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Effects of Methanol Extract of *Uncariae Ramulus et Uncus* on Ibotenic Acid-Induced Amnesia in the Rat

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Abstract. In the present study, we investigated the effects of *Uncariae Ramulus et Uncus* (UR) on learning and memory in the Morris water maze task and the central cholinergic system of rats with excitotoxic medial septum (MS) lesion. In the water maze test, the animals were trained to find a platform in a fixed position during 6 days and then received a 60-s probe trial in which the platform was removed from the pool on the 7th day. Ibotenic lesion of the MS showed impaired performance of the maze test and severe cell losses in the septohippocampal cholinergic system (SHC), as indicated by decreased choline acetyltransferase-immunoreactivity and acetylcholinesterase-reactivity in the hippocampus. Daily administrations of UR (100 mg /kg, i.p.) for 21 consecutive days produced significant reversals of ibotenic acid-induced deficit in learning and memory. These treatments also reduced the loss of cholinergic immunoreactivity in the hippocampus induced by ibotenic acid. These results demonstrated that impairments of spatial learning and memory may be attributable to degeneration of SHC neurons and that UR ameliorated learning and memory deficits partly through neuroprotective effects on the central acetylcholine system. Our studies suggest that UR may be useful in the treatment of Alzheimer's disease.

Keywords: Alzheimer's disease, *Uncariae Ramulus et Uncus*, learning and memory, central cholinergic system, neuroprotection

Introduction

Alzheimer's disease (AD) is accompanied by pronounced neurodegenerative changes in the brain. The consistent findings of AD patients are impairment in cognitive performances, such as attention, learning and memory, and loss of cholinergic markers, including levels of acetylcholine (ACh) and choline acetyltransferase (ChAT) (1 – 3). The damage to cholinergic system is also involved in alcoholism (4) and aging (5) as well as dementia (6). The cholinergic approach to treatment of AD involves counteracting this loss in cholinergic activity by pharmacological intervention to increase cholinergic transmission (7).

Cholinergic neurons originating in the nucleus basalis of Meynert (NBM) and the medial septum (MS) project to areas such as the cortex and hippocampus, which play a role of ACh in cognition (8, 9). Lesioning these pathways in rodents produces a decrease in ACh release and an impairment of memory-related task performance (10). The observations that impairment can be reversed by cholinergic agonists indicate that the cholinergic system is compromised in AD and that drugs which stimulate cholinergic activity may provide treatment of AD (11).

For testing of putative, cognition-enhancing agents, the establishment and standardization of animal cognitive deficit models are required. Lesion of the MS in

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animals has been widely accepted as animal model of memory loss (12, 13). Cognitive dysfunctions after lesioning the MS are mainly considered to be due to deafferentiation of the hippocampus, which plays a pivotal role in learning and memory (14, 15). The interaction of acetylcholine and glutamate is important for formation of memory. ACh functions to facilitate glutamate activity by coordinating states of acquisition and recall in the cortex and hippocampus (16) and the activation of *N*-methyl-D-aspartate (NMDA) receptors is prerequisite for induction of long-term potentiation (LTP) in the septohippocampal cholinergic system, which is involved in memory performance of rats in some learning tasks that depend on hippocampal functions (17). It has been demonstrated that the changes in NMDA and AMPA receptor binding in cortical regions were highly correlated with reduced cholinergic activity resulting from ibotenic acid or cholinergic innnunolesion (18, 19). Intra-hippocampal administration of the muscarinic acetylcholine receptor antagonist scopolamine or the selective and competitive NMDA receptor antagonists such as (±)-3-(2-carboxypiperazin-4-yl) propyl-1-phosphonic acid (CPP) produced a dose-dependent disruption of working memory performance, indicating that memory processes depend to some extent on the excitatory neurotransmission via muscarinic and NMDA receptors in the hippocampus (20).

Ibotenic acid induces neuronal necrosis by a hyperstimulation of the NMDA glutamate receptors leading to calcium overload. Its excitotoxic properties are confined to somata of various neuron types and therefore axons and blood vessels that course through the target areas remain intact (21). Injections of ibotenic acid into the MS in rats leads to profound deficit in the Morris water maze, which has been designed to measure spatial learning and memory (22), and provides a more advanced stage of the neurodegeneration as an animal model of AD (10).

Pharmacological treatments might ameliorate cognitive functioning. Ancient Korean physicians have used several oriental herbs to cure dementia and these effects were described in the Korean herbal books. Among these several cognitive-enhancing oriental herbs, recent pharmacological studies have proved the effectiveness of herbs such as Panax ginseng and its compounds (23 – 26). However, the facilitative effects of many Korean cognitive-enhancing herbs on learning and memory are limited.

