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Tamoxifen and toremifene cause impairment of learning and memory function in mice

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Abstract

Tamoxifen (TAM) and toremifene (TOR) are two antiestrogen agents frequently used in the treatment of breast cancer. They are currently being assessed as the prophylactic for patients at high risk of developing tumors. However, the side effects of these drugs on memory function have drawn attention in clinical usage. In the present study, it is demonstrated in mice that TAM and TOR significantly shortened the escaping latency or increased the number of errors, respectively, by using the step-down and step-through passive avoidance tests. By using an appetitively motivated task in T-maze, it is demonstrated that TAM and TOR significantly delayed the latency of finding food in well-trained mice. TAM appeared to impair memory consolidation and retrieval processes, rather than acquisition of memory, whereas TOR appeared to impair acquisition, consolidation, and retrieval processes. These results provide experimental support for the clinical findings that have shown that these drugs impaired memory function in patients routinely taking the drugs and suggest that caution should be taken for using these drugs as the prophylactics for those at risk of developing tumors.

Keywords: Tamoxifen; Toremifene; Learning; Memory; Acquisition; Consolidation; Retrieval; Step-down; Step-through; T-maze

1. Introduction

The nonsteroidal antiestrogen drugs tamoxifen (TAM) and toremifene (TOR) have been proved to be valuable adjuncts in the treatment of breast cancer. Both drugs have similar chemical structure (Haynes and Dowsett, 1999), efficacy, and adverse effects (Cohen et al., 1997; Pyrhonen et al., 1997; Mitlak and Cohen, 1999). Currently, TAM and TOR are used as the choice of the endocrine treatment for advanced breast cancer as well as for adjuvant therapy for surgery, radiation, and chemotherapy in earlier disease stage (van den Koedijk et al., 1994; Biegon et al., 1996). In the adjuvant therapy of breast cancer, TAM is recommended for use for at least 5 years (Early Breast Cancer Trialist Collaborative Group, 1998). However, the drugs have shown some side effects during clinical application, such as increasing the risk of venous thromboembolism (Fisher et al., 1998), causing vasomotor symptoms (i.e., hot flashes) (Love et al., 1991), as well as neurological side effects such as depression, irritability, and memory problem (Zhang et al., 1994). It is reported that patients treated with adjuvant chemotherapy for operative primary breast carcinoma have significant problems with concentration and memory (van Dam et al., 1998; Brezden et al., 2000). Cognitive impairment following such chemotherapy was noticed in a broad domain of functioning, including attention, mental flexibility, speed of information processing, visual memory, and motor function (Schagen et al., 1999). The adverse effects on memory function have drawn attention of neuroscientists because of the possible induction of senile dementia in the patients with long-term administration of these drugs.

Alzheimer's disease (AD) is the most common cause of dementia. It is reported that AD affects 1.5–3 times more women than men. Recognition that AD may affect women more often than men and that cognitive deficits differ between women and men with AD highlights the need for research to unravel underlying genetic and environmental contributions to these associations (Henderson, 1997). After menopause, estrogen levels are very low, and one important question is the extent to which observed

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differences in AD might be mediated by sex hormones (Carr et al., 1997; Henderson, 1997). Clinical studies indicate that estrogen and other sex hormones affect cognitive skills. Most recent case-controlled and cohort studies have suggested that prior and current estrogen replacement therapy reduce the risk for AD or dementia. For example, it has been reported that five of six women who had mild AD and wore an estrogen patch for 2 months showed noteworthy improvements in verbal memory and attention (Brenner et al., 1994; Henderson et al., 1994; Mortel and Meyer, 1995).

Thus, despite of the importance of using antiestrogen drugs for preventing breast cancer, concurrent adverse actions such as memory impairment induced by these drugs may also threaten the quality of life of the patients. In order to provide more experimental information for reasonable use of these drugs, the characteristics of the memory impairing properties of TAM and TOR were investigated by using passive avoidance and appetitively motivated tasks in mice.

