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Combination Effects of ZSET1446/ST101 With Memantine on Cognitive Function and Extracellular Acetylcholine in the Hippocampus

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Abstract. In the novel object recognition task, ZSET1446 (also coded as ST101) enhanced object recognition memory in mice and ameliorated cognitive impairment caused by scopolamine in rats. The enhancement induced by ZSET1446 in mice was abolished by injection of mecha-mylamine, a nonselective antagonist of nicotinic acetylcholine (ACh) receptors, or dihydro-β-erythroidine, a selective antagonist against the α4 subunit of nicotinic ACh receptors. These results suggest that the procognitive effect of ZSET146 is probably mediated by stimulation of nicotinic receptors. Memantine was also effective in these tests and concomitant administration of subeffective doses of ZSET1446 and memantine significantly ameliorated the cognitive performance in the novel object recognition task in both mice and rats. Moreover, oral administration of ZSET1446 or memantine increased the extracellular level of ACh in the hippocampus as compared with the control. Further, concomitant administration of subeffective doses of ZSET1446 and memantine significantly increased the extracellular level of ACh as compared with the group of ZSET1446 or memantine alone. These results suggest that these two compounds have a synergistic effect on the cognitive function possibly by synergistic increase in the extracellular level of ACh in the hippocampus, and that the combination therapy of these compounds might be effective in clinical settings.

Keywords: ZSET1446, memantine, novel object recognition, acetylcholine, hippocampus

Introduction

A wide variety of pathological changes are involved in Alzheimer's disease (AD) (1). AD is related to the increase in amyloid β (Aβ) level and hyperphosphorylated tau, along with loss of neurons and synapses (2). Therefore, it is likely that the dysfunction of AD brain does not arise from a single cause but from multiple causes. However, the most widely used strategy for development of anti-dementia drugs is to target a single molecule. Unfortunately, drugs for AD that were developed through this strategy have almost all failed in clinical trial at the present moment, perhaps because one target is not sufficient or because such targets are also critical for normal brain function so that their inactivation results in severe toxicities. Thus, the principled approach is thought to aim at multiple targets by a single drug or combination of drugs with different mechanisms of action.

The acetylcholinesterase inhibitors (AChEIs) including galantamine and rivastigmine (both approved for use in mild to moderate AD) and donepezil (approved for use in mild to severe AD), and the non-competitive *N*-methyl-D-aspartate (NMDA)-receptor antagonist memantine (approved for use in moderate to severe AD) are currently used for the treatment of AD (3). Availability of the AD drugs with different mechanisms of action introduces the prospect of prescribing drug combinations to amplify their therapeutic efficacy (4). In severely demented patients with AD, who received a stable dose of donepezil, the addition of memantine has resulted in significantly better outcomes than placebo (5). However, at this time, a drug combination other than that of AChEIs and an NMDA-receptor antagonist is unavailable to treat AD. In addition, it has been reported that co-administration of memantine and donepezil potentiates the neurotoxic reaction of memantine, which becomes lethal to

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neurons and disseminated throughout many brain regions in the rat (6). Therefore, combination therapy of multiple drugs with different modes of action is theoretically useful for treatment of AD, but is still at a tentative stage at the present time.

ZSET1446 (also coded as ST101), spiro[imidazo-[1,2-*a*]pyridine-3,2-indan]-2(3H)-one, has been reported to exert ameliorating effects on cognitive impairments in various animal models pharmacologically, operatively, and spontaneously induced by the following: scopolamine or dizocilpine (7), a single intracerebroventricular injection of A$\beta_{25\text{-}35}$ or nucleus basalis magnocellularis lesions by ibotenic acid (8), A$\beta_{1\text{-}40}$ (9), methamphetamine (10), and olfactory bulbectomy (11, 12) and in senescence-accelerated mice (SAMP8) (13). Further, it has been reported that ZSET1446 induces cleavage of APP protein at a novel site, generating a 17-kDa C-terminal fragment, and robustly reduces brain Aβ in 3xTg AD mice and non-human primate (14). This Aβ-reducing effect of ZSET1446 may be involved, in at least some models, with the mechanism not completely elucidated.

Recently, Moriguchi et al. (15) has revealed that mibefradil, a $Ca_v3.1$ (T-type calcium channel) inhibitor, completely blocks ZSET1446-induced enhancement of long-term potentiation in the cortex and that ZSET1446 stimulates the voltage-gated Ca^{2+} current in T-type calcium channel over-expressed neuro2A cells. In addition, acetyhlcholine (ACh) release enhancement by ZSET1446 is abolished by mibefradil in the hippocampus (16). These results suggest that stimulation of T-type calcium channel is important for the ZSET1446-induced enhancement of the cognitive function and ACh release. The above-mentioned findings suggest that the mechanism of ZSET1446 is quite different from those of AChEIs and memantine.

We have previously reported that co-administration of subthreshold doses of ZSET1446 and donepezil caused increase in the extracellular ACh level in the hippocampus and ameliorated cognitive impairment induced by scopolamine in the passive avoidance task, as a consequence of ACh release (ZSET1446) and inhibition of ACh degeneration (donepezil) (17). In the present study, combination effects of ZSET1446 and memantine, which has the different mode of action to donepezil, were examined on cognitive function in the novel object recognition test and on the extracellular level of ACh in the hippocampus.

