Ameliorative Effects of Azaindolizinone Derivative ZSET845 on Scopolamine-Induced Deficits in Passive Avoidance and Radial-Arm Maze Learning in the Rat

Yoshimasa Yamaguchi*, Masaya Higashi, Toshiyuki Matsuno and Seiichiro Kawashima

Research Laboratory, Zenyaku Kogyo Co., Ltd., 2-33-7 Ohizumi-machi, Nerima-ku, Tokyo 178-0062, Japan

Received July 16, 2001 Accepted September 28, 2001

ABSTRACT—Effects of ZSET845 (3,3-dibenzylimidazo[1,2-a]pyridin-2(3H)-one), a newly synthesized cognitive enhancer, and donepezil and tacrine on the scopolamine-induced cognitive deficits in rats were examined in passive avoidance and radial-arm maze tasks. ZSET845 (0.01 mg/kg) showed a greater ameliorative effect than donepezil (0.1 mg/kg) or tacrine (1 mg/kg) in the passive avoidance task. In the radial-arm maze task, ZSET845 (0.1 mg/kg) also showed a greater effect than donepezil (10 mg/kg) or tacrine (10 mg/kg). ZSET845 induced an increase in the choline acetyltransferase (ChAT) activity in the hippocampus, suggesting that the ameliorative effects of ZSET845 are related to the increase in the ChAT activity in the hippocampus.

Keywords: Passive avoidance, Radial-arm maze, Choline acetyltransferase

Alzheimer’s disease (AD) is the most common cause of senile dementia and is characterized by the deposition of amyloid plaques and neurofibrillary tangles in the brain and by the alterations in some neurotransmitter systems. Among the neurotransmitter system alterations, the most striking is the degeneration of forebrain cholinergic neurons, which is claimed to have a close correlation with the severity of dementia (1). In this context, a marked decrease in choline acetyltransferase (ChAT) activity was found in the brain of patients suffering from AD, when compared with age-matched controls (2, 3).

With the aim to reverse the neurochemical and functional alterations characteristic of dementia, a number of investigators have tried to develop drugs that stimulate central cholinergic function. To this purpose, several drugs with different pharmacological properties have been developed such as acetylcholinesterase (AChE) inhibitors (4), cholinergic receptor agonists (5) and choline uptake enhancers (6). The AChE inhibitors are the only family of drugs currently approved for treating AD. Discovery of drugs that have other modes of action will certainly be beneficial.

In a series of primary evaluations for chemicals with ameliorative effects on scopolamine-induced cognitive deficits, we found that ZSET845 is an effective new compound, which enhances ChAT activity in the hippocampus in the rat. In the present paper, we propose that ChAT could be a target for treatment of AD.

Male rats of the Sprague-Dawley strain (Charles River, Kanagawa) at 8 weeks of age (260–330-g body weight) were used in the experiments.

ZSET845 (3,3-dibenzylimidazo[1,2-a]pyridin-2(3H)-one) and donepezil ((±)-2-{[1-benzylpiperidin-4-yl]methyl}-5,6-dimethoxyindan-1-one monohydrochloride) were synthesized in our Department of Organic Synthesis of Zenyaku Kogyo Co., Ltd. ZSET845 and donepezil were suspended in 1% carboxymethyl cellulose. Tacrine (Sigma, St. Louis, MO, USA) was dissolved in distilled water. Scopolamine hydrobromide (Merck, Darmstadt, Germany) was dissolved in 0.9% NaCl.

The passive avoidance apparatus (Neuroscience, Tokyo) consisted of a small illuminated chamber and a larger dark chamber. The two chambers were separated by a guillotine door. On the first and second days of testing, each rat was placed in the apparatus and left for 3 min to habituate to the apparatus. On the third day, an acquisition trial was performed. Oral administration of ZSET845 (0.001, 0.01, 0.1 or 1 mg/kg), donepezil or tacrine (0.01, 0.1, 1 or 10 mg/kg) was given 60 min before the acquisition trial. Scopolamine (2 mg/kg) was intraperitoneally (i.p.) injected 20 min before the acquisition trial. Matched control rats received vehicle only. These experimental schedules
were determined by our preliminary observation that ZSET845 reached a peak in its plasma and brain tissue levels 1 h after the oral administration (data not shown). In the acquisition trial, each rat was placed in the illuminated chamber and immediately after entering the dark chamber, the door was closed and an inescapable scrambled electric shock (100 V, 0.4 mA, 0.8-s once) was delivered through the floor grid. Twenty-four hours later, each rat was again placed in the illuminated chamber (retention trial). The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as latency in both the acquisition and retention trials (maximum 300 s).