2. Method

2.1. Animals

Female and male Swiss mice with body weight 20–22 g were supplied by the Experimental Animal Center of Shenyang Pharmaceutical University. The animals were housed under standard conditions with food and water ad libitum. The mice were used for the behavioral experiments after they had adapted to laboratory conditions at least for 5 days.

2.2. Drugs

Tamoxifen citrate (Shanghai Hualian Pharmaceutical, Shanghai, China) was dissolved in sterile saline and TOR (Department of Organic Chemistry, Shenyang Pharmaceutical University, Shenyang, China) was suspended in 5% hydroxypropyl-β–cyclodextrin solution. Scopolamine hydrochloride (Sigma, St Louis, MO, USA) and $NaNO_2$ (Shenyang Xincheng Chemicals, Shenyang, China) were dissolved in sterile saline. TAM or TOR suspension was intraperitoneally administered in volume of 0.2 ml per 10 g body weight. Scopolamine was intraperitoneally administered 10 min before training and $NaNO_2$ was subcutaneously administered immediately after the training. Ethanol (30% in saline, w/v) was administered orally (10 ml/kg) 10 min before the test.

2.3. Locomotor activity

Thirty minutes after drug administration, each animal was individually placed in a black circular chamber 25 cm in diameter and 10 cm in height. Locomotor activity was measured using an optical animal activity monitoring system that employs a horizontal bank of photo beam sensors to monitor animal movement with time. Locomotor activity was monitored continuously for 30 min and the number of movement was recorded every 5 min via a computer connected to the monitor.

2.4. One-way step-down passive avoidance task

Mice were trained in an apparatus previously placed on a platform (4.3 cm) in a lighted box ($12 \times 10 \times 30$ cm) with a grid floor through which an electric shock of 36 V (55 Hz) was delivered. When the mice stepped down the platform, a constant and continuous electric shock was applied. The normal reaction of the mice was to jump back onto the platform. In the first day, the mice were placed in the box for 3 min and then trained for 5 min. After 24 h each mouse was placed once again on the platform and tested for step-down escaping latency and the number of errors (stepped down the platform) in 5 min. TAM or TOR was administered intraperitoneally 30 min before the training for testing acquisition of memory, or immediately after the training for testing memory consolidation, or 30 min before the test for memory retrieval (Chugh et al., 1991). Each group had 12–14 mice.

2.5. One-way step-through passive avoidance task

A one-trial step-through passive task was performed as described previously (Li et al., 1999). The apparatus consisted of two compartments, an illuminated box and a dark box separated by a guillotine door. The size of both boxes was $20 \times 10 \times 15$ cm. During the training, the mouse was placed in the illuminated compartment and allowed to enter the dark compartment through the door. Immediately after entry, a scrambled foot shock (36 V, 55 Hz) was delivered through the grid floor. The mouse could escape from the shock only by stepping back into the safe illuminated compartment. Twenty-four hours after the training, the mouse was again placed in the safe illuminated compartment. The response latency to enter the dark compartment and numbers of errors (enter the dark compartment) in 5 min were measured. The latency of not entering the dark room during the 5-min observation period was regarded as 300 s. TAM or TOR was administered intraperitoneally 30 min before the training for testing acquisition of memory, or immediately after the training for testing memory consolidation, or 30 min before the test for memory retrieval. Each group had 12–14 mice.

2.6. Appetitively motivated task in T-maze

The T-maze had a start arm and a left and right arm ($50 \times 13 \times 25$ cm) and all painted black. At the extremity of each arm, a food dish (5 cm in diameter, 0.5 cm in

depth) was located on the floor. The T-maze was located in a dimly illuminated room with a weak light (25 W). The animals were familiarized with the maze, food, and food containers for two consecutive days before the start of the experiments. On these days, two trials were carried out for each mouse. Then, the animals were deprived of food for 20 h. At the start of the experimental session, 15 trials per mouse were carried out on the first day. On the next day 10 consecutive trials were carried out for each mouse and TAM or TOR was administered intraperitoneally 30 min before the trial began in the second day. Only one arm of the T-maze was baited with food. The correct choice was the left arm for half of the mice, while it was the right arm for the rest. The mouse was put into the start arm. When the mouse reached one or the other arm, it was removed from the maze and put into a separate waiting box for 10 s and then returned to the maze as before. A correct trial ended with the mouse eating the food. An incorrect trial (error) ended with the mouse reaching the empty food dish. The number of errors and the average time taken for each correct trial (latency) were recorded (Sudha et al., 1995). Ten mice per group were used.