Materials and Methods

Animals

Male mice of the ICR strain (Charles River Laboratories Japan, Inc., Yokohama) at the age of 8 weeks were used in the experiment of a novel object recognition task as a memory-impaired model because our previous studies indicated that these mice had poor long-term memory in this task, whereas rats showed good memory (data not shown). Male rats of the Wistar strain (Charles River Laboratories Japan, Inc.) at the age of 8 weeks were used in the experiments of novel object recognition task for cognitive impairment caused by scopolamine and of microdialysis. They were housed in a cage in a group of 4 mice and a group of 2 or 3 rats, in a room maintained at around 22°C with a 12-h light/dark cycle. Food and water were available ad libitum. All animal care and treatments were conducted in accordance with the Guideline for the Care and Use of Laboratory Animals established at the Central Research Laboratory, Zenyaku Kogyo Co., Ltd.

Drugs

ZSET1446 was synthesized in our Department of Organic Synthesis of Zenyaku Kogyo Co., Ltd. Memantine hydrocloride was purchased from Tocris Bioscience (Bristol, UK). These drugs were suspended in 1% carboxymethyl cellulose (CMC) and orally administered in a volume of 10 mL/kg for mice and 1 mL/kg for rats. For the co-administration studies of ZSET1446 and memantine, both drug suspensions were mixed together and this mixed suspension was prepared immediately before use. Mechamylamine hydrochloride (a nonselective antagonist of nicotinic ACh receptors), dihydro-β-erythroidine hydrobromide (DHβE, a selective antagonist against the α4 subunit of nicotinic ACh receptors), and scopolamine hydrobromide were purchased from Sigma-Aldrich (St. Louis, MO, USA). These drugs were dissolved in saline and intraperitoneally administered in a volume of 10 mL/kg for mice and 1 mL/kg for rats.

Novel object recognition task for mice

This task is based on the behavior of rodents which prefer a novel object to a familiar object and spend more time in exploring the novel object (18). The experimental apparatus consisted of a Plexiglas open-field box [25 cm (width) × 41 cm (length) × 17 cm (depth), model TP-105; Toyo Riko Co., Ltd., Tokyo], the floor of which was covered with sawdust. The apparatus was located in a sound-attenuated room. The procedure for the novel object recognition task consisted of three different sessions: habituation, training, and retention sessions. Each mouse was individually habituated to the box, with 10 min of exploration in the absence of objects (day 1: habituation session). ZSET1446 at doses of 0.001, 0.003, 0.01, and 0.03 mg/kg and/or memantine at doses of 3 and 10 mg/kg was orally administered 60 min before the training trial. In the experiment of injection of nicotinic

receptor antagonists, oral administration of ZSET1446 and i.p. injection of mechamylamine or DHβE at each dose of 1 mg/kg were given 60 min before the training trial. During the training session, two different novel objects were symmetrically fixed to the floor in the box, and each animal was allowed to explore in the box for 10 min (day 2: training session). These objects were different in shape and color but similar in size. The mice were considered to be exploring the object when the mouse was facing, touching, or sniffing the object. The time spent for exploring each object was manually measured by a stopwatch. In the training session, we also simultaneously measured locomotor activity for a period of 10 min automatically, using an Animex Auto (MK-101; Muromachi Kikai Co., Ltd., Tokyo) placed under the open-field box. After the training trial, mice were immediately returned to their home cages.

Twenty-four hours after the training session, one of the familiar objects used during training was replaced by a novel object and then the animals were allowed to explore freely for 5 min and the time spent for exploring each object was recorded (day 3: retention session). A discrimination index, the ratio of the difference in time spent for exploring the novel and familiar object to the total time spent for exploring both objects, was used as a parameter of cognitive function.

For the effect of ZSET1446, the statistical significance of differences among groups was calculated by one-way analysis of variance (ANOVA) followed by post hoc analysis by Dunnett's tests. For the results of the experiments for mechamylamine and DHβE, the statistical significance of differences was calculated by Student's *t*-test. For the results of the experiments with concomitant administration of ZSET1446 and memantine, the statistical significance of differences among groups was calculated by one-way ANOVA in the training session and by two-way ANOVA followed by post hoc analysis by Dunnett's tests in the retention session. The analysis was conducted using Excell Tokei ver 6.0 (Esumi Co., Ltd., Tokyo).

Novel object recognition task for rats

The procedure was the same as that for the task in mice, unless otherwise described. The experimental apparatus consisted of a Plexiglas open field box (60 × 50 × 40 cm, custom box; Takara Co., Ltd., Tokyo), the floor of which was covered with sawdust. ZSET1446 at doses of 0.001 and 0.01 mg/kg and/or memantine at doses of 3 and 10 mg/kg was orally administered 60 min before the training trial. Scopolamine or saline was injected i.p. at dose of 0.5 mg/kg 30 min after the administration of testing drugs. After a 10-min training trial, rats were immediately returned to their home cages. Sixty min after the training trial, the retention trial was carried out for 5 min. Locomotor activity was not measured in this experiment.