Uncariae Ramulus et Uncus (UR) is one of the herbal plants that has been prescribed for a long time to treat stroke and vascular dementia. UR has been described in a medical classic as having the ability to relieve dizziness, headache, and tremors resulting from hypertension (27). Many studies have reported potent antihypertensive or vasodilator actions of UR in animal models of hypertension (28 – 30). Several studies have also demonstrated that UR or its components had a neuroprotective effect against glutamate-induced cell death (31, 32) and Ca^{2+}-blocking inhibitory activity (30). A recent study demonstrated that UR protected hippocampal neurons against global forebrain ischemia, suggesting that it is effective in the treatment of vascular dementia (33).

In the present study, we examined the effect of UR on learning and memory ability in ibotenic acid induced-amnesia rats using the Morris water maze, and the relationship between the cholinergic markers in the MS and the hippocampus and the neural mechanism underlying its improving effect on memory was discussed.

Materials and Methods

Animals

Adult male Sprague-Dawley rats weighing 260 to 280 g were obtained from Samtaco Corp. (Kyungki-do, Korea). The experimental procedures were carried out according to the animal care guidelines of the NIH and the Kyung Hee University Institutional Animal Care and Use Committee. All animals were housed in groups of five or six with continuous access to food and water ad libitum and were maintained on a 12-h light/dark cycle regulated at 23°C room temperature. The experiments began at least 7 days after their arrival in individual home cage.

Preparation of methanol extract of UR

UR was purchased from an oriental drug store (Jungdo, Inc., Seoul, Korea). The voucher specimens (No. HP210002) are deposited at the herbarium located in the College of Oriental Medicine, Kyung Hee University. UR (100 g) was cut into small pieces and extracted three times in a reflux condenser for 24 h each with 85% methanol. The solution was combined, filtered through Whatman No. 1 filter paper, and concentrated using a rotary vacuum evaporator followed by lyophilization. The yield of UR was 10.1% (w/w).

Lesioning procedure and administration of UR

The general procedures for surgery were the same for each group, except that artificial cerebrospinal fluid (CSF) was microinjected into the MS in the sham group, whereas ibotenic acid (Sigma, St. Louis, MO, USA) was microinjected into rats in the lesion group at a concentration of 4 μg/μl of CSF. The anesthetized rat under pentobarbital (50 mg/kg, i.p.) was placed in a stereotaxic apparatus. The skin over the rat's skull was shaved

and cleaned with betadine, an incision was made through the skin and muscle to expose the skull, and the skin was retracted. Two holes were drilled using stereotaxic coordinates based on the Paxinos and Watson brain atlas (34) in the MS (AP : −0.2, L : ±0.3, DV : −6.2 referenced to the bregma). A 22-gauge Hamilton syringe (Reno, NV, USA) filled either with artificial CSF or ibotenic acid was slowly infused at 0.02 μl/min using micro-injection pump (Pump 22; Harvard Apparatus, South Natick, MA, USA) for 5 min and the syringe was left for a further 5 min.

UR was dissolved in saline (100 mg/ml). The day after surgery, a suspension of UR or saline was administered intraperitoneally for 3 weeks. In experiment, sham and ibotenic lesioned + saline groups (Ibo-saline) received saline (1 ml/kg per day), and the ibotenic lesioned + UR group (Ibo-UR) received UR (100 mg /kg per day).

Morris water maze test

The water maze was a circular pool (painted white, 2.0 m in diameter, 0.35 m high) constructed from fiberglass. The pool contained water that was maintained at a temperature of 22 ± 2°C. The water was made opaque by the addition of 1 kg of powered milk. During testing in the water maze, a platform, 15 cm in diameter, was located 1.5 cm below the water in one of four locations in the pool, approximately 50 cm from the sidewalls. The pool was surrounded by many cues external to the maze. A video-camera was mounted to the ceiling above the pool and was connected to a video-recorder and tracking device (S-MART; Pan-Lab, Barcelona, Spain), which permitted on- and off-line automated tracking of the path taken by the rat. The animals received four trials per session. The rats were trained to locate the hidden escape platform, which remained in a fixed location throughout testing. Trials lasted a maximum of 180s and the latency to find the submerged platform was recorded. The animals were tested in this way for 6 days, and then they received a probe trial on the 7th day. For the probe trial, the platform was removed from the pool and then the animal was released from the quadrant opposite to where the platform would have been located. The length of the trial was 60 s, after which the rat was removed from the pool. The proportion of time the rat spent searching for the platform in the training quadrant; i.e., the previous location of the platform was recorded and used as a measure of retention.

ChAT immunohistochemistry

At the end of the behavioral observation, rats were deeply anesthetized with a sodium pentobarbital (80 mg /kg, i.p.) and then perfused through the ascending aorta with normal saline (0.9%), followed by 900 ml of 4% paraformaldehype in 0.1 M phosphate-buffered saline (PBS). The brains were removed, postfixed overnight, and cryoprotected in 20% sucrose with PBS. Brains were cut by a cryostat as 30-μm coronal sections, which were processed immunohistochemically as free-floating sections.