2.7. Statistics

Results of each group were calculated and were expressed as mean ± S.E.M. Data were statistically analyzed via general linear models followed by least significant difference method using SAS statistical package. Statistical significance was set at $P < .05$.

3. Results

3.1. Spontaneous locomotor activity

TAM, at the dose range of 1–10 mg/kg ip, and TOR, at the dose of 3–30 mg/kg ip, showed no significant effect on locomotor activities compared with that of the control group (Fig. 1).

3.2. One-way step-down passive avoidance task in female mice

Fig. 2 shows the effects of TAM and TOR on acquisition of memory. The number of errors in the TOR-treated group, at the doses of 3, 10, and 30 mg/kg ip, was significantly increased and the latencies were significantly shortened when compared with the control group. Neither the number of errors nor the latency in the TAM-treated group had significant difference compared with that of the control (Fig. 2A and B).

Fig. 3 shows the effects of TAM and TOR on memory consolidation. The number of errors in the TAM-treated groups (1 and 10 mg/kg ip), rather than in the TOR-treated groups, was significantly increased compared with that of the control group. The latency in the TAM-treated group (10 mg/kg ip) and in the TOR-treated group (30 mg/kg ip) was significantly shorter than that of the control group (Fig. 3A and B).

Fig. 4 shows the effects of TAM and TOR on memory retrieval. The number of errors in the TAM-treated group, at the dose of 10 mg/kg ip, was significantly increased and the latency was significantly shortened when compared with that of the control group. Neither the number of errors nor the latency in the TOR-treated group showed significant difference compared with that of the control (Fig. 4A and B).

3.3. One-way step-through passive avoidance task in female mice

In the trials on acquisition of memory, the number of errors in the TOR-treated group, at the dose of 30 mg/kg ip, was significantly increased and the latency was significantly shortened when compared with that of the control group. Neither the number of errors nor the latency in the TAM-treated group was significantly different compared with those of the control (Fig. 5A and B).

Fig. 6 shows the effect of TAM and TOR on memory consolidation in step-through test in mice. The number of errors in the TAM-treated group (10 mg/kg ip), rather than the TOR-treated group, was significantly increased com-

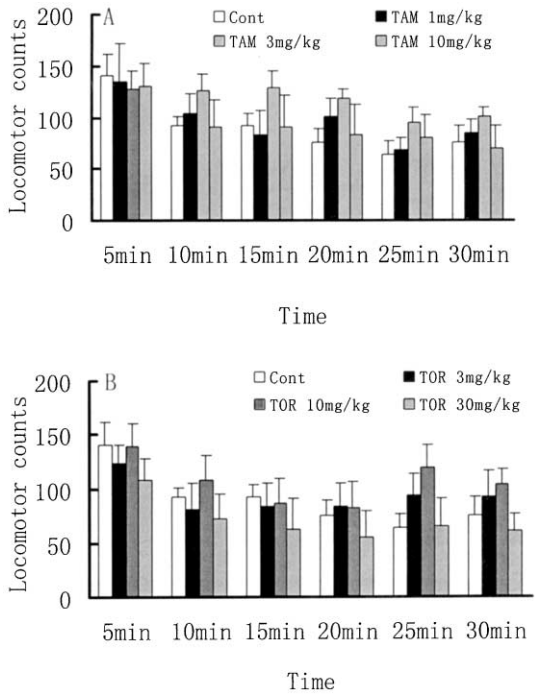

Fig. 1. Effects of TAM (A) and TOR (B) on locomotor activity in female mice. The activity was measured after 30 min of administration. Each column represents the mean ± S.E.M. of 10 animals (Cont = control).

