ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Apr; 23 (4): 355 - 360
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Reversal of scopolamine-induced spatial memory deficits in rats by TAK-147[1]

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KEY WORDS TAK-147; donepezil; tacrine; memory disorders; maze learning; cholinesterase inhibitors

ABSTRACT

AIM: To evaluate effect of TAK-147 on spatial memory deficit induced by scopolamine. **METHODS**: Morris water maze was used to measure spatial memory in rats and open field test was used to analyse locomotor activity. **RESULTS**: In the acquisition memory process, scopolamine (0.4 mg/kg, ip) markedly increased the escape latency to the platform. Ip injection of both TAK-147 and donepezil ameliorated scopolamine-induced deficit, dose-related and significant effect was obtained at doses of 0.1 - 1.0 mg/kg. In the memory retrieval process, increased latency induced by scopolamine (1.5 mg/kg, ip) was also significantly reversed by treatment with TAK-147 (0.1, 0.3, and 1.0 mg/kg), donepezil (0.3 and 1.0 mg/kg), and tacrine (3 and 5 mg/kg), respectively. TAK-147 has a little potent efficacy to donepezil, and was more potent than tacrine. In the locomotor test, both TAK-147 and donepizil created no appreciable change of locomotor activities, compared with scopolamine or saline. **CONCLUSION**: TAK-147 plays an important role in spatial cognition, and this result provides additional evidence that TAK-147 is an ideal AChE inhibitor and is useful for the treatment of Alzheimer's disease.

INTRODUCTION

Alzheimer's disease (AD) is a slowly progressive neurodegenerative disorder characterized by severe memory loss and cognitive impairment. It has become the fourth leading cause of death in the developed nations[1]. It is well known that the most striking and consistent neurochemical changes in the brain of patient with AD are significant decrease of choline acetyltransferase (ChAT) activity in the cerebral cortex, amygdala, and hippocampus[1]. A significant correlation has been observed between the deterioration of these cholinergic activity and the cognitive impairment in AD[1,2]. It is demonstrated that cholinergic drugs affect cognitive function, including attention and memory in both rodent and human, such as lesion of NBM and scopolamine-induced memory deficit in various memory paradigm[1,3-5]. Therefore, these findings have led to the hypothesis that enhancement of cholinergic neurotransmission with cholinergic agents may ameliorate cognitive impairment in AD, which means a replacement therapy strategies for decline of basal forebrain cholinergic activity in AD.

Many attempts have been made to reverse the memory deficit using cholinergic agents. AChE inhibitors such as physostigmine and tacrine have been evaluated on a large scale in AD[6]. However, their excessive peripheral side effects, low bioavailability, serious hepatotoxicity, and the short half-lives limited their wide applications[6,7]. In addition, though donepezil may overcome the problem of physostigmine and tacrine, which was reported causing no hepatotoxicity, and be initially in clinical use for treatment for AD, it still results in vomiting, nausea, diarrhea, and muscle cramp[6,8,9]. Recently, many new AChE inhibitors have been developed for treatment of AD, and some are under clinical use or have been submitted for approval, such as huperzine A and rivastigmine[7-10].

TAK-147 {3-[1-(phenylmethyl)-4-piperidinyl]-1-(2,3,4,5-tetrahydro-1*H*-1-benzazepin-8-yl)-1-propanone fumarate} has recently been introduced to be another selective AChE inhibitor, which is indicated to selectively inhibit activity of AChE[11,12]. It has been demonstrated

[1] Project supported by the National Natural Science Foundation of China (№ 30000019) and Zhejiang Provincial Natural Science Foundation of China (№ 300062).
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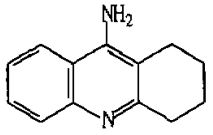
Received 2001-06-25 Accepted 2002-01-08

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Apr; 23 (4)
E-mail aps@mail.sbcnc.ac.cn Phn/Fax 86-21-6474-2629



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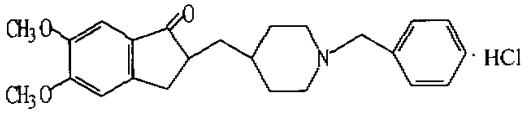