The sections were washed in PBS containing 0.3% Triton X-100 and 1% rabbit serum and were then incubated in the ChAT primary antibody (Cambridge Research Biochemicals, Wilmington, DE, USA) diluted 1:2000 in the same buffer at 4°C for 72 h. After washing, the sections were incubated in biotinylated anti-sheep serum and ABC complex (Vectastain Elite Kit; Vector Lab., Burlingame, CA, USA) for 2 h. The ABC complex was visualized with 0.05% diaminobenzidine with 0.02% H_2O_2. Images were captured using an Axio Vision 3.0 imaging system (Zeiss, Oberkochen, Germany) and processed in Adobe Photoshop. For measuring cells of ChAT, the grid was placed on the MS, hippocampal CA1 and CA3 area according to the atlas of Paxinos and Watson (34). Number of cells was counted at 100× magnification using a microscope rectangle grid measuring 100 × 100 μm.

AChE histochemistry

The sections were washed in PBS and incubated in the solution with 25 mg acetylthiocholine iodine for 1 h. The solution was composed of 32.5 ml 0.1 M sodium hydrogen phosphate buffer ($NaH_2PO_4 \cdot H_2O$, pH 6.0), 2.5 ml 0.1M sodium citrate, 5 ml 30 mM copper sulfate, 5 ml 5 mM potassium ferricyanide, and 5 ml distilled water. The color of mixing solution is pretty green. The density of stained nuclei of hippocampal cells was measured using Scion image program (Scion Corp., Frederick, MD, USA).

Statistical analyses

The data were expressed as means ± S.E.M. Group differences in the escape latency in the Morris water maze task were analyzed using one-way analysis of variance (ANOVA) with repeated measures. One-way ANOVA followed by the Tukey post hoc test multiple group comparison was used to analyze group differences of the data collected during successive training days, probe trials, immunohistochemical assay, and image analysis. A difference between groups was considered as statistically reliable if the associated probability (*P*-value) was below 0.05.

Results

Morris water maze test

The results of acquisition of Morris water maze task are depicted in Fig. 1. The escape latency differed among the groups when averaged over all sessions [$F_{2,25} = 4.403$, $P<0.05$]. Post hoc comparisons revealed that the Ibo lesion group needed more time to locate the platform than the Ibo-UR group did. During the experiment, the latency to escape diminished over the time [$F_{5,125} = 29.313$, $P<0.001$] over the time, but there was no interaction between the group and day [$F_{10,125} = 0.613$, $P>0.801$]. Tukey post-hoc test revealed that the Ibo-UR ($P<0.05$ on day 3 and 4) significantly reduced the latency of swimming time, compared with those of the Ibo-saline group. Analysis of the performance on the probe trial comparing the percentage of time spent swimming in the platform is illustrated in Fig. 2. The time spent around the platform among groups differed [$F_{2,27} = 5.936$, $P<0.05$] and sham and Ibo-UR groups spent more time around the platform than the Ibo-saline group ($P<0.05$ for the sham group, $P<0.05$ for the Ibo-UR group). Ibotenic acid lesion severely impaired spatial cognition in the water maze task, and the UR treatment group displayed attenuated ibotenic acid-induced learning and memory damage in the water maze.

ChAT immunohistochemistry

The results of the ChAT immunoreactive analysis in the MS are shown in Figs. 3 and 4. The number of neurons of ChAT was 12.4 ± 0.6 (mean $\pm$ S.E.M.) in the sham group and 9.0 ± 0.7 in the Ibo-saline group. This reduction exceeded 40% and the Ibo-UR group had significant effect compared to the Ibo-saline group [$F_{2,40} = 7.496$, $P<0.01$]. The results of ChAT immuno-

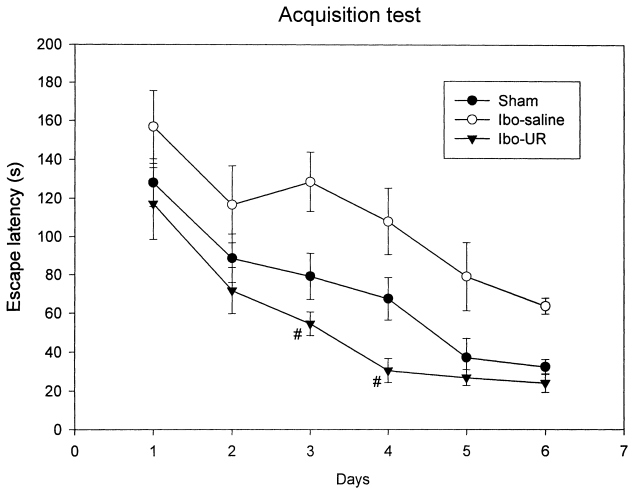

Fig. 1. Comparison of acquisition performance on the Morris water maze task among the three groups of rats. Mean swimming time traveled per trial. Mean values of the four trials per day for 6 days for each of the three groups are shown. Repeated measures of ANOVA of swimming time among the groups followed by Tukey test. $^{\#}P<0.05$, as compared with the corresponding data of the Ibo-saline group.