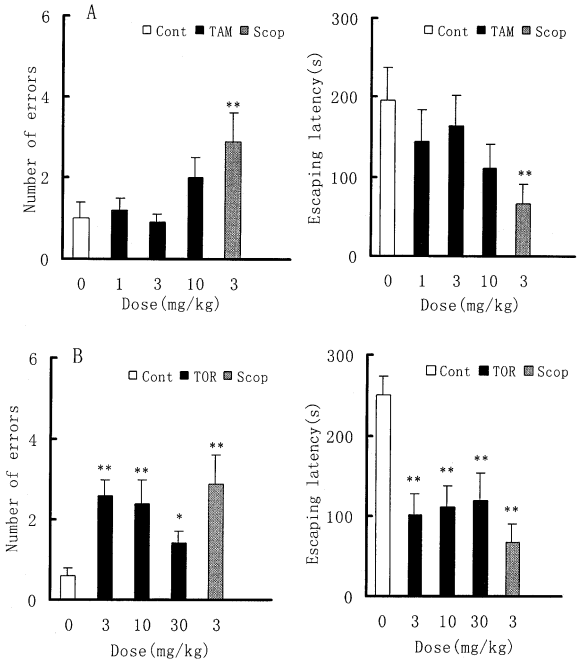

Fig. 2. Effects of TAM (A) and TOR (B) on acquisition of memory in step-down test in female mice. TAM or TOR was administered intraperitoneally 30 min before the training. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control and Scop = scopolamine). * $P < .05$, ** $P < .01$ vs. the control group.

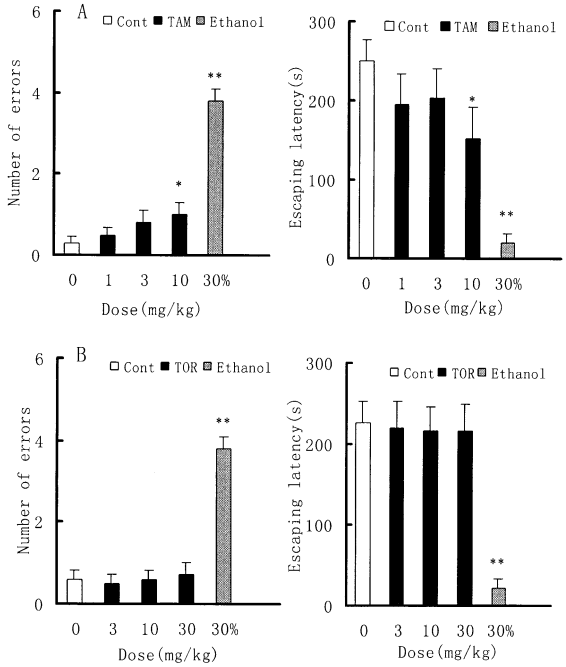

Fig. 4. Effects of TAM (A) and TOR (B) on memory retrieval in step-down test in female mice. TAM or TOR was administered 30 min before test. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

pared with that of the control group. Neither the TAM-treated group nor the TOR-treated group showed significant difference in latency compared with the control (Fig. 6A and B).

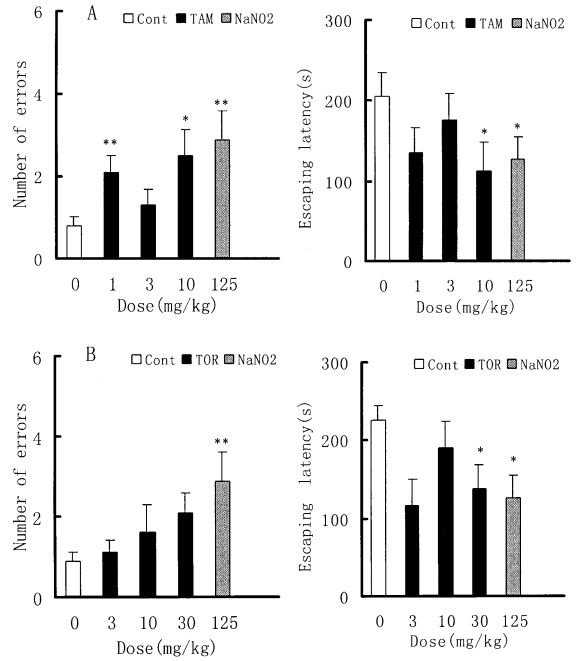

Fig. 3. Effects of TAM (A) and TOR (B) on memory consolidation in step-down test in female mice. TAM or TOR was administered immediately after the training for testing memory consolidation. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

