As shown in Fig. 4, ibotenic acid (4–20 μg/rat) significantly extended goal latencies from 2 to 6 sessions in the water maze task, as compared with the sham-operated group [F(4,270) = 12.78, P < 0.01].

As shown in Fig. 5, 2-weeks subacute administration of tacrine (0.3 mg/kg) shortened the prolonged goal latency induced by ibotenic acid (12 μg/rat) [F(5,252) = 4.35, P < 0.01]. The dose-response curve for tacrine was bell-shaped. There was no significant difference between the control and other drug-treatment groups.

Fig. 4. Effect of ibotenic acid on acquisition of the water maze task in rats. Overall F(4,270) = 12.78, P < 0.01 (2-way ANOVA). *P < 0.05, **P < 0.01 vs. vehicle-treated group (Mann-Whitney’s U-test). N = 10. Ibotenic acid, 0 μg/rat (○); 4 μg/rat (▲); 8 μg/rat (▲); 12 μg/rat (■); 16 μg/rat (■); 20 μg/rat (●).

Fig. 5. Effect of subacute (2 weeks) administration of tacrine on basal forebrain lesion-induced amnesia in rats in the water maze task. Overall F(5,252) = 4.35, P < 0.01 (2-way ANOVA). *P < 0.05 vs. vehicle-treated group, **P < 0.01 vs. ibotenic acid (12 μg/rat)-treated group (Mann-Whitney’s U-test). N = 5–8. Ibotenic acid, 0 μg/rat + tacrine, 0 mg/kg (○). Ibotenic acid, 12 μg/rat + tacrine, 0 mg/kg (●); 0.1 mg/kg (▲); 0.3 mg/kg (▲); 0.5 mg/kg (■); 3 mg/kg (■).
Table 2. Effect of tacrine on ChAT activity of the frontal cortex, anterior occipital cortex and occipital cortex in BF-lesioned rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ChAT activity (ACh nmol/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>frontal cortex</td>
</tr>
<tr>
<td>Sham</td>
<td>119.82 ± 4.30 (7)</td>
</tr>
<tr>
<td>BF-lesion</td>
<td>108.92 ± 4.29 (8)</td>
</tr>
<tr>
<td>+ Tacrine (0.1 mg/kg)</td>
<td>103.69 ± 4.46 (7)</td>
</tr>
<tr>
<td>+ Tacrine (0.3 mg/kg)</td>
<td>111.82 ± 8.01 (5)</td>
</tr>
<tr>
<td>+ Tacrine (0.5 mg/kg)</td>
<td>110.10 ± 3.00 (5)</td>
</tr>
</tbody>
</table>

*P < 0.05 vs. vehicle-treated group (Bonferroni’s test).

Effect of subacute tacrine administration on the BF lesion-induced decrease of ChAT activity

At the dose used, ibotenic acid significantly decreased the ChAT activity in the anterior occipital cortex (P < 0.05 one-way ANOVA). Tacrine (0.1–0.5 mg/kg) failed to ameliorate the decrease of the ChAT activity (Table 2).

DISCUSSION

In the present study, cycloheximide induced amnesia in the passive avoidance task. It has usually been interpreted as resulting from an inhibition of the synthesis of cerebral proteins during the critical post-training consolidation period which prevents the manufacture of proteins necessary for long-term memory (17, 18). The training-induced elevation in muscarinic receptor density was blocked by cycloheximide administration (19). We have also reported that cycloheximide decreases the density of muscarinic receptors (20) and cycloheximide-induced amnesia is ameliorated by physostigmine, a choline esterase inhibitor (21). These results indicate the possibility that the reduction of AChergic neuronal activity, such as inhibition of biosynthesis of receptor protein, is responsible for the cycloheximide-induced amnesia in the passive avoidance task. The subacute treatment with tacrine ameliorated the cycloheximide-induced amnesia. Therefore, it is suggested that tacrine inhibited AChE activity in the brain and activated the AChergic neuronal system; and as a result, tacrine improved the cycloheximide-induced amnesia.

In the clinical state, it is well-known that there is a dysfunction of the basal magnocellular cholinergic system in Alzheimer’s disease (22, 23). A major AChergic input to the cerebral cortex originates in the BF which is known as the basalis magnocellularis or nucleus basalis of Meynert in primates (24, 25). The BF is an important region for memory function, since there are many reports describing BF lesion-induced amnesia (24, 26–28). We have confirmed that ibotenic acid injection into the BF decreased ChAT activity in the fronto-parietal cortex and induced amnesia in the water maze task (29, 30). In the present experiment, the BF lesion also decreased ChAT activity and impaired memory function in the passive avoidance and water maze tasks. Tacrine ameliorated the BF lesion-induced amnesia in both the passive avoidance and water maze tasks, although it failed to ameliorate the decrease of ChAT activity. These facts suggested that the tacrine-induced amelioration of amnesia in the passive avoidance and water maze tasks may be interpreted as an inhibition of AChE activity. Furthermore, tacrine may be effective for patients with Alzheimer’s disease.

In this study, we attempted to examine whether subacute treatment with tacrine at low doses improves memory deficit or not, since previous reports have indicated that acute administration of tacrine at high doses...
(1–5 mg/kg) improves cognitive function (4–7). The present results showed that subacute treatment with tacrine for 2–3 weeks at a low dose (0.3 mg/kg) was the most effective for improving the BF lesion-induced amnesia. Since it is reported that tacrine produces some side effects such as hepatotoxicity, a subacute administration of tacrine at low doses may be useful for the therapy of Alzheimer’s disease.

Tacrine inhibits AChE activity in a dose-dependent manner in vitro (9). However, the present results indicated that the dose (0.1–3 mg/kg)-response curves for tacrine were typically bell-shaped, especially in the BF lesion-induced amnesia model, although it ameliorated cycloheximide-induced amnesia in a dose-dependent fashion (until 10 mg/kg).Tacrine (0.5–3 mg/kg) failed to ameliorate the amnesia in the water maze task. The discrepancy of the dose-response curves may be due to the administration schedule and animal models: BF-lesioned animals were administered tacrine 3 times (3 weeks subacute administration) more than cycloheximide-treated animals (1 week subacute administration). As we have reported, cycloheximide affects not only the AChergic neuronal system, but also the GABAergic (21), serotonergic (31) and opioidergic (32) neuronal systems, while ibotenic acid impairs only the AChergic neuronal system. Furthermore, there is a possibility that the bell-shaped dose-response curve is related to the toxicity of tacrine, since tacrine interacts with the N-methyl-D-aspartate type of excitatory amino acid receptor as a mixed agonist/antagonist and produces neurotoxicity (33).

Finally, our results are interesting in view of the recent reports demonstrating a rehabilitation of memory function in Alzheimer patients by tacrine (10), lending additional empirical support for the use of tacrine and other cholinomimetics in clinical trials of treatment strategies for Alzheimer’s disease.

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