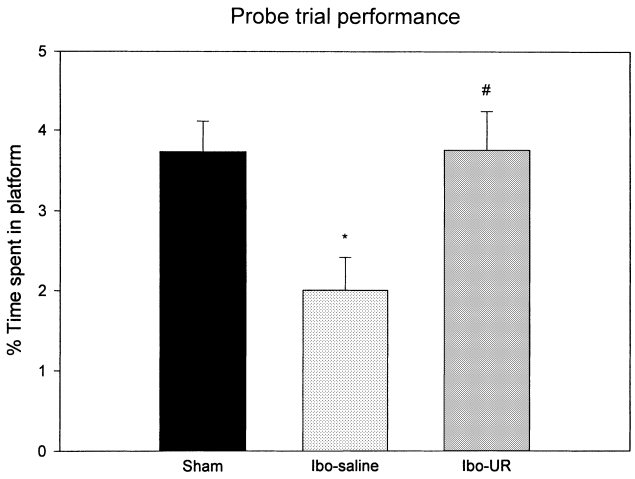

Fig. 2. Comparison of retention performance on the Morris water maze task among the three groups of rats. Mean percentage time of platform spent swimming per trial. Mean values of the four trials for each group are shown. Separate measures of one-way ANOVA of swimming time among the groups followed by Tukey test. $^{*}P<0.05$, as compared with the corresponding data of the sham group; $^{\#}P<0.05$, as compared with the corresponding data of the Ibo-saline group.

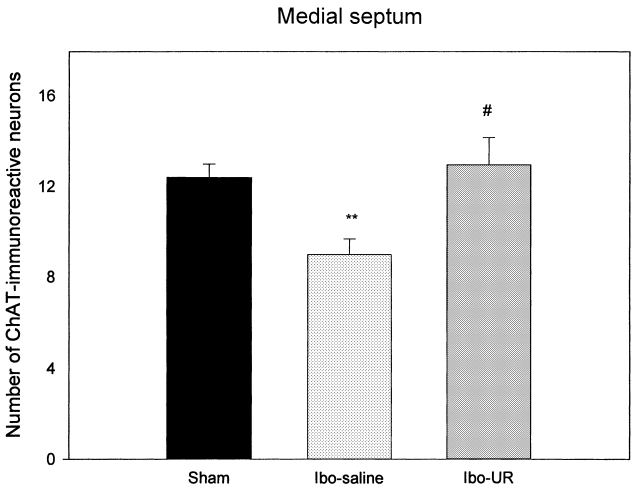

Fig. 3. The mean (±S.E.M.) values of quantities of choline acetyltransferase (ChAT) immunostained nuclei in the medial septum of the experimental groups after water maze learning task 7 days post-operatively. Immunohistochemical data of ChAT were analyzed by performing separate one-way ANOVA of neurons among the groups followed by the Tukey test. $^{**}P<0.01$, as compared with the corresponding data of the sham group; $^{\#}P<0.05$, as compared with the corresponding data of the Ibo-saline group.

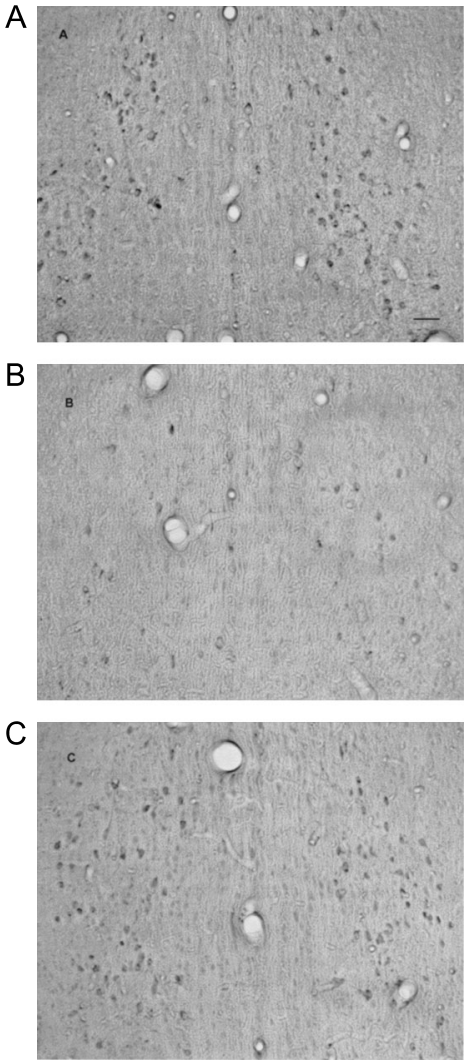

Fig. 4. Photographs showing the distribution of ChAT immunoreactive cells in the medial septum of sham (A), Ibo-saline (B), and Ibo-UR (C) rats after water maze learning task 7 days post-operatively. Sections were cut coronally at 30 μm and the scale bar represents 50 μm.