For the results of the training session, the statistical significance of differences among groups was calculated by one-way ANOVA. For the retention session, the results were compared between the control group and the group administered with scopolamine alone using the Mann-Whitney U-test. When there was a significant difference, we considered that scopolamine induced cognitive impairment. For the results of the novel object recognition task with the cognitive impairment induced by scopolamine in rats, the statistical significance of differences among groups treated with scopolamine was calculated by two-way analysis of variance (ANOVA) followed by post hoc analysis by Dunnett's tests. The analysis was conducted using Excell Tokei ver 6.0 (Esumi Co., Ltd.).

Surgery

Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and fixed in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). The skull was exposed and a stainless-steel guide cannula (AG-8; Eicom, Kyoto) was implanted into the hippocampus (A, −5.8; L, 4.8; V, 4.0 mm) according to the atlas of Paxinos and Watson (19). On the day after the operation, microdialysis probes with 3-mm-long cellulose membrane tubing (A-I-8-03, Eicom) were inserted into the hippocampus through the implanted guide cannula.

ACh measurement

The probes were perfused with Ringer's solution (147 mM NaCl, 4.02 mM KCl, and 2.25 mM $CaCl_2$) at a flow rate of 1.0 μL/min. Dialysates were collected every 20 min and ACh level was detected by an HPLC system with electrochemical detection (ECD). ACh was separated from the dialysates by a column (Eicompac AC-Gel, 2.0 × 150 mm; Eicom). The enzymatic reactor contains acetylcholinesterase (AChE) and choline oxidase that catalyzes the formation of hydrogen peroxide from ACh and choline. The resultant H_2O_2 was detected by an ECD (ECD-300, Eicom), with a platinum electrode (WE-PT, Eicom) at 450 mV.

The ACh levels in the perfusate are shown as percentages of individual basal ACh release, which was calculated from the mean of 4 pre-administration control values. The statistical significance of differences among groups was calculated by two-way repeated measure ANOVA, which was followed by Dunnett's multiple comparison tests. The analysis was conducted using Excell Tokei ver 6.0.

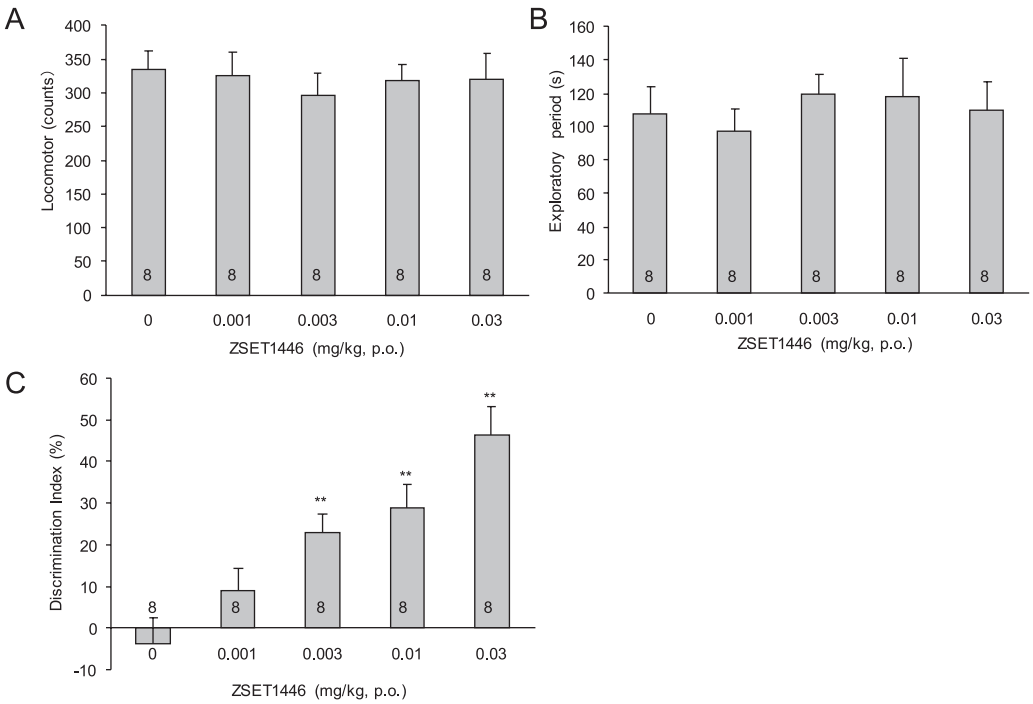

Fig. 1. Effects of oral administration of ZSET1446 on performance in the novel object recognition task. Each column represents the mean locomotor activity (A) and total exploratory period (B) in the training trial and the discrimination index in the retention trial (C). Vertical bars show the S.E.M. The number above or within each column shows the number of mice used. $^{**}P < 0.01$, compared with the control (Dunnett's *t*-test).

Results

Effect of ZSET1446 on mice in the novel object recognition task

In the training session, there were no significant differences in locomotor activity counts [$F(4, 39) = 0.21$, $P > 0.05$] and total exploring time [$F(4, 39) = 0.30$, $P > 0.05$] among all groups in each experiment (Fig. 1: A and B). The retention session was carried out 24 h after the training session. There was a significant group effect on the discrimination index in the retention trial [$F(4, 39) = 11.14$, $P < 0.01$]. Control mice, with the discrimination index near zero, seemed not to have the memory of the familiar object at all (Fig. 1C). A single oral administration of ZSET1446 at doses of 0.003, 0.01, and 0.3 ($P < 0.01$), but not 0.001 mg/kg, significantly increased the discrimination index as compared with that in the control group (Fig. 1C).