Other rats were tested in a radial-arm maze (Muromachi Kikai, Tokyo). It is well known that passive avoidance response can be affected by fear, where animals are negatively reinforced by electric shock. Therefore, we further evaluated the effect of test drugs using radial arm maze task, where animals were positively reinforced by bait. Body weights at the beginning of training trials were reduced to about 80% of the starting weights by reduction of daily ration of food (CE-7; Clea Japan, Tokyo). On the first day of pretraining, rats were placed in groups of 6 animals in the maze without any reward bait (crystal sugar, about a 50-mg piece) and left to explore the arms for 10 min. On the second and third days of pretraining, they were placed in the maze with bait scattered on the platform and arms including food cups, and then they were left for 10 min. The training trials started 3 days after pretraining and were carried out for 18 times in order to allow the rats to learn how to perform the radial-arm maze task. Each trial was continued until the bait of all 8 wells had been consumed, until 16 choices had been made, or until 10 min had elapsed, whichever occurred first. On the next day following the last training trial, the test trial was carried out. In the test trial, ZSET845, donepezil or tacrine (0.01, 0.1, 1 or 10 mg/kg) was given orally 60 min before the placement of rats in the maze. Scopolamine (0.5 mg/kg) was injected i.p. 20 min before the test trial. Matched control rats received vehicle only. In each trial, a rat was placed on the central hub of the maze and allowed to visit the wells at the end of each of 8 arms, each of which had been baited with one sugar crystal. Running paths were tracked with a camera fixed on the ceiling of the room and the records were stored in a computer (BTA-2 system, Muromachi Kikai). The number of consecutive correct choices prior to re-entry into a previously visited arm (initial correct responses) and the running speed were considered as indices of performance.

ChAT activity in the basal forebrain, medial septum, cortex and hippocampus was assayed as described by Fonnum (7), 60 min after the administration of ZSET845 (0.01, 0.1 or 1 mg/kg, p.o.), donepezil or tacrine (0.1, 1 or 10 mg/kg, p.o.). Brain regions were dissected out according to the previously described procedure (8). Protein concentration was measured by a Protein Assay Kit I (Bio-Rad Laboratories, Hercules, CA, USA). ChAT activity is expressed as nmol ACh synthesized per mg protein per hour.

For the results of passive avoidance, data were analyzed using the Kruskal-Wallis analysis of variances by ranks, which was followed by the non-parametric analysis of the Mann-Whitney U-test. For the results of the radial-arm maze and ChAT, the statistical significance of differences between groups was calculated by one-way analysis of variance, which was followed by Dunnett’s multiple comparison test.

In the acquisition trial of the passive avoidance task, we found no difference between the vehicle-administered control and test samples-treated rats in the step-through latency (Fig. 1). Either ZSET845, donepezil or tacrine in-
duced no significant changes in the latency in control rats given no scopolamine. Scopolamine given 20 min before the acquisition trial significantly reduced the latency determined 24 h later. The latency was significantly prolonged by treatments with ZSET845 [H = 14.91, P<0.01], donepezil [H = 9.61, P<0.05] and tacrine [H = 11.58, P<0.05]. Treatment with ZSET845 at the dose of 0.01 (P<0.01), 0.1 (P<0.05) and 1 (P<0.01) mg/kg significantly ameliorated impaired performance caused by scopolamine (Fig. 1A). Donepezil at the dose of 0.1 mg/kg (P<0.01) and tacrine at the doses of 1 (P<0.01) and 10 (P<0.05) mg/kg significantly ameliorated performance impaired by scopolamine (Fig. 1: B and C, respectively). It is well known that passive avoidance response can be affected by pain threshold or fear. However, we found no differences between vehicle-treated controls and ZSET845-treated rats in the three parameters relevant to shock sensitivity (data not shown), indicating that the increase in latency by oral administration of ZSET845 was not due to the changes in pain threshold or fear, but due to the amelioration in cognitive function.

