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Evaluation of acute tacrine treatment on passive-avoidance response, open-field behavior, and toxicity in 17- and 30-day-old mice

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Abstract

The potential of tacrine in altering cognitive/behavioral function as well as in causing toxicity was evaluated in mice of 17 and 30 days of age. Cognitive and behavioral studies were performed using a step-through passive avoidance task and a habituation open-field test with a 24-h retention interval. Tacrine was subcutaneously injected (1.25−80 μmol/kg) 30 min prior to the first session of both tests. During the training session in step-through task, tacrine treatment dose-dependently decreased the number of footshocks, with IC_{50} values being 7.8 and 23.3 μmol/kg in 17- and 30-day-old mice, respectively. Treatment with tacrine at a low dose (5 μmol/kg) significantly prolonged the retention latency in 17-day-old mice only, but it shortened the retention latency at high doses of 20 and 40 μmol/kg in 17- and 30-day-old, respectively. During the acquisition session in the open-field test, tacrine treatment dose-dependently decreased the locomotor activity in 17- and 30-day-old mice, with IC_{50} values being 15.1 and 24.7 μmol/kg, respectively. High doses of tacrine invariably increased the locomotor activity during the recall session. Tacrine treatment at a dose of 40 μmol/kg caused a significant increase in serum alanine aminotransferase activity in 17- and 30-day-old mice at 6 h post-dosing, with the extent of stimulation in 30-day-old mice being more prominent. In conclusion, tacrine was more potent in enhancing/disrupting the cognitive function, inhibiting locomotor activity as well as in causing hepatotoxicity in 17-day-old than in 30-day-old mice.

Keywords: Tacrine; Passive-avoidance response; Open-field memory; Locomotor; Defecation; Acute toxicity; Hepatotoxicity; Developmental changes

1. Introduction

Drugs augmenting cholinergic transmission in the brain have long been known to induce both promnestic and amnestic effects (Sabolek et al., 2005, 2004; Diez-Ariza et al., 2003). Previous experimental and clinical studies have shown that while learning processes are more sensitive to the enhancing action of cholinergic mimetic drugs, memory processes are more susceptible to the inhibition by anti-cholinergic drugs (Potamianos and Kellett, 1982; Nakahara et al., 1988; Pan and Han, 2000, 2004). Experimental investigations showed that cholinergic receptors in neonatal rat brain were increased during the course of development, with the number of receptors reaching an adult level around 35 days postnatal (Zhu et al., 2000, Daws and Overstreet, 1999). Moreover, the sensitivity of muscarinic receptor-stimulated phosphoinositide metabolism in brain areas, such as hippocampus, cerebral cortex, cerebellum, and brainstem, was enhanced in neonatal rats, and immature rats are more sensitive than adult rats to the lethal effect produced by chlorpyrifos, an organophosphorus insecticide (Balduini et al., 1990, 1987; Zheng et al., 2000). While the higher sensitivity of immature animals to organophosphorus insecticides is causally related to a slower rate of insecticide inactivation in the liver, it is also found that the developing nervous system may be more sensitive to the non-cholinesterase action of acetylcholinesterase inhibitors (AChEIs), as assessed by *in vitro* studies using brain slices from 7-, 21-, and 90-day-old rats (Oliver et al., 2001). In this connection, studies in our laboratory showed that young mice were more sensitive than their adult counterparts to muscarinic cholinergic antagonists-induced behavioral changes and amnesia (Pan et al., 1998).