Ameliorative effects of ZSET1446 is abolished by mechamylamine or DHβE

Mechamylamine and DH*β*E administered solely or co-administered with ZSET1446 did not affect locomotor activity and an exploratory period in the training session (Fig. 2: A – D). The increase in the discrimination index induced by ZSET1446 at a dose of 0.03 mg/kg was reduced by mechamylamine and DH*β*E at a dose of 1 mg/kg, and the reduction by DH*β*E was significant (Fig. 2: E and F).

Combination effect of ZSET1446 and memantine in the novel object recognition task for mice

Treatment with ZSET1446 and/or memantine affected neither locomotor activity [$F(5, 47) = 0.16$, $P > 0.05$] nor an exploratory period [$F(5, 47) = 2.23$, $P > 0.05$] in the training session (Fig. 3: A and B). Two-way ANOVA with all of the groups revealed significant effects of ZSET1446 [$F(1, 47) = 13.31$, $P < 0.01$], memantine [$F(2,47) = 14.57$, $P < 0.01$], but no significant interaction between ZSET1446 and memantine [$F(2,47) = 0.82$, $P > 0.05$] (Fig. 3C). These results suggest that both ZSET1446 and memantine increase the discrimination index and the effects of these drugs are additive. Especially, co-administration of subeffective doses of ZSET1446 (0.001 mg/kg) and memantine (3 mg/kg) markedly increased the discrimination index (Fig. 3C).

Combination effect of ZSET1446 and memantine in the novel object recognition task for rats

Since rats showed good memory in the novel object

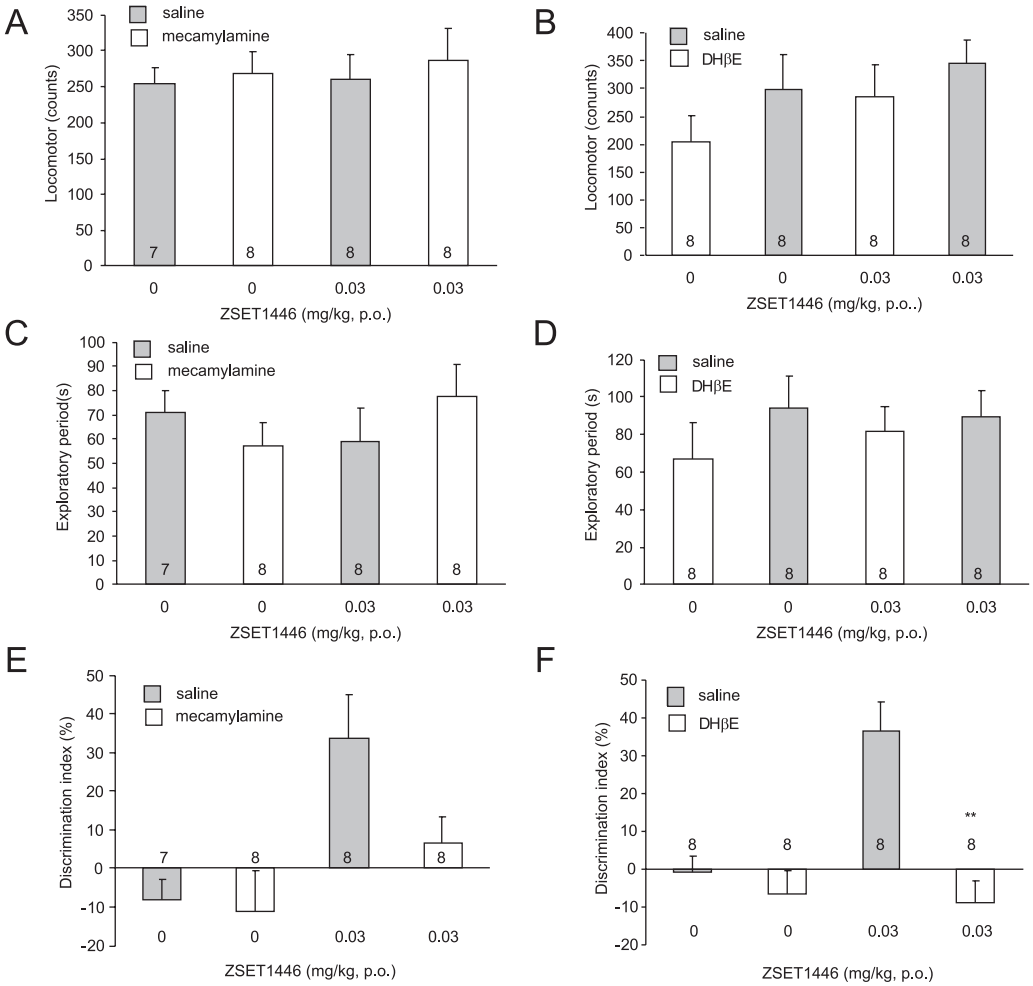

Fig. 2. Injection of mechamylamine or DH*β*E prevents facilitation of novel object recognition memory induced by ZSET1446. Each column represents the mean locomotor counts (A and B) and exploratory period (C and D) in the training trial and the discrimination index in the retention session (E and F). Vertical bars show S.E.M. The number above or within each column shows the number of mice used. $^{**}P < 0.01$, compared with treatment of ZSET1446 alone (Student's *t*-test).

recognition test, scopolamine was administered to rats in order to induce cognitive impairment (data not shown). Treatment with ZSET1446 and/or memantine did not affect the exploratory period in the training session [$F(6, 4) = 0.61$, $P > 0.05$] (Fig. 4B). Two-way ANOVA with all of the groups administered with scopolamine revealed significant effects of ZSET1446 [$F(2, 47) = 15.81$, $P < 0.01$] and memantine [$F(2,47) = 4.22$, $P < 0.05$] and significant interaction between ZSET1446 and memantine [$F(4,47) = 13.21$, $P < 0.01$] (Fig. 4A). These results suggest that both ZSET1446 and memantine ameliorate the cognitive impairment caused by scopolamine and the effects of these drugs are synergistic.