ZSET845, donepezil and tacrine induced no significant changes in the number of initial correct responses in control rats given no scopolamine. The number of initial correct responses was significantly decreased by scopolamine treatment as compared to the vehicle controls in the radial-arm maze task (Fig. 2). However, the number of initial correct responses was significantly increased by oral administration of ZSET845 [F(4,35) = 3.06, P<0.05], donepezil [F(4,35) = 9.53, P<0.01] and tacrine [F(4,40) = 3.70, P<0.05]. Statistical significance for the ameliorative performance was detected in the ZSET845 (0.1 mg/kg, P<0.01, Fig. 2A), in the donepezil (10 mg/kg, P<0.01, Fig. 2B) and tacrine (10 mg/kg, P<0.01, Fig. 2C). Furthermore, the number of total errors were significantly decreased by ZSET845 (0.1 mg/kg, P<0.05) and donepezil (10 mg/kg, P<0.01), but not by tacrine (10 mg/kg, P>0.05) (data not shown). These ameliorative effects were not the consequences of enhanced locomotor activity, because the running speed of rats treated with ZSET845 [F(4,35) = 0.94, P>0.05], donepezil [F(4,35) = 0.91, P>0.05] or tacrine [F(4,40) = 1.40, P>0.05] was not significantly deviated from the level of controls given no test samples (Fig. 2).

Oral administration of ZSET845 caused an increase in ChAT activity in the hippocampus [F(3,28) = 4.10, P<0.05], but not in the basal forebrain [F(3,28) = 0.26, P>0.05], medial septum [F(3,28) = 1.83, P>0.05] and cortex [F(2,28) = 1.65, P>0.05] as compared with vehicle-injected controls (Fig. 3A). In the hippocampus, ZSET845 (0.01, 0.1 or 1 mg/kg) significantly increased ChAT activity (112%, 113.8% or 108.7%, respectively) compared with matched vehicle-injected control rats (P<0.05). ChAT activity was not changed in any of these regions by donepezil or tacrine (Fig. 3: B and C).

Studies using pharmacological antagonists and brain lesions have indicated that the disturbance of central cholinergic transmission is associated with cognitive impairment (9, 10). In the present study a cholinergic receptor antagonist scopolamine that is widely accepted as an agent causing cognitive deficits in experimental animals with induced performance impairment in both passive avoidance and radial-arm maze tasks, in agreement with the previous reports (5, 11). ZSET845 ameliorated performance impaired by scopolamine in both tasks. Donepezil and tacrine also ameliorated impaired performance caused by scopolamine in both tasks, consistent with the previous report (5, 12). These results provide the view that ZSET845 has a cognition enhancing action in rats. In our preliminary study, ZSET845 had no inhibitory action on AChE activity and enhanced ChAT activity in NB-1 cells in vitro (data not shown). Donepezil and tacrine have been claimed to act through AChE inhibition. Therefore, ZSET845 has an
action different from that of donepezil and tacrine. Furthermore, in the present study, a single oral administration of ZSET845 caused an increase in ChAT activity in the hippocampus. These results suggest that ZSET845 may contribute to the ameliorative effects through the activation of ChAT in the hippocampus.

The minimum effective doses of ZSET845, donepezil and tacrine were 0.01, 0.1 and 1 mg/kg in the passive avoidance task and 0.1, 10 and 10 mg/kg in the radial-arm maze task, respectively. These results indicate that the minimum effective doses of these drugs in passive avoidance are smaller than those in the radial-arm maze. The reason for the dose differences between the two tasks is currently unknown. At any rate, when minimum effective doses were compared, the potency of ZSET845 was 10 times greater than that of donepezil and 100 times greater than tacrine in the passive avoidance task, and it was 100 times greater than donepezil or tacrine in the radial-arm maze task. Furthermore, ZSET845 showed a broader effective dose range (0.01 – 1 mg/kg) than the other two drugs in the passive avoidance task. According to Heise (13), the ‘therapeutic window’ of effective dose range should be broad enough, if any compound is potentially beneficial for human use. In this regard, the broader ‘window’ observed in ZSET845 may be advantageous. ZSET845 in none of the tested doses produced any apparent adverse effects, and the acute preliminary toxicity test by much greater doses showed no toxicity (data not shown). Further study is necessary to elucidate the effect of drugs on scopolamine-induced deficits in the radial-arm maze task with different ratio of reinforcement, such as a 4-out-of-8 baiting procedure in order to assess the working memory of test rats.