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Tacrine (9-amino-1,2,3,4-tetrahydroaminoacridine hydrochloride), a reversible inhibitor of acetylcholinesterase (AChE), was first synthesized in 1945. It has been demonstrated that tacrine could improve the cognitive function in naturally aged mice and experimental model of aging, both of which exhibited learning and memory deficits, as well as in young cognitively impaired rats (Marighetto et al., 2000, Van Dam et al., 2003; Sabolek et al., 2004). In 1993, this drug was approved by the FDA (USA) for clinical use as a cognitive enhancer in mild to moderate Alzheimer's disease (Wagstaff and McTavish, 1994; Qizilbash et al., 1998). However, if tacrine also affects the cholinergic nerves in the body, it may produce undesirable side effects. In this regard, prolonged use of tacrine has proven to be hepatotoxic in about 30% of the patients. While the exact mechanism involved in the development of tacrine-induced hepatic injury in clinical situations remains unclear (Salmon et al., 2001; Watkins et al., 1994), experimental studies showed that the acute hepatotoxicity caused by a single, high dose of tacrine could result from hypoxia–reoxygenation injury secondary to the activation of the sympathetic nervous system (Stachlewitz et al., 1997). In the present study, we, therefore, endeavored to examine and compare the tacrine-induced changes in cognitive and behavioral functions as well as toxicity in 17- and 30-day-old mice that were presumably free of cognitive deficit.

2. Materials and methods

2.1. Animal care

Male ICR mice of 17-day-old (13−15 g) and 30-day-old (23−25 g) were used in all experiments. Animals were purchased from Vital River Lab Animal Co. Ltd. (grade II, Certificate No. SCXK-2002-0003) and eight animals were housed per cage. Mice were maintained on a 12-h light/dark cycle (light from 07:00 to 19:00 h) at 20−22 °C with a relative humidity of 50−55%, and given food and water *ad libitum* in an animal care facility. All experimental protocols were approved by the University Committee of Research Practice in Beijing University of Chinese Medicine.

2.2. Drug treatment

Tacrine hydrochloride was purchased from Sigma Chemical Co., USA. The drug was dissolved in distilled water and administered by subcutaneous injection (1.25−80 μmol/kg or 0.32−20.22 mg/kg). Control mice received the vehicle (10 ml/kg). In behavioral studies, mice of both age groups were administered with tacrine or vehicle 30 min prior to the training session in the step-through task or the acquisition session in the open-field test. No drug was given before the retention session in step-through task or the recall session in open-field test. Animals in various experimental groups were randomized according to a Latin square design. Experiments were carried out between 08:00 and 13:00 h. In the acute toxicity test, tacrine was administered subcutaneously at increasing doses in both 17- and 30-day-old mice and the mortality rate was determined within 24 h post-dosing. LD_{50} values were then estimated using the Probit method.

2.3. Step-through task

Step-through task experiment for measuring passive-avoidance response was conducted in a box with two symmetrical compartments [14 cm (long)×5 cm (wide)×8 cm (high)] being separated by a dark board with a door of 3 cm in diameter near the floor. The grid floor of the two chambers consisted of copper bars (0.3 cm in diameter, separated by 1 cm center to center) that could be electrified with 34 V in the dark, but not in the light (i.e., safe) compartment. The experiment was begun with a training session in which each mouse, with face away from the door, was gently put inside the safe compartment of the conditioning box, with two 40-W bulbs suspended 200 cm above the top of the chamber. Upon stepping from the safe room to the dark compartment, the mouse would receive a footshock. In response to the punishment, the mouse would return to the safe compartment and avoid entering the dark one again. During the training session, the number of footshocks for each mouse was recorded within 5 min and then the animal was returned to its home cage. The retention session was conducted 24 h after the training session, in which each mouse was placed in the safe room as in training session and the time lapse (latency) for crossing from the safe place to the electrified chamber was recorded for 3 min. If the mouse did not cross to the dark compartment within 3 min, the session would be ended and a score of 180 was assigned. The enhancement of learning ability in the step-through task was reflected in the decrease in the number of footshocks in the training session. The drug-induced enhancement or impairment of passive avoidance memory was indicated by the prolonged or shortened retention latency, respectively, when compared with the untreated control.

