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Tamoxifen and toremifene impair retrieval, but not acquisition, of spatial information processing in mice

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Abstract

The present study examines the effects of tamoxifen (TAM) or toremifene (TOR), two triphenylethylene antiestrogen agents, on spatial information in mice by using Morris water maze. In a 30-s free swim trial, the TAM- or TOR-treated mice (intraperitoneally, 30 min before test) spent shorter time than the blank control mice in target quadrant. Compared to saline control group, animals exposed to TAM (1–10 mg/kg ip, once a day for 5 days) or TOR (3–30 mg/kg ip, once a day for 5 days) did not show significant difference on the acquisition of place task in Morris water maze. These results suggest that TAM, at the doses of 1–10 mg/kg, and TOR, at the doses of 3–30 mg/kg, impair the retrieval, but not the acquisition, of spatial information task in Morris water maze. It seems, however, that TOR is more potent than TAM on impairing memory retrieval.

Keywords: Tamoxifen; Toremifene; Morris water maze; Spatial information; Memory retrieval; Memory acquisition

1. Introduction

For decades, estrogen has been thought of as a "sex hormone," as it plays a fundamental role in regulating behavioral and physiological events that are essential for successful procreation. In recent years, estrogen receptors have also been detected in the brain regions, including the rat cerebellum, cerebral cortex, hippocampus and the nuclei of basal forebrain. Estrogen bound on the estrogen receptor has been shown to regulate neurotransmitter production and release, enzyme activities, membrane potentials, dendritic arborization and synaptogenesis in the nonhypothalamic regions of the rodent brain (Shughrue et al., 2000). The estrogen receptor in the central nervous system and peripheral systems demonstrates a variety of activities.

Estrogen influences the development of memory function in human and rodents, and can modulate memory in adults (Mortel and Meyer, 1995; Kuller, 1996). It is reported that estrogen can empower brain cells involved in thinking process in many ways: It boosts the cell's chemical function, spurs their growth and even keeps them alive by shielding them from toxins (Wickelgren, 1997). Now there is increasing interest in estrogen replacement therapy for the treatment and prevention of mental illnesses of late life, including impaired cognition, mood disorders and Alzheimer's disease (Schneider and Finch, 1997; Simpkins et al., 1997; McCormick and Abrass, 1998).

Clinically, it is well demonstrated that overexpression of estrogen receptor is involved in the development of breast cancer. Some patients with breast cancer often receive antiestrogen drugs as adjuvant chemotherapy.

Tamoxifen (TAM) and toremifene (TOR) are triphenylbutylene derivatives. Several or various studies have shown that TAM and TOR work as antiestrogen drugs. They bind to estrogen receptors and show varieties of action, including the inhibition of protein kinase C (O'Brian et al., 1988; Grainger and Metcalfe, 1996), working as a calmodulin antagonist (Furr and Jordan, 1984; Lam, 1984; Allen et al., 1998), blocking various chloride channels (Zhang et al., 1994) and acting as a histamine antagonist (Kroeger and Brandes, 1985). All these actions may directly or indirectly affect memory functions.

Currently, TAM and TOR are not only frequently used as adjuvant chemotherapy in the treatment of breast cancer, but also being assessed as a prophylactic for those at high risk of

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developing tumors (van den Koedi jk et al., 1994; Mitlak and Cohen, 1999). However, the side effects induced by antiestrogen agents on different systems especially on memory function have been reported (Biegon et al., 1996). Patients treated with adjuvant chemotherapy for operative primary breast carcinoma had significant problems with concentration and memory (van Dam et al., 1998; Schagen et al., 1999; Brezden et al., 2000). Cognitive impairment following such chemotherapy was noticed in a broad domain of functioning, including attention, mental flexibility, the speed of information processing, visual memory and motor function (Schagen et al., 1999).

In spite of the clinical reports on the memory-impairing effects of adjuvant chemotherapy of breast cancer, a few studies have demonstrated the adverse actions of these drugs in experimental animals. In the previous studies, we have observed that TAM and TOR significantly impaired learning and memory abilities in passive avoidance tests in mice (Chen et al., 2002). In order to further evaluate the properties of the antiestrogen agents on memory function, the present study tested the effect of TAM and TOR on spatial memory function by using Morris water maze in mice.

2. Materials and methods

2.1. Animals

Female Swiss mice with body weight of 20–22 g were supplied by Experimental Animal Center of Shenyang Pharmaceutical University. The animals were housed in plastic cages in groups of five and maintained under standard conditions with a 12–12 h light–dark cycle (lights on 0600 h) and free access to food and water. The mice were used for the behavioral experiments after they had been adapted to laboratory conditions for at least 5 days.