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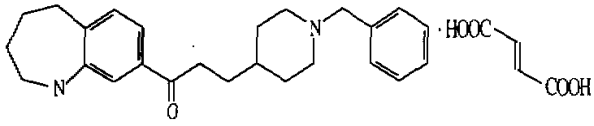

TAK-147

that TAK-147 is more potent and brain-specific than tacrine and physostigmine to inhibit AChE[12], and has been approval potentiating ChAT activity in cultured rat septal neurons more potent than donepezil, tacrine, and revastigmine[13]. In addition, initial behavioral studies have found that the treatment with TAK-147 ameliorates learning and memory impairment in passive avoidance test and plus-maze learning behaviour[12]. In these studies, however, the memory parameter used was escape latency, which can be affected by behavioral toxicity such as decreases in locomotor activity or muscle relaxant activity, less is known about the characteristics of pharmacological effects of TAK-147 and whether the drug is effective in spatial cognitive in rodent or not, have not yet been evaluated.

Therefore, in the present study, compared with tacrine and donepezil, we use Morris water maze to further investigate effects of TAK-147 on spatial memory deficit caused by scopolamine in rats.

MATERIALS AND METHODS

Animals The animals used in this study were male Sprague-Dawley rats (♂, 200 – 280 g, $n = 200$, Grade Ⅱ, Certificate No 22-9601018, Experimental Animal Center, Zhejiang University), maintained in individual cages with a 12-h light-dark cycle (lights on from 8 : 00 – 20 : 00). Water was given *ad libitum*. Experiments were carried out each day between 13 : 00 – 18 : 00.

Morris water maze task The Morris water maze is widely used to evaluate spatial navigation scores in rodents. A circular pool (150 cm in diameter, 50 cm in high) was filled 24-cm deep with clear water at a temperature of (22 ± 1) ℃. The water was made opaque with addition of powdered milk. A clear glass platform (15 cm in diameter) was submerged 1 cm below the water surface, on which rodents could escape. The pool was conceptually divided into four quadrants of equal area: NE, NW, SE, and SW. The experimental room contained different extra-maze cues, and the illumination was provided by a 40 W white light placed 1.6 m above the center of the pool.

After familiarizing the rats with the Morris water maze for one day, all rats were trained with three trials per day for 4 consecutive days (acquisition test). For each training trial, the rats were placed in the water facing the pool wall at one of 3 quadrants (NW, SE, SW) in different order each day, and allowed to swim until they reached the platform. The time and track to reach the platform was recorded by a video camera overhead. Latency to reach the platform was recorded up to a cutting time of 120 s. On the platform they remained for 10 s before removal. If the rat did not find the platform on time, it was placed on the platform for 10 s by the experimenter. After each training trial, the rat was dried with a towel and allowed to remain in a cage, and with a 10-min inter-trial interval. After the acquisition test, rats were trained once a day continually until they satisfied a criterion of reaching the platform within 10 s for 3 consecutive days. The animals were treated with scopolamine (1.5 mg/kg) to impair their recall process, and then tested with either drug or vehicle. Eleven of 200 rats which failed to climb on platform, and 8 of 200 were not able to satisfied the criterion of Morris water maze were excluded. Fifteen of 181 rats which created tolerable effects to scopolamine (1.5 mg/kg) by repeated administration were also

excluded from the statistical analysis.

Open-field test The open field test was used for testing the behavioral responses of rats to a novel environment. It consisted of a black wooden square enclosure with 30-cm high walls and a 50 cm × 35 cm floor in a separate sound insulation box. Illumination was provided by a 40 W fluorescent lamp positioned 1.8 m above the floor of the apparatus. The animal was placed in the center of the arena and left free to explore the environment for 5 min, and the activities data (meter, m) was collected by an on-line video tracking device designed to track the object in its field with the highest contrast, which was always the white rat on the black background. The tracker's digitized coordinate values were sampled in turn using a computer analysis system.

Drugs TAK-147 was kindly donated by Dr Yuji ISHIHARA and Dr Masaomi MIYAMOTO (Takeda Chemical Industries, Ltd, Japan). Donepezil was a gift from Dr Takashi KOSASA (Eisai Chemical Industries, Ltd, Japan). Scopolamine (Sigma) and tacrine (Sigma) were used in the study. Drugs were dissolved in saline and injected ip 30 min before test trial. Studies of memory recall process for drug effect were carried out once every five days.