Combination effect of ZSET1446 and memantine in the extracellular ACh in the hippocampus

Two-way repeated measure ANOVA with all of the treatment groups revealed significant main effects of groups [$F(4, 324) = 50.56$, $P < 0.01$], time [$F(12, 324) = 5.67$, $P < 0.01$] and interaction between groups and time [$F(48, 324) = 2.30$, $P < 0.01$]. The extracellular level of ACh in concomitant administration of ZSET1446 (0.001 mg/kg) and memantine (10 mg/kg) was significantly higher than that in memantine at 10 mg/kg as well as ZSET1446 at 0.001 mg/kg (Fig. 5).

Discussion

In the present study, mice treated with ZSET1446 at doses of 0.003, 0.01, and 0.03 mg/kg, but not 0.001 mg/kg, were capable of discriminating between a familiar object and a novel object after an intertrial interval of 24 h, whereas this discrimination was difficult for the vehicle-treated control mice. Further, also in rats, ZSET1446 at

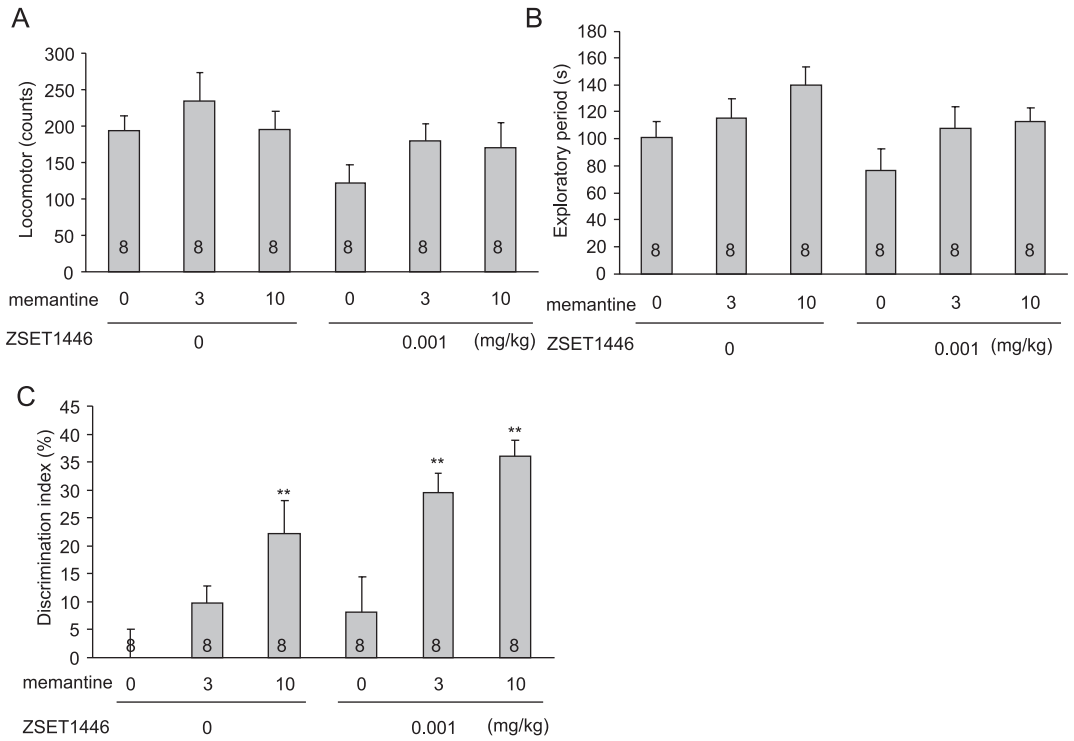

Fig. 3. Combination effects of ZSET1446 and memantine on the discrimination index in the object recognition test. Each column represents the mean locomotor count (A) and exploratory period (B) in the training trial and discrimination index in the retention trial (C). Vertical bars show S.E.M. The number above or within each column shows the number of mice used. $^{**}P < 0.01$, compared with the control (Dunnett's multiple comparison test).

the dose of 0.01 mg/kg, but not 0.001 mg/kg, ameliorated the cognitive impairment caused by scopolamine. On the other hand, oral administration of memantine at 10 mg/kg, but not 3 mg/kg, ameliorated novel object recognition memory in both mice and rats. In the training session, there were no significant differences in total exploring time and/or locomotor activity counts among all treated groups. These results suggest that the ameliorative effects of ZSET1446 and memantine are not due to the changes in motor and motivational functions.