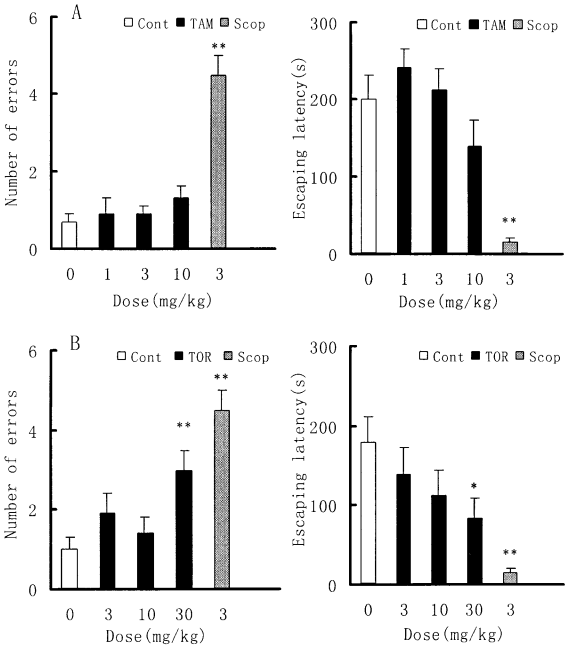

Fig. 5. Effects of TAM (A) and TOR (B) on acquisition of memory in step-through test in female mice. TAM or TOR was administered intraperitoneally 30 min before the training. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control and Scop = scopolamine). * $P < .05$, ** $P < .01$ vs. the control group.

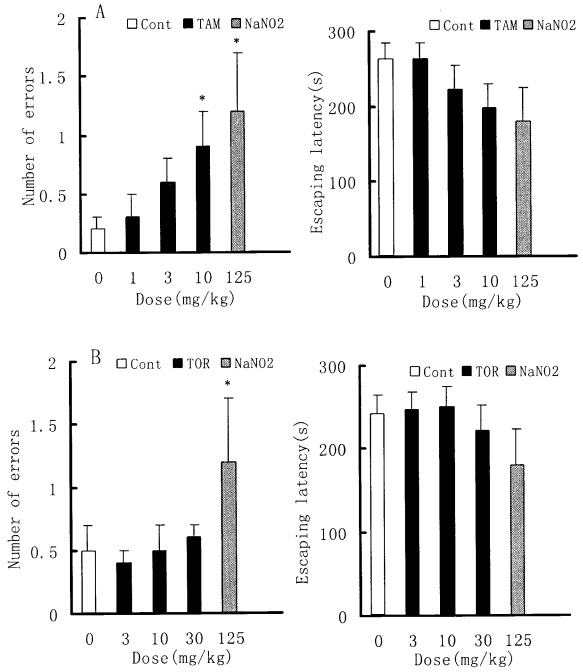

Fig. 6. Effects of TAM (A) and TOR (B) on memory consolidation in step-through test in female mice. TAM or TOR was administered immediately after the training for testing memory consolidation. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control). * $P < .05$ vs. the control group.