Statistical analysis Differences were analyzed by paired t test, and one-way analysis of variance with Dunnett's test using computer software (SigmaStat 1.01 for Windows 95, 1992, Jandel Corp, USA). Values are shown as $\bar{x} \pm s$. Significant difference are expressed as $P < 0.05$ *vs* control group, respectively. ED_{50} (95 % confidence limits) values of the test compounds for decreases in scopolamine (1.5 mg/kg)-induced prolongation of escape latency were calculated and compared by weighted probit analysis of Bliss method.

RESULTS

Effects of TAK-147, donepezil, and tacrine on spatial memory deficit of acquisition process induced by scopolamine in Morris water maze Saline-treated rats showed a marked reduction in escape latency from the 1 to 4 d and reached stable latency after the fourth trial. Ip injection of scopolamine (0.4 mg/kg) significantly increased the latency to reach the platform from the 2 to 4 test trial ($P < 0.05$). On the other hand, TAK-147 dose-dependently attenuated the scopolamine-induced increase of latency. As shown in Tab 1, TAK-147 at a dose of 0.1 mg/kg significantly antagonized the effect of scopolamine on escape latency from the 3 to 4 test trial ($P < 0.05$), and at doses of 0.3 and 1.0 mg/kg markedly reversed scopolamine action from the 2 to 4 test trial ($P < 0.05$). Similarly, donepezil at doses of 0.1 – 1.0 mg/kg, and tacrine at doses of 3.0 and 5.0 mg/kg resulted in significant effects on escape latency of Morris water maze from the 2 to 4 test trial ($P < 0.05$).

Effects of TAK-147, donepezil, and tacrine on spatial memory deficit of retrieval process induced by scopolamine in Morris water maze As shown in Fig 1, scopolamine (1.5 mg/kg) significantly increased the escape latency to reach the platform after the rodents have trained to satisfy a standard criterion. Both TAK-147 and donepezil reversed scopolamine effect dose-relatedly. TAK-147 at doses of 0.1, 0.3, and 1.0 mg/kg, and donepezil at doses of 0.3 and 1.0 mg/kg significantly attenuated scopolamine-induced increase of latency. The ED_{50} values for TAK-147 and donepezil were 0.110 mg/kg (0.044 – 0.275 mg/kg) and 0.184 mg/kg (0.088 – 0.383 mg/kg), respectively. In addition, tacrine also decreased escape latency, and it created a dose-bell-shape actions. It greatly antagonized the effect of scopolamine on escape latency at a dose of 3 mg/kg ($P < 0.05$), a lower or higher dose of tacrine was less effective. The ED_{50} value for tacrine is 4.06 mg/kg (2.25 – 7.33 mg/kg).

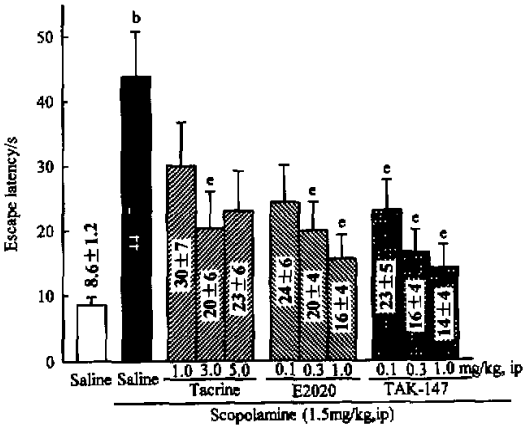

Fig 1. Effects of TAK-147, donepezil, and tacrine on spatial memory deficit of retrieval process induced by scopolamine (1.5 mg/kg) as evaluated by Morris water maze in rats. $n = 13 - 16$ rats. $\bar{x} \pm s$. $^bP < 0.05$ *vs* saline-treated group. $^eP < 0.05$ *vs* scopolamine + saline-treated group.