It has been reported that oral administration of memantine at doses of 10, 20, and 30 mg/kg, but not 3 mg/kg, enhances object recognition memory in the rat (20, 21). In addition, it has been also reported that memantine enhances spatial learning in the Morris water maze task (22) and decreases the number of re-entry errors in a delayed radial-arm maze task (23). These studies (20 – 23) and the present study in mice revealed that memantine could enhance cognitive function in the absence of pharmacological, genetic, or neurological induction of cognitive impairments.

In the present study, oral administration of memantine ameliorated the cognitive impairment caused by scopolamine in the object recognition task. These results are consistent with the previous report that memantine reverses scopolamine-induced learning deficit in mice in the water maze task and increases cholinergic signaling and excitability in the mouse hippocampus (24). Our results also showed that memantine increased the extracellular ACh in the hippocampus. It has been reported that the extracellular ACh increases in the ventral tegmental area and nucleus accumbens after acute subcutaneous injection of memantine (25) and that a single i.p. injection of memantine significantly increases extracellular ACh in the hippocampus and cortex due to increased synaptic release of ACh in the rat (26). These results suggest that memantine has procognitive activity via cholinergic stimulation by increased extracellular ACh in the brain of the rodents. The mechanism of memantine to enhance hippocampal ACh release is unknown at present. However, it has been suggested that changes of hippocampal ACh release following NMDA-receptor antagonists, such as MK-801, are mediated by dopaminergic (27), GABA, and/or α2-adrenergic systems (28). Therefore, it is likely that memantine enhances ACh release mediated by these systems. Further studies are necessary to evaluate this possibility.

We have previously shown that ZSET1446 increases ACh levels in the hippocampus (7) and cortex (8). Moreover, the present study confirmed that ZSET1446 increases ACh levels in the hippocampus. It is unclear whether the cognitive enhancing effect of ZSET1446 is due to increase in ACh release in the brain. At least, the Aβ-reducing effects of ZSET1446 (14) is unlikely to contribute to the cognitive enhancement by ZSET1446 in the present acute study, because such effects need long-term treatment. In our previous study, ZSET1446 at doses from 0.003 to 0.01 mg/kg increases ACh level in the hippocampus of rats (7). It is noteworthy that the same doses of ZSET1446 showed cognitive enhancing effect in the object recognition test in the present study in rats and mice. Further studies are required to elucidate the effect of ZSET1446 on ACh levels in the mice.

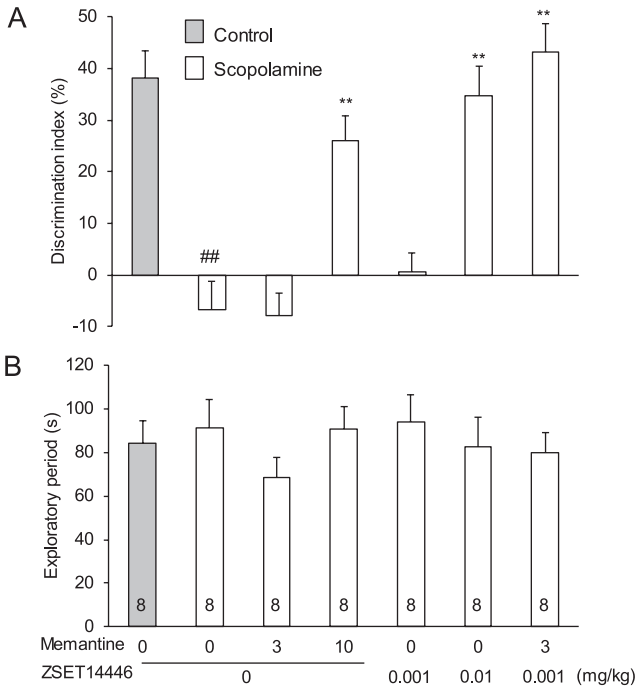

Fig. 4. Combination effects of ZSET1446 and memantine on the cognitive impairment caused by scopolamine in the object recognition test. Each column represents the discrimination index in the retention trial (A) and exploratory period in the training trial (B). Vertical bars show S.E.M. The number within each column shows the number of rats used. $^{\#\#}P < 0.01$, compared with the control. $^{**}P < 0.01$, compared with the group treated with scopolamine alone (Dunnett's multiple comparison test).

In the present study, procognitive effect of ZSET1446 was prevented by two nicotinic receptor antagonists, mechamylamine hydrochloride and DHβE, although the mechamylamine's antagonism did not reach a significant level. Shioda et al. (12) has reported similar results that chronic mecamylamine infusion into the brain prevents amelioration of ZSET1446 on depressive and memory-related behavior in olfactory bulbectomized mice. These results suggest that procognitive effects of ZSET1446 are mediated by nicotinic ACh receptors. Actually, nicotinic signaling has an important role in cognitive function. It has been reported that systemic administration of nicotine enhances object recognition memory in the rat (29). Further, nicotine is well known to increase ACh levels by a stimulation of nicotinic receptors on cholinergic nerve terminals in the brain (30, 31). Therefore, procognitive effect of ZSET1446 on novel object recognition behavior is probably via stimulation of nicotinic ACh receptor, especially of the α4 subunit (DHβE's target). It is likely that those effects are indirectly elicited by this compound, since ZSET1446 has no binding affinity for the nicotinic acetylcholine receptors (9).