2.2. Drugs

Tamoxifen citrate (Shanghai Hualian Pharmaceutical, Shanghai, China) was dissolved in sterile saline and TOR (purity >99%; offered by the Department of Organic Chemistry, Shenyang Pharmaceutical University, Shenyang, China) was suspended in 5% hydroxypropyl-β-cyclodextrin solution. TAM solution or TOR suspensions were intraperitoneally administered to mice in a volume of 0.2 ml/10 g body weight.

2.3. The ability of swimming test

This experiment was performed in an iron pool ($86 \times 17 \times 37$ cm) filled with water to a depth of 20 cm. Water temperature was maintained at 25 ± 1 °C. At the end of the pool, there is a platform on which there is food and where mice could climb. The location of the platform was made visible by a blue-colored picture mounted above the platform. The tests started 30 min after intraperitoneal administration of TAM (1, 3 or 10 mg/kg), TOR (3, 10 or 30 mg/kg) or saline. During the test, the mouse was put into the water at the starting point. The swimming time from the starting point to the end of the pool was recorded (Li et al., 2001).

2.4. Morris water maze test

2.4.1. Apparatus

The water maze used was a circular swimming pool measuring 100 cm in diameter and 40 cm in height, filled with water to a depth of 20 cm. The water was kept at 25 ± 1 °C and colored black with a nontoxic dye to make the platform invisible. Four equally spaced points around the edge of the pool were designed as: east (E), south (S), west (W) and north (N). An escaping platform (diameter is 7 cm) was set 1 cm below the surface of the water and placed in a constant position in the middle of the SW quadrant. The mouse in the pool was trained to find the platform using a variety of extra maze cues, including the desk, wall, window, experimenter, etc. The experimenter always sat at the same position.

2.4.2. Place training procedure

The mice were required to perform a water escape task that was modified from the standard version of the Morris water maze (Morris, 1984; Li et al., 2001). A trial started by placing a mouse by hand into the water facing the wall of the circular pool, at the midpoint of the sign of S and E, S and W, N and E, or N and W around the edge of the pool. Mice were allowed to swim to the hidden platform and the escape latency (time to find the hidden platform) was recorded. If the platform was not found within 60 s, the mouse was manually placed onto the platform and permitted to remain there for 20 s and gave a maximum score of 60 s, then the next trial began. This procedure was repeated with each mouse from starting position in all four quadrants for four times.

2.4.3. Acquisition of memory

The trials started 30 min after intraperitoneal administration of TAM (1, 3 or 10 mg/kg), TOR (3, 10 or 30 mg/kg) or saline to different groups of mice everyday. Each mouse was trained four times daily at intervals of 20 s for five consecutive days. The escaping latencies of four times daily of each mouse were recorded by the observer who stayed at the same place during the experiment. Daily swimming ceased after 5 days (Petrie et al., 1991). The mean escaping latency of four times daily was calculated.

2.4.4. Retrieval of memory

In order to evaluate the effect of TAM or TOR on the retrieval of spatial information, another eight groups of mice were trained to locate a hidden platform according to

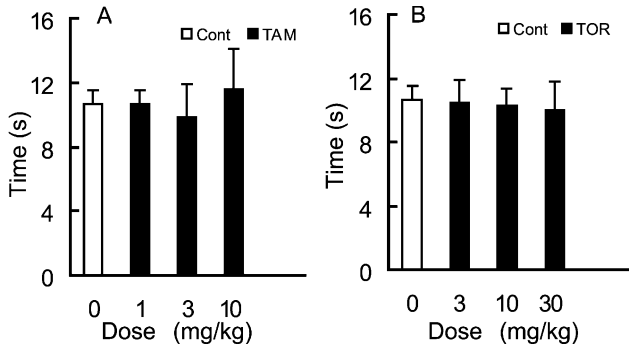

Fig. 1. Effect of TAM (A) and TOR (B) on swimming ability in mice. TAM or TOR was administered 30 min before the test. Each column represents the mean ± S.E.M. of 10 animals. Cont = control, TAM = tamoxifen, TOR = toremifene.

the place training procedure outlined above. Animals received 20 trials (eight times daily each mice for 2 1/2 days) of training under saline intraperitoneally, 30 min before training everyday, and were able to escape in less than 10 s (mean value) during the last four trials. One day after the training was completed, animals were subjected to a 30-s free swim trial under the influence of saline, TAM (1, 3 or 10 mg/kg), vehicle (5% hydroxypropyl-β-cyclodextrin solution) or TOR (3, 10 or 30 mg/kg), respectively. The limits of the four quadrants were marked with a thread at the test. Mice were put into the water at the left adjacent quadrant. The quadrant time (i.e., the time spent by the mouse in each of the four quadrants in 30 s) was recorded and calculated. In this design, the effect of TAM or TOR on retrieval of spatial information on well-trained mice was evaluated (Brioni and Arolfo, 1991).