ISSN 1671-4083 Acta Pharmacol Sin 中国药理学报 2002 Apr; 23 (4)
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Tab 1. Effect of TAK-147, donepezil, and tacrine on spatial memory deficit of acquisition process induced by scopolamine (0.4 mg/kg) as evaluated by Morris watet maze. $\bar{x} \pm s$. $^bP < 0.05$ *vs* **saline-treated group.** $^cP < 0.05$ *vs* **scopolamine + saline-treated group.**

Drugs	Dose/ mg·kg^{-1}	n	Escape latency/s d 1	d 2	d 3	d 4
Saline	–	18	96 ± 26	44 ± 21	22 ± 13	16 ± 10
Scopolamine	0.4	16	115 ± 10	97 ± 22^b	75 ± 19^b	60 ± 20^b
Scopolamine + saline	–	16	109 ± 11	99 ± 17	178 ± 17	64 ± 15
Scopolamine + tacrine	1.0	14	105 ± 24	100 ± 22	61 ± 27	51 ± 20
	3.0	14	111 ± 17	70 ± 29	48 ± 17^c	40 ± 14^c
	5.0	15	106 ± 21	58 ± 22^c	42 ± 15^c	37 ± 16^c
Scopolamine + donepezil	0.1	14	91 ± 37	60 ± 22^c	44 ± 15^c	32 ± 13^c
	0.3	15	98 ± 37	63 ± 20^c	39 ± 14^c	25 ± 9^c
	1.0	15	96 ± 32	41 ± 18^c	21 ± 8^c	17 ± 7^c
Scopolamine + TAK-147	0.1	15	113 ± 12	78 ± 27	43 ± 18^c	34 ± 10^c
	0.3	14	99 ± 14	60 ± 20^c	36 ± 11^c	25 ± 11^c
	1.0	15	107 ± 11	55 ± 13^c	25 ± 9^c	20 ± 9^c

Influence of drug treatment in locomotor activities of rats as evaluated by open field test

As shown in Tab 2, compared with vehicle group, either scopolamine (0.4 and 1.5 mg/kg) or TAK-147 (0.3 and 1.0 mg/kg), donepezil (0.3 and 1.0 mg/kg) created no appreciable changes of locomotor activities. Tacrine at a dose of 3.0 mg/kg showed no marked effect, however at a dose of 5.0 mg/kg it significantly decreased locomotor activities ($P < 0.05$).

Tab 2. Influence of drugs on locomotor activities of rats. Locomotor activities were measured in an open field test. $\bar{x} \pm s$. $^bP < 0.05$ *vs* **scopolamine (1.5 mg/kg)-treated group.**

Drugs	Dose/ mg·kg^{-1}	n	Locomotor activities/ m·min^{-1}
Saline	–	18	0.94 ± 0.14
Scopolamine	0.4	18	1.02 ± 0.14
	1.5	18	1.06 ± 0.20
Tacrine	3.0	14	0.70 ± 0.16
	5.0	15	0.36 ± 0.08^b
Donepezil	0.3	15	1.02 ± 0.20
	1.0	15	0.94 ± 0.10
TAK-147	0.3	15	1.08 ± 0.12
	1.0	16	0.92 ± 0.16

DISCUSSION

It has been generally known that the cholinergic system in the basal forebrain plays an important role in learning and memory[1-3]. Scopolamine interferes with memory and cognitive function in both human and rodents by blocking muscarinic receptor in the brain[3-5]. It is found that scopolamine (0.4 and 1.5 mg/kg) resulted in an increase in the escape time to platform in either acquisition or recall process as evaluated by the Morris water maze. Bejar[10] has also reported that scopolamine (0.5 and 1.0 mg/kg) cause a serious impairment of either working or reference memory in the Morris water maze. On the other hand, the locomotor activity of rodents in the open field test was not influenced by scopolamine. Therefore, it is reasonable to presume the inhibition of spatial cognition induced by scopolamine was essentially unrelated to an increase in locomotor activity.

It has been demonstrated that TAK-147 is a highly selective and brain-specific AChE inhibitor. Previous finding has shown that TAK-147 enhances ChAT activity more potent than donepezil, physostigmine, tacrine, and rivastigmine[13]. Our results showed that TAK-147 were effective in antagonizing the memory deficit induced by scopolamine in both the acquisition and recall memory process in the Morris water maze without affecting motor activity. The ameliorating effect against scopolamine-induced memory deficit is little potent than that of donepezil, and more potent than that of tacrine. These results are in agreement with the previous ones, in which TAK-147 has been reported to reverse scopolamine- or AF64A-induced memory deficits as evaluated by passive avoidance test in rats[12]. Therefore, the ameliorating effects of TAK-147 are likely to be due to activation of

the cholinergic system in the brain.