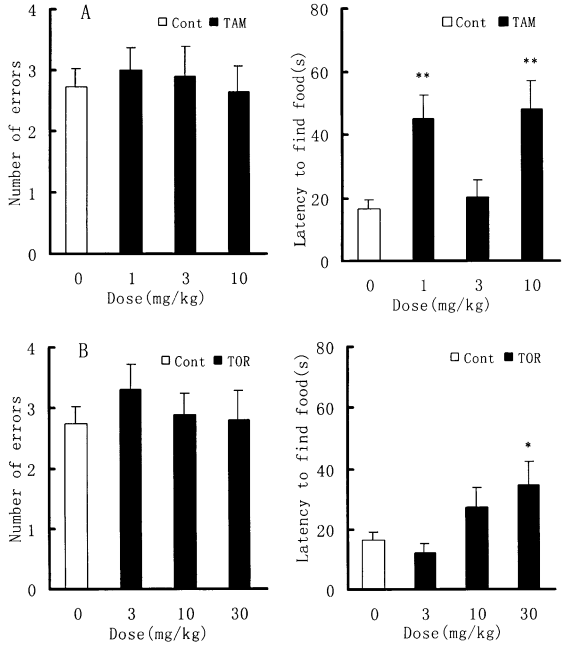

Fig. 8. Effects of TAM and TOR on memory retrieval on an appetitively motivated task in T-maze in female mice. TAM or TOR was administered 30 min before test. Each column represents the mean ± S.E.M. of 10 animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

Fig. 7 shows the effect of TAM and TOR on memory retrieval in step-through test in mice. The number of errors in the TOR-treated group (10 mg/kg ip), rather than TAM-treated group, was significantly increased compared with that of the control group. Neither the TAM-

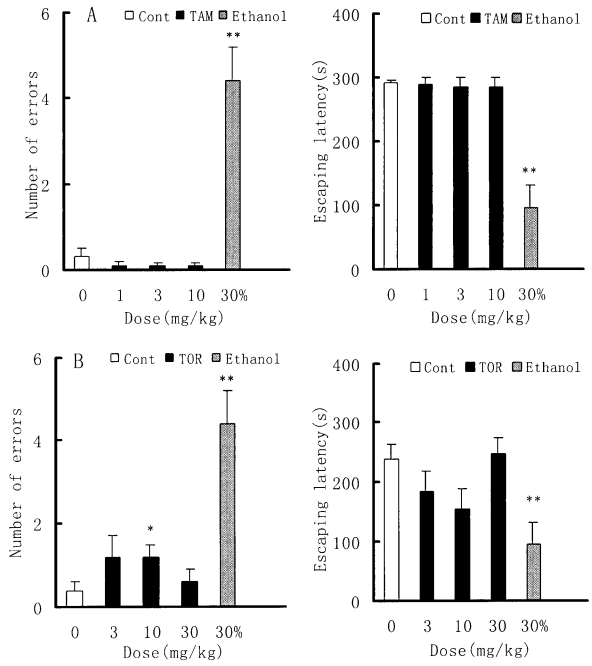

Fig. 7. Effects of TAM (A) and TOR (B) on memory retrieval in step-through test in female mice. TAM or TOR was administered 30 min before test. Each column represents the mean ± S.E.M. of 12–14 animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

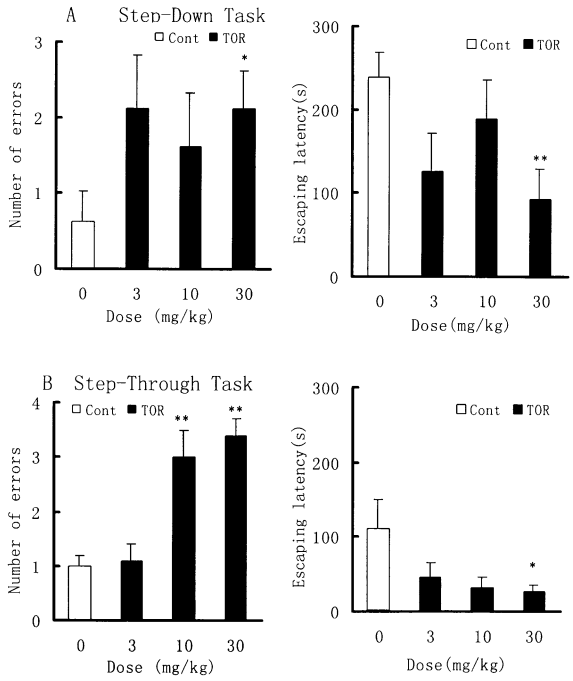

Fig. 9. Effect of TOR on memory acquisition in step-down (A) and step-through (B) in male mice. TOR was administered intraperitoneally 30 min before the training. Each column represents the mean ± S.E.M. of seven to eight animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

treated group nor the TOR-treated group showed significant difference in latency compared with the control (Fig. 7A and B).