2.4. Open-field test

An open-field test apparatus was used for measuring the extent of locomotion and defecation within a fixed period of time. The apparatus consisted of a rectangular chamber [38 cm (long)×20 cm (wide)×24 cm (high)], with the field being illuminated with two 40-W light bulbs over-hanging at 200 cm above the center of the field. In the experiment, each mouse was gently placed in the field for 5 min in 2 consecutive days. The first day and the second day were referred to as acquisition session and recall session, respectively. In each session, when mouse was put in the chamber, the locomotor activity was counted immediately in an automatic manner for 5 min using Activity Meter (MK-ANIMEX, Muromachi Kikai Co., Japan). During the 5-min monitoring period, the extent of defecation was measured by noting the number of fecal boluses deposited by the mouse. In the open-field test, the drug-induced behavioral inhibition was indicated by the reduction in locomotor activity, when compared with the untreated control, during the acquisition session. The drug-induced impairment of open-field memory was indicated by the increase in locomotor activity during the recall session relative to that in the acquisition session and vehicle-treated control.

2.5. Assessment of hepatotoxicity

Mice were subcutaneously administered with increasing doses (5−80 μmol/kg) of tacrine and orbital blood samples were obtained at 6 h post-dosing. A previous study indicated that a maximum elevation of plasma alanine aminotransferases (ALT) activity was observed at 6 h after tacrine administration (Pan et al., 2002). Serum samples were obtained by centrifuging the whole blood at 2000×*g* at 4 °C for 8 min. Serum ALT activity was measured using an assay kit from Zhongsheng Beikong Bio-technology and Science Inc. (Beijing, China).

2.6. Statistical analysis

Values given are means±S.E.M. Data were analyzed either by one-way analysis of variance (ANOVA) followed by Dunnett's multiple-range test or by Student's *t* test for paired comparisons, using SPSS12.0 software. Significant inter-group difference was detected when $P<0.05$.

3. Results

3.1. Passive-avoidance response

As shown in Fig. 1, tacrine treatment (1.25−20 μmol/kg) caused a dose-dependent decrease (33−67%) in the number of footshock in 17-day-old mice, with the IC_{50} value estimated to be 7.8 μmol/kg. In 30-day-old mice, tacrine treatment (5−40 μmol/kg) reduced the number of footshock (by 12−59%) in a dose-dependent manner, with the IC_{50} value estimated to be 23.3 μmol/kg.

During the retention session, tacrine treatment (1.25−5.0 μmol/kg) resulted in increases in retention latency, with all 17-day-old mice pretreated with tacrine at 5.0 μmol/kg not crossing to the dark room in 3 min and the retention latency being prolonged by 53% when compared with the vehicle-treated control. However, further increases in the dose of tacrine (up to 20 μmol/kg) shortened the retention latency by 65%. No significant changes in retention latency were observed up to a dose of 20 μmol/kg in 30-day-old mice, but the retention latency was significantly shortened by 58% at a dose of 40 μmol/kg, when compared with the vehicle-treated control (Fig. 2). There was no difference between the vehicle and tacrine-treated groups in the average latency for entering the dark compartment during the training session (data not shown).