The most important finding in the present study was that concomitant administration of ZSET1446 and

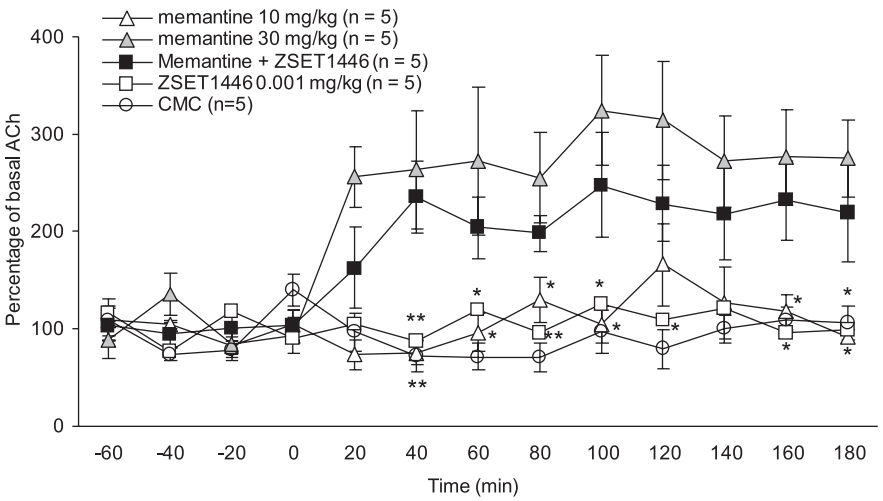

Fig. 5. Combination effects of ZSET1446 and memantine on the extracellular level of ACh in the hippocampus of rats. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the group of concomitant administration with ZSET1446 and memantine (Dunnett's multiple comparison test).

memantine at subeffective doses for each drug synergistically ameliorated the cognitive impairment caused by scopolamine in rats in the novel object recognition task, enhanced cognitive function for mice, and increased the extracellular level of ACh in the hippocampus in rats. The primary mechanism of the pharmacological action of ZSET1446 is suggested to be the stimulation of ACh release in the hippocampus and cortex (7, 8) without inhibitory action on AChE (9). We have also reported that ZSET1446 has no affinity for NMDA receptors (9), the target of memantine. These results suggest the synergistic interaction between ZSET1446 and memantine through different mechanisms, which might be beneficial for AD patients. Therefore, it is likely that the stimulated ACh release by ZSET1446 and memantine through different mechanisms increases the ACh concentration and/or duration of ACh presence in the synaptic cleft to a greater extent than that by each drug alone in the brain.

In conclusion, ZSET1446 and memantine synergistically enhanced cognitive function in the novel object recognition task, possibly due to synergistic increase in the extracellular level of ACh. In our previous study, ZSET1446 and donepezil produces a synergic effect to reverse cognitive impairment induced by scopolamine in the passive avoidance task via synergistic increase in ACh level in the hippocampus (17). These results suggest that ZSET1446 in combination with the other antidementia drugs is promising as a therapeutic interaction for the treatment of AD.

References

1 Selkoe DJ. The molecular pathology of Alzheimer's disease. Neuron. 1991;6:487–498.

2 Takata K, Kitamura Y. Molecular approaches to the treatment, prophylaxis, and diagnosis of Alzheimer's disease: Tangle formation, amyloid-β, and microglia in Alzheimer's disease. J Pharmacol Sci. 2012;118:331–337.

3 Atri A. Effective pharmacological management of Alzheimer's disease. Am J Manag Care. 17 (Suppl 13);S346–S355.

4 Xiong G, Doraiswamy PM. Combination drug therapy for Alzheimer's disease: what is evidence-based, and what is not? Geriatrics. 2005;60:22–26.

5 Cummings JL, Schneider E, Teriot PN, Graham SM. Memantine MEM-MD-02 study group. Behavioral effects of memantine in Alzheimer's disease patients receiving donepezil treatment. Neurology. 2006;67:57–63.

6 Creeley CE, Wozniak DF, Nardi A, Farber NB, Olney JW. Donepezil markedly potentiates memantine neurotoxicity in the adult rat brain. Neurobiol Aging. 2008;29:153–167.

7 Yamaguchi Y, Takano M, Saito K, Matsuno T, Kawashima S. Enhancement of acetylcholine release by ZSET845 and ZSET1446 in the rat hippocampus. Neurosci Res. 2002;6 (Suppl):S131.

8 Yamagichi Y, Takano M, Saito K, Matsuno T, Kawashima S. Effects of ZSET845 and ZSET1446 on acetylcholine release in the cortex and learning impairments in the rat. In: Hanin I, Fisher A, Cacabelos R, editors. New trends in Alzheimer and Parkinson related disorders. Bolonga: Monduzzi Editore; 2003. p. 145–148.

9 Yamaguchi Y, Miyashita H, Tsunekawa H, Mouri A, Kim H-C, Saito K, et al. Effects of a novel cognitive enhancer, spiro[imidazo-[1,2-a]pyridine-3,2-indan]-2(3H)-one (ZSET1446), on learning impairments induced by amyloid-β_{1-40} in the rat. J Pharmacol Exp Ther. 2006;317:1079–1087.

10 Ito Y, Mizoguchi H, Nagai T, Yamada K. A novel azaindolizinone derivative ZSET1446 (spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one) improves methamphetamine-induced impairment of recognition memory in mice by activating extracellular signal-regulated kinase 1/2. J Pharmacol Exp Ther. 2007;320:819–827.