reative cells per section from different hippocampal formation are shown in Fig. 5 – 7. The number of neurons of ChAT in the CA1 area was 19.1 ± 1.1 in the sham group, 13.2 ± 0.9 in the Ibo-saline group, and 18.0 ± 1.2 in the Ibo-UR group [$F_{2,36} = 8.768$, $P<0.01$]. The immunoreactive cells in the CA3 area were 21.0 ± 1.0 in the sham group, 13.4 ± 0.7 in the Ibo-saline group, and 17.8 ± 0.9 in the Ibo-UR group [$F_{2,36} = 21.353$, $P<0.001$]. The Tukey post-hoc test revealed that the Ibo-UR significantly reduced the number of ChAT neurons, compared with those of the Ibo-saline group ($P<0.05$ in the MS, CA1 and CA3 area).

AChE histochemistry

As shown in Figs. 8 and 9, the density of AChE

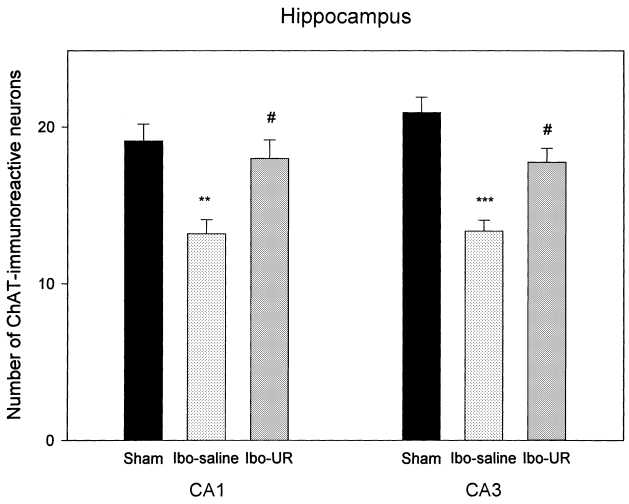

Fig. 5. The mean (±S.E.M.) values of quantities of choline acetyltransferase (ChAT) immunostained nuclei in different hippocampal areas of the experimental groups after the water maze task 7 days post-operatively. Immunohistochemical data of ChAT were analyzed by performing separate one-way ANOVA of neurons among groups followed by the Tukey test. $^{**}P<0.01$, $^{***}P<0.001$, as compared with the corresponding data of the sham group; $^{\#}P<0.05$, as compared with the corresponding data of the Ibo-saline group.

fibers in the hippocampal formation was decreased in the Ibo-saline group compared to the sham group. The density of AChE neurons in the CA1 area was 24.0 ± 1.6 (100 ± 6.5%) in the sham group, 10.2 ± 2.1 (42.5 ± 8.6%) in the Ibo-saline group, and 23.0 ± 5.0 (96.0 ± 21.0%) in the Ibo-UR group [$F_{2,24} = 10.848$, $P<0.01$]. The density of AChE neurons in the CA3 area was 47.8 ± 2.5 (100 ± 5.2%) in the sham group, 30.5 ± 1.8 (63.8 ± 3.7%) in the Ibo-saline group, and 43.2 ± 3.9 (90.4 ± 8.2%) in the Ibo-UR group [$F_{2,24} = 15.061$, $P<0.001$]. The Tukey post-hoc test revealed that the density of AChE neurons of the Ibo-UR group in the hippocampus increased markedly compared to the Ibo-saline group ($P<0.01$ in the CA1, $P<0.05$ in the CA3).

Discussion

The present studies demonstrated that infusion of ibotenic acid into the MS induced marked amnesic effects along with signs of neurodegeneration, including decreased ChAT and AChE activity in the hippocampus in rats. In addition, treatment of UR significantly improved performance on the Morris water maze and attenuated the decrease in cholinergic neurons by ibotenic acid lesion.

AD is a neurodegenerative disease, characterized by cognitive impairment and personality changes, that affects from 5 to 10% of the adult population over 65 years of age. One of the consistent findings in brains of