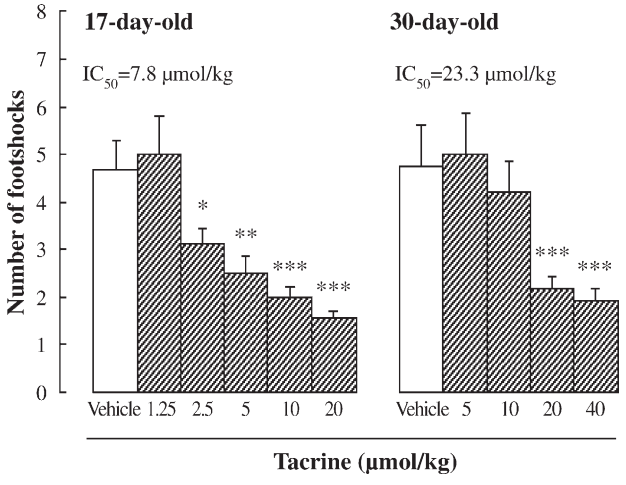

Fig. 1. Effects of tacrine treatment on the number of footshocks in passive-avoidance response in 17- and 30-day-old mice. Mice were subcutaneously administered with tacrine at increasing doses (1.25−40 μmol/kg) or vehicle 30 min prior to the training session. The number of footshocks within 5 min in each mouse during the training session was recorded. Each bar represents the mean±S.E.M., with $n=11$. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs. the respective vehicle-treated group, using one-way ANOVA followed by Dunnett's test.

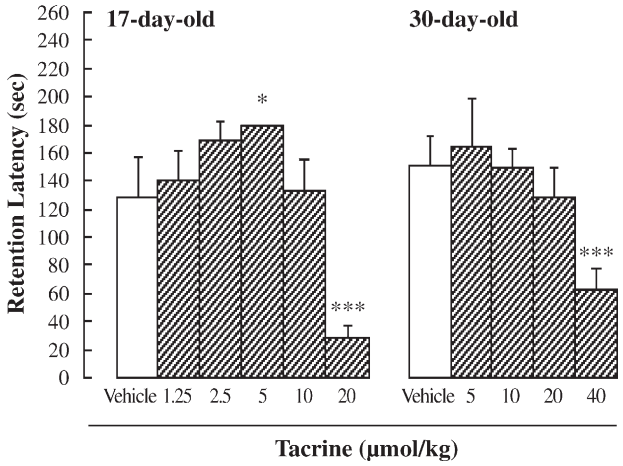

Fig. 2. Effects of tacrine treatment on the retention latency in passive-avoidance response in 17- and 30-day-old mice. Mice were subcutaneously administered with tacrine at increasing doses (1.25−40 μmol/kg) or vehicle 30 min prior to the training session. The retention session was conducted 24 h after the training session and the retention latency was recorded within 3 min. Each bar represents the mean±S.E.M., with $n=11$. *$P<0.05$, ***$P<0.001$ vs. the respective vehicle-treated group, using one-way ANOVA followed by Dunnett's test.

3.2. Locomotor activity

Tacrine treatment (2.5−40 μmol/kg) dose-dependently decreased the locomotor activity (by 7−90%) during the acquisition session in 17-day-old mice, with the IC_{50} value estimated to be 15.1 μmol/kg. In tacrine-treated 30-day-old mice (5−80 μmol/kg), a dose-dependent decrease (6−81%) in locomotor activity was also observed, with the IC_{50} value estimated to be 24.7 μmol/kg (Fig. 3a). The locomotor activity counts recorded in the recall session were reduced by 49% ($P<0.001$) and 43% ($P<0.001$), respectively, in vehicle-treated (control) 17- and 30-day-old mice, when compared with the respective value in the acquisition session (Fig. 3b). While there were no detectable changes in locomotor activity in both 17- and 30-day-old mice treated with low doses of tacrine in the recall session, the locomotor activity was significantly increased by 60% ($P<0.01$) and 64% ($P<0.001$), respectively, in 17- and 30-day-old mice at a dose of 40 and 80 μmol/kg, when compared with the vehicle-treated control (Fig. 3b).

3.3. Defecation

The number of boluses deposited by mice in 5 min, a measure of the extent of defecation, was recorded during the acquisition