2.4.5. Statistics

Results of each group were calculated and were expressed as mean ± S.E.M. Data were statistically analyzed via General Linear Models (GLM) followed by Least Significant Difference (LSD) method using SAS statistical package. Statistical significance was set at $P < .05$.

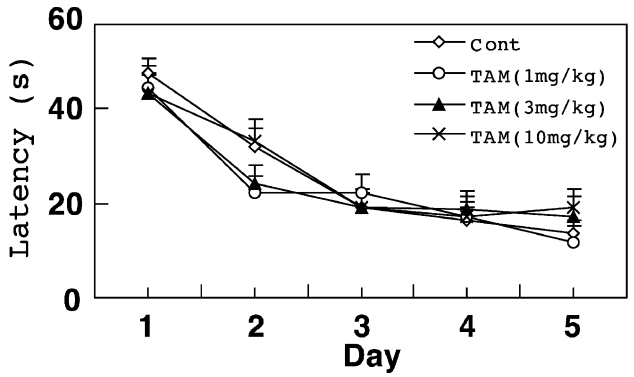

Fig. 2. Effect of TAM on acquisition of spatial memory in Morris water maze in mice. TAM or saline was administered 30 min before training for 5 days. Each point represents the mean ± S.E.M. of 10–11 animals. Cont = control, TAM = tamoxifen.

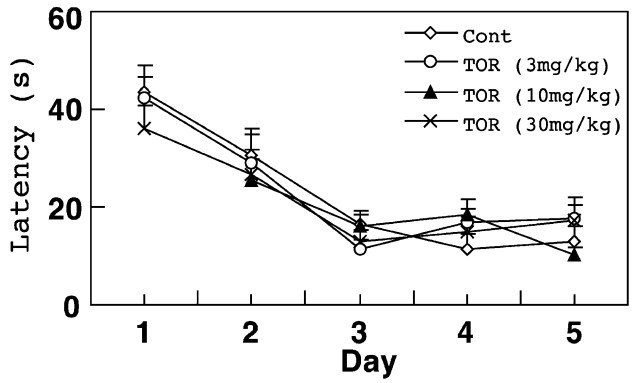

Fig. 3. Effect of TOR on acquisition of spatial memory in Morris water maze in mice. TOR or saline was administered 30 min before training for 5 days. Each point represents the mean ± S.E.M. of 10–11 animals. Cont = control, TOR = toremifene.

3. Results

3.1. Swimming ability test

In order to test whether TAM or TOR influences the swimming ability, different doses of the drugs were intraperitoneally administered, respectively. The results showed that at the doses used, none of the drugs inhibits the swimming ability of the mice (Fig. 1). Therefore, these doses were used in the following experiments.

3.2. Effect of TAM or TOR on memory acquisition

Neither TAM at the doses of 1, 3 and 10 mg/kg nor TOR at the doses of 3, 10 and 30 mg/kg, which was administered consecutively for 5 days, showed any significant effect on the time of reaching the platform (Figs. 2 and 3).

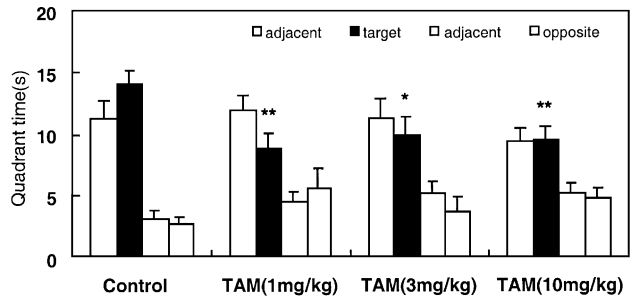

Fig. 4. Effect of TAM on retrieval of spatial memory in Morris water maze in mice. TAM or saline was administered 30 min before test. Each column represents the mean ± S.E.M. of 10 animals. Cont = control, TAM = tamoxifen. * $P < .05$, ** $P < .01$ compared with the time spent by the control animals in the target quadrant.

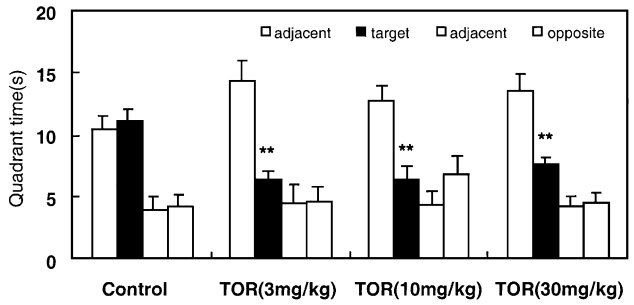

Fig. 5. Effect of TOR on retrieval of spatial memory in Morris water maze in mice. TOR or saline was administered 30 min before test. Each column represents the mean ± S.E.M. of 10 animals. Cont = control, TOR = toremifene. * $P < .05$, ** $P < .01$ compared with the time spent by the control animals in the target quadrant.