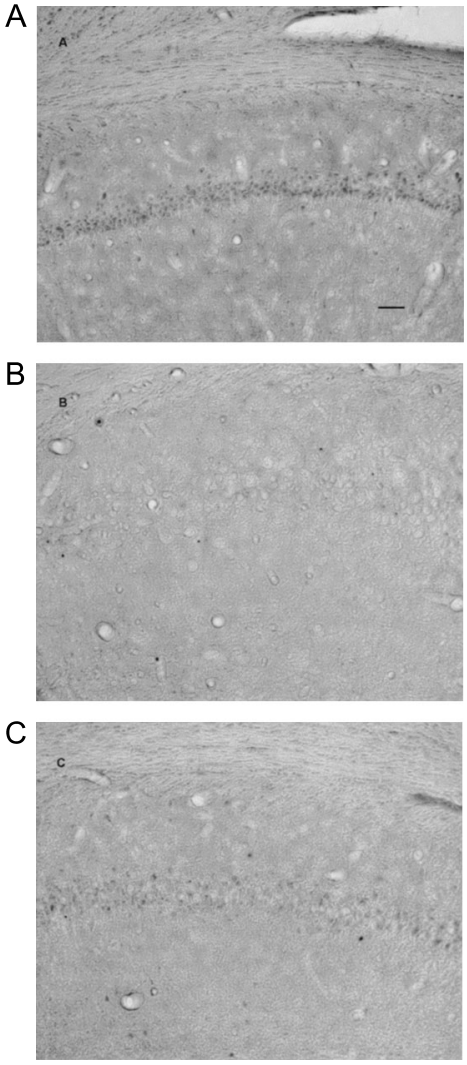

Fig. 6. Photographs showing the distribution of ChAT immunoreactive cells in the hippocampal CA1 region of sham (A), Ibo-saline (B), and Ibo-UR rats after the water maze learning task 7 days postoperatively. Sections were cut coronally at 30 μm and the scale bar represents 50 μm.

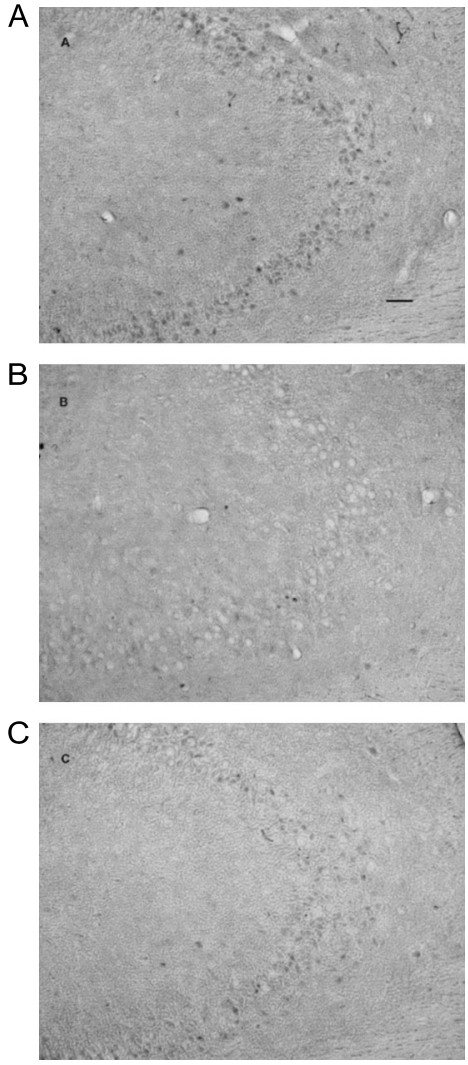

Fig. 7. Photographs showing the distribution of ChAT immunoreactive cells in the hippocampal CA3 region of sham (A), Ibo-saline (B), and Ibo-UR (C) rats after water maze learning task 7 days postoperatively. Sections were cut coronally at 30 μm and the scale bar represents 50 μm.

AD patients is loss of cholinergic markers, including levels of ACh and ChAT. The cholinergic approach for the treatment of AD involves counteracting this loss in cholinergic activity by pharmacological intervention to increase cholinergic transmission (1). The relevant animal model of AD is an important research tool in ongoing attempts to understanding the pathology and therapeutics of AD. Although no current model develops the full pathologic spectrum of the disease, injection of excitotoxin into the MS has been shown to impair memory and elicit a degree of Alzheimer-type neurodegeneration (12).

Ibotenic acid, a rigid structural analogue of glutamate, is a neuroexcitatory compound and is also a pharmacologic tool used for studies of rat models involving lesion of cholinergic neurons by stereotaxic injections into the brain (35, 36). After ibotenate lesions to the MS, at the source of the hippocampal branches of the forebrain cholinergic projection system, rats displayed long-lasting stable impairment in reference and working memory in both spatial (place) and associative (cue) radial maze tasks (12, 13). Injections of ibotenic acid into the MS or the NBM in rats produced decreased activities of ChAT in the hippocampus and frontal cortex, respectively, followed by impairment in memory acquisition (37). Consistent with the previous reports, the present study demonstrated that injection of ibotenic acid produced a loss of spatial working memory and