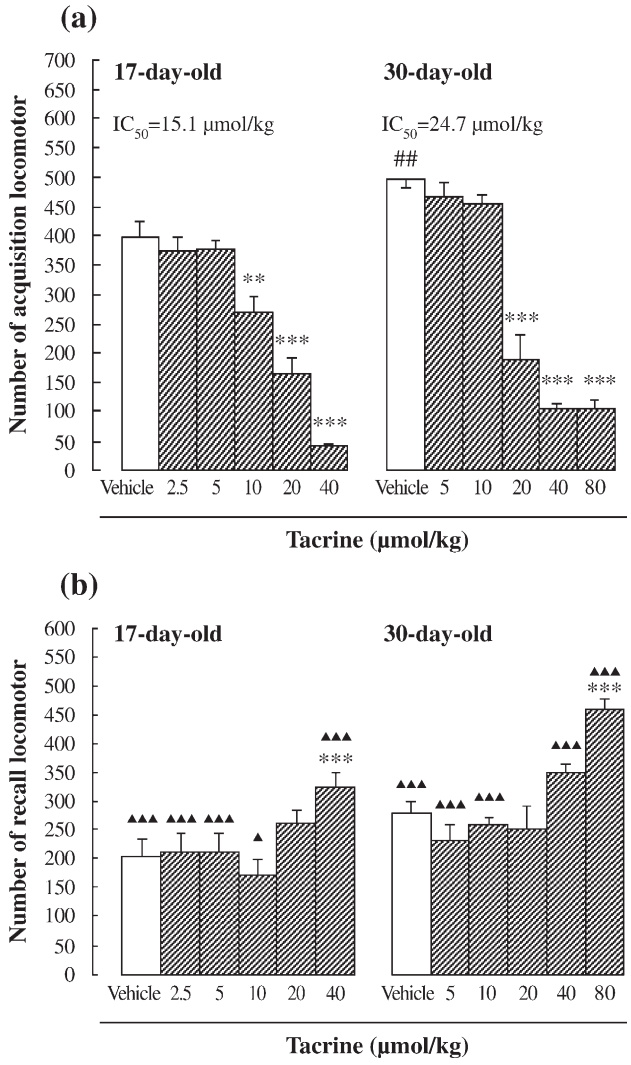

Fig. 3. Effects of tacrine treatment on locomotor activity *in open-field test* in 17- and 30-day-old mice. Mice were subcutaneously administered with tacrine at increasing doses (2.5−80 μmol/kg) or vehicle 30 min prior to the acquisition session. The locomotor activity in each mouse was monitored for 5 min in (a) acquisition session and (b) recall session, with the latter being conducted 24 h after the former. The locomotor activity was expressed as the number of photo-beam detected during the monitoring period. Each bar represents the mean±S.E.M., with $n=11$. $^{**}P<0.01$, $^{***}P<0.001$ vs. vehicle-treated control, using one-way ANOVA followed by Dunnett's test. $^{\#\#}P<0.01$ vs. vehicle-treated 17-day-old mice; $^{\blacktriangle}P<0.05$, $^{\blacktriangle\blacktriangle}P<0.01$, $^{\blacktriangle\blacktriangle\blacktriangle}P<0.001$ vs. the corresponding group in the acquisition session, using Student's *t* test.

and recall sessions in the open-field test (Table 1). During the acquisition session, tacrine treatment at a dose of 40 μmol/kg decreased the extent of defecation by 80% ($P<0.001$) and 61% ($P<0.01$), respectively, in 17- and 30-day-old mice. During the recall session, the extent of defecation was increased by 57% ($P<0.01$) and 112% ($P<0.01$), respectively, in 17- and 30-day-old control (vehicle-treated) mice, when compared with the corresponding value in acquisition session. Tacrine-treated animals also showed increases in the extent of defecation relative to the corresponding values in the acquisition session, but there were no detectable differences between the control and tacrine-treated groups during the recall session (Table 1).