It is well known that among the neurochemical abnormalities described for the brains of AD, the decrease in the ChAT activity is the most prominent and provides an excellent biochemical correlate for the severity of dementia (2, 14). To conclude, ZSET845 has ChAT-enhancing effect in the hippocampus and might be of therapeutic value for the cognitive and memory disorders such as AD.

REFERENCES

1 Perry EK, Tomlinson BE, Blessed G, Bergmann K, Gibson PH and Perry RH: Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J 25, 1457 – 1459 (1978)
2 Bartus RT, Dean RL III, Beer B and Lippa AS: The cholinergic hypothesis of geriatric memory dysfunction. Science 217, 408 – 417 (1982)
3 Coyle JT, Price DL and DeLong MR: Alzheimer’s disease: a disorder of cortical cholinergic innervation. Science 219, 1184 – 1190 (1983)
4 Benzi G and Moretti A: Is there a rationale for the use of acetylcholinesterase inhibitors in the therapy of Alzheimer’s disease? Eur J Pharmacol 346, 1 – 13 (1998)
5 Wanibuchi F, Nishida T, Yamashita H, Hidaka K, Koshiba K, Tsukamoto S and Usuda S: Characterization of a novel muscarinic receptor agonist, YM796; comparison with cholinesterase inhibitors in in vivo pharmacological studies. Eur J Pharmacol 265, 151 – 158 (1994)
6 Bessho T, Takashima K, Tabata R, Ohshima C, Chaki H, Yamabe H, Egawa M, Tobe A and Saito K: Effect of the novel high affinity choline uptake enhancer 2-(2-oxopyrrolidin-1-yl)-N-(2,3-dimethyl-5,6,7,8-tetrahydrofuro[2,3-b]quinolin-4-yl) acetamide on deficits of water maze learning in rats. Arzneimittelforschung 46, 369 – 373 (1996)
7 Fonnum F: A rapid radiochemical method for the determination of choline acetyltransferase. J Neurochem 24, 407 – 409 (1975)
8 Yamaguchi Y and Kawashima S: Effects of amyloid-β-(25 – 35) on passive avoidance, radial-arm maze learning and choline...
acetyltransferase activity in the rat. Eur J Pharmacol 412, 265 – 272 (2001)
9 Sala M, Braid D, Calcatera P, Leone MP, Comotti FA, Giannola S and Gori E: Effect of centrally administered atropine and pirenzepine on radial arm maze performance in the rat. Eur J Pharmacol 194, 45 – 49 (1991)
10 Dutar P, Bassant M-H, Senut M-C and Lamour Y: The septo-hippocampal pathway: structure and function of a central cholinergic system. Physiol Rev 75, 393 – 427 (1995)
11 Wirsching BA, Beninger RJ, Jhamandas K, Boegman RJ and El-Defrawy SR: Differential effects of scopolamine on working and reference memory of rats in the radial maze. Pharmacol Biochem Behav 20, 659 – 662 (1984)
12 Wang T and Tang XC: Reversal of scopolamine-induced deficits in radial-arm maze performance by (–)-huperizine A: comparison with E2020 and tacrine. Eur J Pharmacol 349, 137 – 142 (1998)
13 Heise GA: Facilitation of memory and cognition by drugs. Trends Pharmacol Sci 8, 65 – 68 (1987)
14 Bierer LM, Haroutunian V, Gabriel S, Knott PJ, Carlin LS, Purohit DP, Perl DP, Schmeidler J, Kanof P and Davis KL: Neurochemical correlates of dementia severity in Alzheimer’s disease: relative importance of the cholinergic deficits. J Neurochem 64, 749 – 760 (1995)