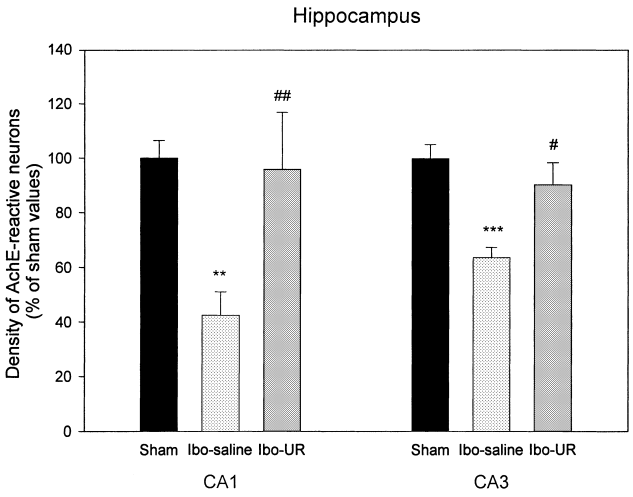

Fig. 8. The percentage (±S.E.M.) of sham values of density of acetylcholinesterase (AChE) stained nuclei in different hippocampal areas of the experimental groups after the water maze learning task 7 days post-operatively. The results of AChE-reactivity were analyzed by performing separate one-way ANOVA of neurons among the groups followed by the Tukey test. $^{**}P<0.01$, $^{***}P<0.001$, as compared with the corresponding data of the sham group; $^{\#}P<0.05$, $^{\#\#}P<0.01$, as compared with the corresponding data of the Ibo-saline group.

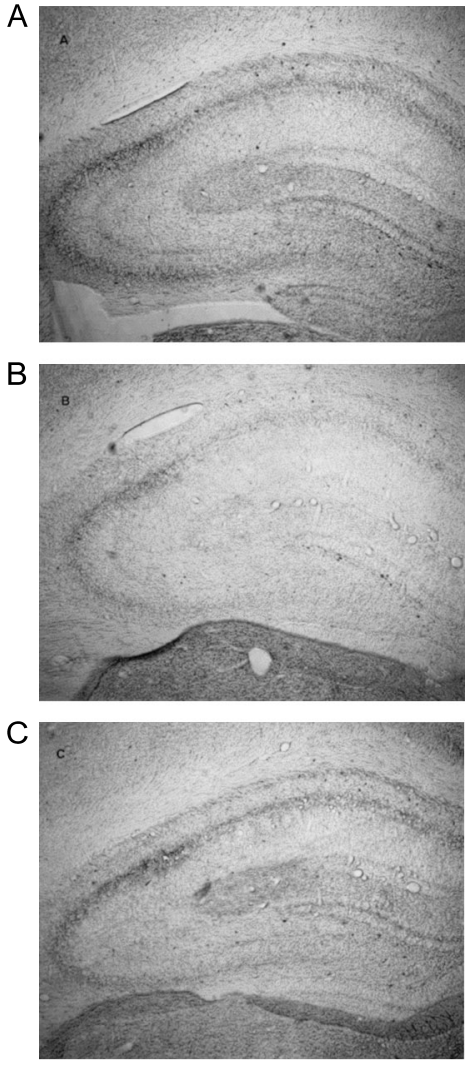

Fig. 9. Photographs showing the distribution of AChE reactive cells in the hippocampus of sham (A), Ibo-saline (B), and Ibo-UR (C) rats after the water maze learning task 7 days post-operatively.

cholinergic markers as indicated by reduction of ChAT and AChE-reactive neurons in the hippocampus, which is a particularly vulnerable and sensitive region of the brain and is also very important for declarative and spatial learning and memory (14, 15). The Morris water maze task used to test relatively pure spatial learning capability and reference memory can determine whether cholinergic depletion is sufficient to produce memory impairment (38, 39).

In the current study, injections of ibotenic acid into the MS in rats affected the performance of rats in the water maze. The Ibo-saline group showed poorer performance of acquisition and retention test than did the sham group. The latencies to find the platform on acquisition trials by the UR-pretreated group were significantly decreased compared to those of the Ibo-saline group. The UR treatment group also spent a greater proportion of the probe trial searching in the training quadrant, demonstrating that treatment with UR for 3 weeks attenuated ibotenic acid-induced learning and memory deficits in the Morris water maze.

Consistent with our results, several studies have implicated that UR improves learning and memory ability. Traditionally, UR has been used for suppression of hyperfunction of the liver, relief of dizziness, and the treatment of hypertension, tremor, and convulsion (40). UR also has been used in ailments of the cardiovascular and central nervous system (32, 41). Treatment of *Choto-san*, a *Kampo* medicine that consists of UR, other medicinal herbs, and gypsum fibrosum, gave pharmacological evidence for an antidementia effect (42). The clinical efficacy of *Choto-san* in patients with vascular dementia has been described by a double blind and placebo controlled study (43). It has been shown that three dominant *Uncaria* plants, *U. rhynchophylla* (UR), *U. sinensis*, and *U. macrophylla*, and their indole and oxinole alkaloid constituents significantly decreased locomotor activity after oral administration to rodents (44). In our study, there was statistically no significant difference between Ibo-UR and sham group, even if Ibo-UR group tended to decrease the latency of swimming distance compared with that of the sham group. In swimming speed, which is an index of motor function, there was no significant difference between the