3.4. Acute hepatotoxicity and toxicity

While tacrine treatment at doses (up to 20 μmol/kg) did not produce any detectable changes in serum ALT activity in 17-day-old mice at 6 h post-dosing, a 130% increase in serum ALT activity, an indirect biochemical index of hepatic injury, was observed after the treatment at a dose of 40 μmol/kg, when compared with the vehicle-treated control. When administered at a dose of 40 μmol/kg, serum ALT activity was also increased (by 66%) in 30-day-old mice. Increasing the dose of tacrine (up to 80 μmol/kg) caused a further elevation of serum ALT activity (175%) in 30-day-old mice. When comparing the serum ALT activity at the same dose of 40 μmol/kg, the tacrine-induced hepatic injury was more severe in 17-day-old mice than that of the 30-day-old mice ($P<0.05$) (Fig. 4). LD_{50} values of tacrine were estimated to be 91.90±8.45 μmol/kg (or 23.24±2.12 mg/kg) and 111.98±8.90 μmol/kg (or 28.30±2.25 mg/kg), respectively, in 17- and 30-day-old mice (Table 2).

4. Discussion

A growing body of experimental and clinical evidence has accumulated suggesting that the cholinergic system in the brain plays a significant role in cognitive function, particularly in memory formation (Rispoli et al., 2004; Baskin et al., 1999; Mesulam, 2004). Cholinergic drugs such as AChEIs and nicotinic receptor agonists, which reverse the cholinergic dysfunction in experimental animals and patients suffering from Alzheimer's disease, can be used as cognition-enhancing

Table 1
Effect of tacrine treatment on the extent of defecation in open-field test in mice

Treatment	Dose (μmol/kg)	Number of boluses	
		17-day-old	30-day-old
Acquisition session			
Control	0	3.18±0.20	2.36±0.16
Tacrine	2.5	3.63±0.23	–
	5	3.36±0.20	2.73±0.26
	10	3.18±0.40	1.91±0.20
	20	2.55±0.34	1.91±0.23
	40	0.64±0.21***	0.91±0.15**
	80	–	1.00+0.11**
Recall session			
Control	0	5.00±0.65[b]	5.00±0.59[b]
Tacrine	2.5	4.72±0.47	–
	5	4.36±0.56	4.82±0.79[a]
	10	4.46±0.61	5.91±0.40[c]
	20	4.92±0.51[c]	5.35±0.64[c]
	40	3.83±0.52[c]	4.20±0.60[c]
	80	–	6.93±1.00[c]

Mice were treated with tacrine at doses of 2.5−80 μmol/kg as described in Fig. 3. Control animals received the vehicle only. The open-field test was performed as described in Materials and methods. Values are given as means±S.E.M., with $n=11$.

$^{**}P<0.01$, $^{***}P<0.001$ vs. vehicle-treated control, using one-way ANOVA followed by Dunnett's test.

$^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ vs. the corresponding group in the acquisition session, using Student's *t* test.

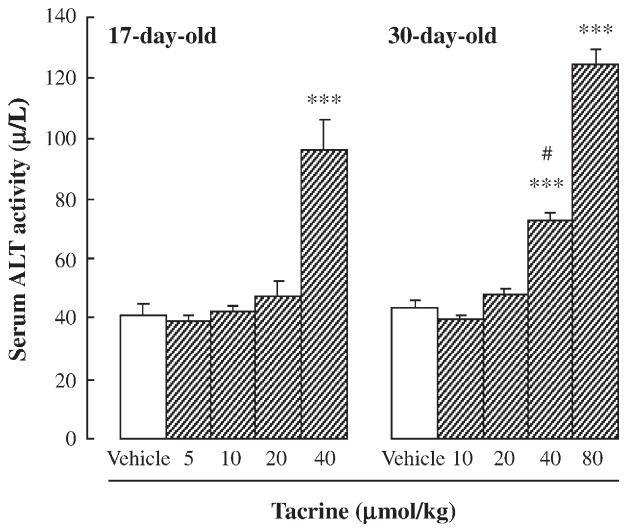

Fig. 4. Tacrine-induced hepatic injury in 17- and 30-day-old mice. Mice were subcutaneously administered with tacrine at increasing doses (5−80 μmol/kg). Control animals received the vehicle only. About 6 h after the drug or vehicle administration, serum alanine aminotransferases (ALT) activity was measured. Each bar represents the mean±S.E.M., with $n=8$. ***$P<0.001$ vs. vehicle-treated control, using one-way ANOVA followed by Dunnett's test; $^{\#}P<0.05$ vs. 17-day-old mice treated with the same dose of tacrine, using Student's t test.