It is generally known that an AChE inhibitor without higher affinity for the central nervous system could inhibit butyrylcholinesterase (BuChE) activity, a nonselective cholinesterase located in the blood and in peripheral organs such as lung and liver, which might result in peripheral side effects[14,15]. In our present study, tacrine showed a typical bell-shaped dose response curve for antagonism of scopolamine-induced retrieval memory impairment, which is similar to that of physostigmine and rivastigmine[10,16,17]. The finding[12,18] that the high dose of tacrine is unselective for its affinity to both AChE and BuChE, might contribute to the phenomena. High dose of tacrine decreased motor activity of rodents, and this might create influence in its cognitive result. In addition, high doses of certain AChE inhibitor such as physostigmine, tacrine, donepezil, and rivastigmine could reduce acetylcholine release by activating presynaptic muscarinic autoreceptor[13,17], and those might also contribute to the bell-shaped phenomena. However, there is still no report of TAK-147 decreasing acetylcholine release. In the present study, TAK-147 did not create bell-shaped phenomenon till at a high dose of 2.5 mg/kg, and donepezil until at a dose of 2 mg/kg (data not shown). It appeared that TAK-147 is more effective with a wide range of doses than that of tacrine and donepezil. TAK-147 has been reported hardly to inhibit activity of BuChE[19], which suggested that TAK-147 might create less peripheral side effect. It has been indicated that TAK-147 induce less behavioral depression and acute toxicity such as behaviors of lacrimation, salivation, and LD_{50} values, than those of physostigmine, tacrine, and donepizil[12]. Therefore, TAK-147, the highly selective AChE inhibitor, may result in more beneficial effects on spatial cognition, compared to those of tacrine and donepezil.

Combined with the present finding, we may conclude that TAK-147, as a novel AChE inhibitor, plays an important role in spatial cognition, and our results provide additional evidence, that TAK-147 should be an ideal AChE inhibitor and useful for the treatment of AD.

ACKNOWLEDGEMENTS We thank Dr Yuji ISHIHARA and Dr Masaomi MIYAMOTO (Takeda Chemical Industries, Ltd, Japan), and Dr Takashi KOSASA (Eisai Chemical Industries, Ltd, Japan) for their kindness of presenting TAK-147 and donepezil.

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TAK-147 逆转东莨菪碱诱发的大鼠空间记忆障碍[1]

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关键词 TAK-147; donepezil; 他克林; 记忆障碍; 迷宫学习; 胆碱酯酶抑制剂

目的: 研究和阐明 TAK-147 对东莨菪碱诱发的大鼠空间记忆损伤的作用. **方法**: 采用 Morris 水迷宫的程序研究大鼠的空间记忆, 利用开场实验方法测定动物自发活动量. **结果**: 在水迷宫的学习过程中, 腹腔内注射东莨菪碱(0.4 mg/kg, ip)明显延长大鼠上台的潜伏期, 而腹腔内注射 TAK-147 或 donepezil (多奈哌齐)能剂量依赖性地改善东莨菪碱诱发的记忆损伤, 两药在 0.1 – 1.0 mg/kg 的剂量时具有显著性差异. 在记忆的再生过程中, 腹腔内注射东莨菪碱(1.5 mg/kg, ip)引起空间记忆再生过程的障碍分别被 TAK-147 (0.1, 0.3 和 1.0 mg/kg)、多奈哌齐(0.3 和 1.0 mg/kg)以及他克林(3 和 5 mg/kg)显著性改善. TAK-147 的作用比多奈哌齐略强却明显强于他克林. 此外, 在开场实验中, TAK-147 和多奈哌齐与生理盐水和东莨菪碱相比, 对大鼠运动量未产生明显改变. **结论**: TAK-147 在空间认知功能上起重要的作用, 进一步证明 TAK-147 能够成为一个治疗阿尔采默病的理想的胆碱酯酶抑制药.

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