groups (data were not shown) and swimming speed did not influence the animal's performance in the Morris water maze test. For example, septohippocampal lesion did not influence the swimming speed, but it influenced the swimming distance in the Morris water maze test in rats. Analysis of data with septohippocampal damage and swim distance demonstrates a clear correlation between reduction in septohippocampal damage and in swim distance but not swim speed (45). However, a complete lesion in the striatum, a major center for the control of motor behavior, was accompanied by a decrease in swim speed, but not by swim distance (46). It was known that there was no correlation between reduction in damage of the striatum and in swim distance, suggesting that the striatum was critically involved in motor function, rather than in spatial memory. In our study, the septohippocampal pathway was lesioned. Therefore, swim speed was not altered by septohippocampal damage and was not affected by drug treatment (45), consistent with our results showing that UR protected Ibo-induced cell damage mainly in the septohippocampus, rather than the cortex or the striatum. Since the septohippocampus plays a more critical role in spatial memory than does the striatum, it is more relevant to suggest that the reduction in septohippocampal damage due to treatment with UR appears to be the basis for the functional improvement in terms of learning and memory. Pretreatment of *Choto-san*, the component herb, UR, and indole alkaloids and phenolic fractions of UR prevented ischemia-induced impairment of spatial learning behavior in water maze performance of mice (42). Thus, UR influences the learning and memory, rather than the motor activity in hippocampal-lesioned rats and the effect of UR on swimming distance may not be directly related with the spontaneous locomotor activity in rats.

Several studies have investigated the neural mechanisms underlying pharmacological effects of UR. In good agreement with our results, the previous studies have shown that UR protected forebrain ischemia-induced hippocampal cell death and cognitive impairments in the Morris water maze (33, 47) with a relatively standard dose of UR (100 mg/kg) chosen in our study that other workers had reported in rodent experiments (33, 47, 48). It was shown that UR has a neuroprotective effect against glutamate-induced cell death and Ca^{2+}-blocking inhibitory activity (31, 32). UR extract also blocked NMDA-evoked currents and reduced NMDA-induced neuronal death (49). NMDA-receptor antagonist-like actions of UR may underlie the neuroprotective effects against cell damage and improvements of learning and memory deficits produced by ibotenic acid lesion in the present study. UR contains a variety of the oxyindole and indole alkaloids including rhynchophylline, isorhynchophylline, corynoxeine, isocorynoxetine, geissoschzine methyl ether, hirsuteine, and hirsutine, all of which have been demonstrated to possess a vasodilator effect (41, 50, 51). These alkaloids also have neuroprotective effects against glutamate-induced neuronal death in cultured cerebellar granule cells in rats (32), suggesting that these major components of UR may have been responsible for the protection against ibotenic acid-induced cell damage and cognitive deficits shown in the present study. Future studies are needed to understand the neuroprotective and behavioral effects of these components in the ibotenic acid lesion model. In addition to the role of the glutamate system, another study showed that *Choto-san* prevented impairment of the passive avoidance learning response produced by ischemia or scopolamine injections in mice through the central serotoninergic system, since this effect was blocked by a serotonin$_{1A}$-receptor antagonist (52). Even though several studies suggest that the actions of UR on the glutamate or serotonin system may contribute to the improvement of neuronal and cognitive deficits, involvement of the cholinergic system seems to be critical. In the present study, we found that the Ibo-saline group had a reduction in ChAT activity in the MS and hippocampus. The density of AChE in the hippocampal CA1 and CA3 regions was also significantly reduced after injection of ibotenic acid into the MS. It is relevant to suggest that the reduction in hippocampal cell loss after treatment with drug appears to be associated with improvement of learning and memory since the Ibo-UR group had greater cholinergic markers, such as ChAT and AChE, than the Ibo-saline group in the septohippocampal pathway. Taken together, it is likely that UR prevents the loss of cholinergic cells in the septohippocampal pathway and improves spatial learning ability and working memory deficits produced by ibotenic acid.

It should be also noted that interaction of cholinergic and other associated neurotransmitter systems should be taken into consideration. Glutamatergic cells are intermingled with cholinergic neurons in all basal forebrain nuclei, and glutamatergic cells in the medial septum receive a cholinergic input via muscarinic cholinergic receptors (53). Thus UR-induced increase in medial septal and hippocampal cholinergic neurons might affect the cholinergic control of glutamatergic neurons, and the changes in the cholinergic neurotransmission following treatment with UR were also effective enough to influence other associated neurotransmitter systems.

Our results provide strong evidence that UR may have potential therapeutic effects on cognitive impair-

ments in clinical patients. The mechanism underlying the beneficial effects of UR on neural damage and cognitive impairment produced by intra-septal injections of ibotenic acid should be investigated further.

In summary, the deficits of ibotenic acid lesions in the MS are pertinent to degeneration of cholinergic neurons in regard to memory impairment. UR significantly improved performance on the spatial memory test and protected against destruction of septohippocampal cholinergic cells by ibotenic acid. Therefore UR might be utilized as one of the cognitive-enhancing and memory-improving therapeutic herbs. Such a protective effect might prove useful in slowing disease progression as opposed to mere symptomatic palliation. Furthermore, UR could also be effective in the treatment of AD due to cholinergic neurochemical abnormalities.

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