agents. However, the cognitive effect of AChEIs is often associated with an inverted U-shaped dose–response curve, and the impairment of memory storage occurs when AChEIs are administered at doses that are higher than the optimal dose for cognition enhancement (Riekkinen et al., 1991; Braida et al., 1996; Ou et al., 2001). Results obtained from the present study indicated that while the memory of passive-avoidance response was maximally enhanced by tacrine treatment at a dose of 5 μmol/kg in 17-day-old mice, it was completely blocked at a dose of 20 μmol/kg. On the other hand, tacrine treatment at all tested doses did not enhance the memory of passive-avoidance response in 30-day-old mice. Conceivably, the inability of tacrine to enhance the memory in 30-day-old mice is likely related to the developmental changes in cholinergic-mediated cognitive function, in which a maximum stage of development might have reached in 30 days of age, thus rendering the failure of tacrine in augmenting the memory process in mice. Consistent with this postulation, the expression of nicotinic receptor mRNAs in the rat brain was found to be much higher during the perinatal development than in adult stage (O'Hara et al., 1999). Moreover, the development of cholinergic basal forebrain innervation, which is crucial for cognitive function, is finished during the second postnatal week of development in rodents (Berger-Sweeney, 1998).

The acute toxicity produced by tacrine treatment was slightly higher in 17-day-old than in 30-day-old mice. However, the 17-day-old mice seemed to be more sensitive than the 30-day-old counterparts to the hepatic injury induced by tacrine treatment. On the other hand, the dose of tacrine required for producing comparable effects on cognitive function and locomotor activity in 30-day-old mice were almost doubled that of the 17-day-old mice. These findings suggest that the developing brain, as is the case for 17-day-old mice, is more sensitive to cholinomimetic drug-induced changes in cognitive and locomotor functions. The memory impairment caused by a high dose of tacrine could be attributed to the over-stimulation of *presynaptic cholinergic autoreceptors* caused by a high concentration of acetylcholine (ACh) resulting from the inhibition of AChE by tacrine in the synaptic cleft. Over-stimulation of presynaptic cholinergic autoreceptors, a lesser amount of ACh is released from the presynaptic sites, which may in turn affect the formation of a longer-term memory (Miranda et al., 2003). Moreover, tacrine, which is an antagonist of M_1 and M_2 receptors, can also block nicotinic receptor in the brain, with the resultant impairment in cognitive function (Pearce and Potter, 1988; Clarke et al., 1994; Newhouse et al., 1992). Consistent with this postulation, tacrine treatment at high doses, as observed in the present study, caused the impairment in passive-avoidance response and open-field memory in both 17- and 30-day-old mice.

The enhancement of learning ability afforded by tacrine treatment at high doses (>5 μmol/kg), as assessed by the step-through task, was accompanied by a reduced locomotor activity. In clinical applications, it is undesirable for cognitive function-enhancing agents such as AChEIs to produce cholinergic toxicity, with the resultant suppression of locomotor activity. Nevertheless, tacrine treatment at low doses (0.25 or 5 μmol/kg), while not causing the suppression of locomotor activity, could improve the cognitive function in 17- but not 30-day-old mice. The effect of tacrine in enhancing cognitive function at low doses may be related to the action unrelated to the inhibition of cholinesterase (Freeman and Dawson, 1991). In this regard, it has been found that tacrine, when administered at a dose that does not elevate the ACh level in the brain, could improve cognitive function in rats showing persistent deficits in cholinergic neurons in the forebrain (Hodges et al., 1990). In the present study, the effective doses of tacrine for enhancing learning/memory were found to be ranging from 2.5 to 20 μmol/kg in mice. However, the effective doses of tacrine for inhibiting AChE ranged from 90 to 120 μmol/kg in rats (Wang et al., 1999).