3.3. Effect of TAM or TOR on memory retrieval

Well-trained mice consistently escaped from water onto the hidden platform in less than 10 s. The quadrant time spent in target quadrant by TAM-treated mice was significantly shorter than the time spent by saline control animals (Fig. 4). TOR, at the doses of 3, 10 and 30 mg/kg, showed a significant effect on decreasing the time in the target quadrant, since the time in the target quadrant was almost the same as the time in the opposite and another adjacent quadrant (Fig. 5). In theory, the mice put into the water should stay in the two adjacent quadrants in a similar time. However, in the present study, the mice stayed longer time in one of the two adjacent quadrants. This may be simply due to the special protocol of the experiment, in which the mice were always put into the water in the same adjacent quadrant.

4. Discussion

In the present study, the mice treated with TAM at the doses of 1–10 mg/kg or TOR at the doses of 3–30 mg/kg failed to attain the same level of competence as the control mice in memory retrieval test in Morris water maze. This lower level of competence was unlikely due to physical defects, but was likely due to the result of memory recall impairment after drug treatment because the mice showed no evidence of any slowness or acceleration in swimming ability in the water or any difficulty in mounting onto the target platform in the water escape task.

In the previous studies, it was observed that TAM and TOR significantly shortened the escaping latency and increased the number of errors, respectively, in the consolidation and retrieval processes of memory in the step-down and step-through passive avoidance tests (Chen et al., 2002). TOR also affected acquisition of memory in the passive avoidance test (Chen et al., 2002). In the present study, the results showed that TAM and TOR failed to affect acquisition of the spatial learning task, but significantly affected memory retrieval in Morris water maze. These studies further confirmed that TAM and TOR impaired not only passive avoidance but also spatial memory. Furthermore, it seems that the memory retrieval function is more susceptible than memory acquisition function to these triphenylbutylene derivatives.

Morris water maze is designed to test spatial memory that is mainly related to the function of hippocampus—a brain structure known to be essential for spatial learning and memory (Jett et al., 1996). Deficits in spatial learning and memory with lesion of hippocampus have been reported (Morris et al., 1982; Jett et al., 1996; Wong et al., 1997). The hippocampus is one of the important targets for estrogen to affect the memory function. Estrogen replacement induces a 30% rise in both NMDA receptors and spines in the hippocampus of ovariectomized female rats (Luine et al., 1980; Wickelgren, 1997). Thus, one may speculate that TAM and TOR may affect the function of hippocampus to impair the memory ability.

When the potencies of TAM or TOR on memory retrieval were compared, it was observed that animals treated with TOR stayed for shorter time in the target quadrant than animals treated with TAM. It is unlikely that this qualitative difference in the effect on memory is due to the lower dose of TAM, since it is reported that TAM shows its antiestrogenic effect three times higher than TOR (Haynes and Dowsett, 1999). Thus, these results suggest that TOR might be more harmful on memory retrieval than TAM.

Close interactions between estrogen and cholinergic function in the central nervous system have been reported (Toran-Allerand et al., 1992; Singh et al., 1995). It has been known that estrogen affects memory behaviors mainly by modulating basal cholinergic function (Singh et al., 1995). An increase in the levels of choline acetyltransferase was observed in certain neurons of the basal forebrain when estrogen was given to the ovariectomized female rats (Singh et al., 1995). Ovarian steroid deprivation (via ovariectomy) alters choline acetyltransferase activity and cholinergic receptor density in rats (Flicher et al., 1983). Dysfunction of cholinergic neurons and lesions of certain basal forebrain nuclei lead to disruptions of learning and memory function (Simpkins et al., 1997). Estrogen replacement therapy reverses the decrease induced by ovariectomy in high-affinity choline uptake and choline acetyltransferase activity in the hippocampus and frontal cortex and improves the impairment of learning and memory behaviors (Luine, 1985; Luine et al., 1975; O'Malley et al., 1987). Since TAM and TOR are antiestrogenic drugs, it is reasonable to assume that the memory impairment induced by TAM and TOR might be due to the blockade of the estrogen actions, which subsequently affects the activity of the cholinergic system. What is still unclear is that TAM and TOR did not show a clear dose-dependent effect on memory retrieval. The similar result was also observed in the previous study by using other experimental models (Chen et al., 2002).

Taken together, our observations give further evidence for the finding that TAM and TOR impair the memory

processing and suggest that the retrieval memory is more sensitive than the acquisition memory to TAM- and TOR-induced memory impairment in spatial information processing. Although TAM and TOR were used at a relatively lower dose range in clinic setting than they are in the present study, these results imply that a caution should be taken when these drugs are used as a long-term therapy, especially for those having a tendency for dementia.

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