11 Han F, Shioda N, Moriguchi S, Yamamoto Y, Raie AY, Yamaguchi Y, et al. Spiro[imidazo[1,2-a]pyridine-3,2-indan]-2(3H)-one (ZSET1446/ST101) treatment rescues olfactory bulbectomy-induced memory impairment by activating Ca^{2+}/calmodulin kinas II and protein kinase C in mouse hippocampus. J Pharmacol Exp Ther. 2008;326:127–134.

12 Shioda N, Yamamoto Y, Han F, Moriguchi S, Yamaguchi Y, Hino M, et al. A novel cognitinve enhancer, ZSET1446/ST101, promotes hippocampal neurogenesis and ameliorates depressive behavior in olfactory bulbectomized mice. J Pharmacol Exp Ther. 2010;333:43–50.

13 Yamaguchi Y, Saito K, Matsuno T, Takeda K, Hino M. Effects of ZSET1446/ST101 on cognitive deficits and amyloid β deposition in the senescence accelerated prone mouse brain. J Pharmacol Sci. 2012;119:160–166.

14 Green KN, Khashwji H, Estrada T, LaFerla FM. ST101 induces a novel 17kDa APP cleavage that precludes Aβ generation in vivo. Ann Neurol. 2011;69:831–844.

15 Moriguchi S, Shioda N, Yamamoto Y, Tagashira H, Fukunaga K. The T-type voltage-gated calcium channel as a molecular target of the novel cognitive enhancer ST101: enhancement of long-term potentiation and CaMKII autophosphorelation in rat cortical slices. J Neurochem. 2012;121:44–53.

16 Yamamoto Y, Shioda N, Han F, Moriguchi S, Fukunaga K. The novel cognitive enhancer ST101 enhances acetylcholine release in mouse dorsal hippocampus through T-type voltage-gated calcium channel stimulation. J Pharmacol Sci. 2013;121:212–226.

17 Yamaguchi Y, Takeda K, Fukuzaki E, Hino M. Combination of ZSET1445 (ST101) with donepezil synergistically ameliorates cognitive deficits mediated by increase in the extracellular level of acetylcholine. Neurodegener Dis. 2009;6 (Supple 1):258.

18 Ennaceur A, Delacour J. A new one-trial test for neurobiological studies of memory in rats. 1: behavioral data. Behav Brain Res. 1988;31:47–59.

19 Paxinos G, Watson C. The rat brain in stereotaxic coordinates. NewYork: Academic Press; 1982.

20 Pitsikas N, Sakellaridis N. Memantine and recognition memory: possible facilitation of its behavioral effects by the nitric oxide (NO) donor molsidomine. Eur J Pharmacol. 2007;571:174–179.

21 Kertész S, Kapus G, Gacsályi I, Lévay G. Deramciclane improves object recognition in rats: potential role of NMDA receptors. Pharmacol Biochem Behav. 2010;94:570–574.

22 Zoladaz PR, Campbell AM, Park CR, Schaefer D, Danysz W, Diamond DM. Enhancement of long-term spatial memory in adult rats by the noncompetitive NMDA receptor antagonists,

memantine and neramexane. Pharmacol Biochem Behav. 2006; 85:298–306.
23 Wise LE, Lichtman AH. The uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist memantine prolongs spatial memory in a rat delayed radial arm maze memory task. Eur J Pharmacol. 2007;575:98–102.
24 Drever BD, Anderson WGL, Johnson H, O'Callaghan M, Seo S, Choi DY, et al. Memantine acts as a cholinergic stimulant in the mouse hippocampus. J Alzheimers Dis. 2007;12:319–333.
25 Shearman E, Rossi S, Szasz B, Juranyi Z, Fallon S, Pomara N, et al. Changes in cerebral neurotransmitters and metabolites induced by acute donepezil and memantine administrations: a microdialysis study. Brain Res Bull. 2006;69:204–213.
26 Ihalainen J, Sarajärvi T, Rasmusson D, Kemppainen S, Keski-Rahkonen P, Lehtnen M, et al. Effects of memantine and donepezil on cortical and hippocampal acetylcholine levels and object recognition memory in rats. Neuropharmacology. 2011; 61:891–899.
27 Hutson PH, Hogg JE. Effects of and interactions between anatagonists for different sites on the NMDA receptor complex on hippocampal and striatal acetylcholine efflux in vivo. Eur J Pharmacol. 1996;295:45–52.
28 Kim SH, Price MT, Olney JW, Farber NB. Excessive cerebrocortical release of acetylcholine induced by NMDA antagonists is reduced by GABAergic and α2-adrenergic agonists. Mol Psychiatry. 1999;4:344–352.
29 Puma C, Deschaux O, Molimard R, Bizit JC. Nicotine improves memory in an object recognition task in rats. Eur Neuropsychopharmacol. 1999;9:323–327.
30 Rowell PP, Winkler DL. Nicotinic stimulation of [^{3}H]acetylcholine release from mouse cerebral cortical synaptosomes. J Neurochem. 1984;43:1593–1598.
31 De Sarno P, Giacobini E. Modulation of acetylcholine release by nicotinic receptors in the rat brain. J Neurosci Res. 1989;22: 194–200.