In the context of cognitive psychology, memory is categorized into three types: sensory memory, short-term memory, and long-term memory. As far as the cognitive concept of learning and memory is concerned, the number of footshocks received during the training session in the step-through task is regarded as a parameter of learning ability,

Table 2
Acute toxicity of tacrine treatment in 17- and 30-day-old mice

	Dose (μmol/kg)	Mortality rate (%)	
		17-day-old	30-day-old
Tacrine	64	0	–
	80	20	0
	100	70	20
	125	–	80

Mice were divided into groups of 10 animals in each and subcutaneously administered with tacrine at doses of 64−125 μmol/kg. The mortality rate in each group was determined within 24 h post-dosing. LD_{50} values (17-day-old – 91.94±8.45 μmol/kg; 30-day-old – 111.98±8.90 μmol/kg) were estimated by Probit method.

whereas the retention latency serves as a parameter of memory function. If a drug could reduce the number of footshocks and/or increase the retention latency, but did not induce motor dysfunction, the drug may be used as a specific learning- and/or memory-enhancing agent. Given that the suppression of locomotor activity is a consequence of inhibition of AChE, the dose–response of AChEI-induced inhibition of locomotor activity can be assessed *in vivo* using the open-field test, with IC_{50} values being estimated and compared among various AChEIs. When compared with data obtained from the acquisition session in the open-field test, the decrease in locomotor activity during the recall session in control (vehicle-treated) mice is a manifestation of open-field memory (Pan, 1995, 1992). In this connection, the microinjection of tacrine or nicotine into the core of the nucleus accumbens after the first exposure to the open field was found to enhance the open-field memory (Schildein et al., 2000, 2002). In the present study, mice receiving a high dose (40 or 80 μmol/kg) of tacrine showed an increase in locomotor activity during the recall session. This observation suggests that the open-field memory formation may be blocked by tacrine at high dose.

AChEIs can cause involuntary defecation, especially during acute intoxication. However, in the present study, the extent of defecation was inhibited by tacrine treatment at a high dose of 40 μmol/kg (approximately 50% lower than the LD_{50} value) during the acquisition session of the open-field test in mice. Moreover, tacrine treatment at high doses was found to delay gastric emptying and inhibit intestinal motility (data not shown). As AChEIs can also block the nicotinic acetylcholine receptors, the gastrointestinal effects of tacrine may therefore result from the blockade of nicotinic receptor located in the parasympathetic nervous ganglia lying in the intestinal wall (Paddle and Dowling, 1996; Zheng et al., 1997). While the mechanism underlying the increase in the extent of defecation during the recall session in vehicle- or tacrine-treated mice remains unknown, the suppressive effect of tacrine, as observed in the acquisition session, seemed to be greatly reduced in the recall session.

Using passive-avoidance response, open-field behavior and liver injury as assessment parameters, it was found that the relative sensitivity of cognitive/behavioral functionality or liver tissue to tacrine-induced changes in mice, with reference to the minimal effective doses, appears to be in a descending order as follows: enhancement of passive-avoidance learning > enhancement of passive-avoidance memory > inhibition of locomotor activity > impairment of passive avoidance memory > inhibition of defecation and induction of hepatotoxicity > impairment of open-field memory.

In summary, the results indicated that tacrine was more potent in enhancing the learning function as well as in inhibiting the locomotor activity in 17-day-old than in 30-day-old mice. While the low dose (5 μmol/kg) of tacrine treatment enhanced the 24-h retention of learning memory in 17-day-old mice only, the high dose (20–40 μmol/kg) disrupted the retention of learning and open-field memory in both 17- and 30-day-old mice. Finally, the 17-day-old mice seemed to be more sensitive to the hepatic injury induced by tacrine treatment.

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