3.4. Appetitively motivated task in female mice

The performance of female mice on an appetitively motivated task after TAM and TOR treatment was tested in T-maze. Neither the TAM-treated nor the TOR-treated animals showed significant difference in the number of errors compared with the saline control. However, the latency of finding food in the TAM-treated group (3 and 10 mg/kg ip) and in the TOR-treated group (30 mg/kg ip) was significantly longer than that of the saline control group (Fig. 8A and B).

3.5. Effects of TAM and TOR on the memory function in male mice

Several groups of male mice were used to test the possible actions of antiestrogens on memory functions in male animals by using step-down and step-through passive avoidance tasks. The results showed that TOR affected memory acquisition in both experimental models (Fig. 9). TOR impaired memory retrieval in step-through test (Fig. 10B) and TAM impaired memory consolidation in step-down test (Fig. 10A).

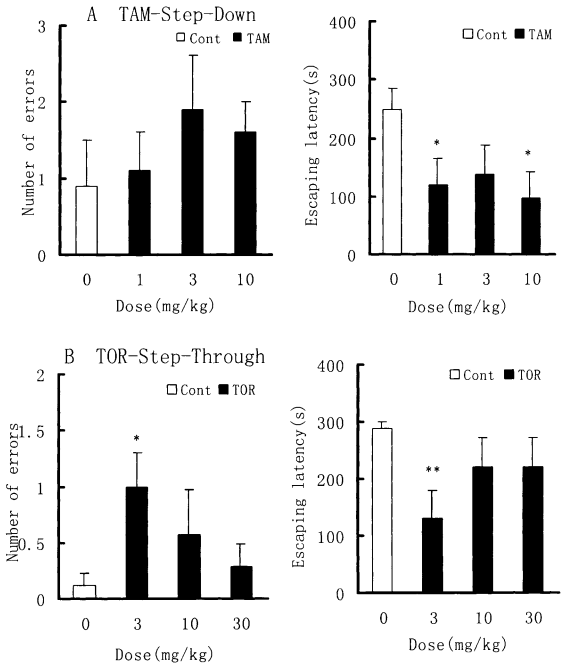

Fig. 10. Effect of TAM on memory consolidation in step-down (A) and effect of TOR on memory retrieval in step-through (B) in male mice. TAM was administered immediately after the training for testing memory consolidation and TOR was administered 30 min before test. Each column represents the mean ± S.E.M. of seven to eight animals (Cont = control). * $P < .05$, ** $P < .01$ vs. the control group.

4. Discussion

Learning and memory abilities include acquisition, consolidation, and retrieval processes. In the present study, the characteristics of TAM and TOR on memory functions were evaluated according to these three processes. The results demonstrated that TAM and TOR impaired all these processes of memory functions in experimental animals. However, the impairing potencies of these drugs are weaker compared with scopolamine, $NaNO_2$, and ethanol, which are commonly used as positive drugs for impairing memory acquisition, consolidation, and retrieval, respectively.

The memory impairing actions of TAM and TOR were confirmed by using three experimental models with different motivational characteristics, although step-down and step-through models are similar passive avoidance tasks. In order to devoid the possible effect of TAM and TOR on motor abilities, a locomotor test was designed in the experiment. The results clearly showed that both TAM and TOR did not affect the locomotor activities of the mice at the doses used in the present study. This suggests that the observed latencies in the performance of mice in the different tasks were due to TAM- and TOR-induced memory impairments.

TOR, at the doses used in the present study, impaired the three processes of memory, i.e., acquisition, consolidation, and retrieval, although the impairing extent showed marginal difference. TAM mainly impaired memory consolidation and retrieval processes. However, this does not mean that TAM has less efficacy than TOR in impairing memory function since the memory consolidation impaired by TAM was more significant than that induced by TOR at the same dose. It is reported that TAM is 1.5–3 times more effective than TOR as a chemotherapeutic agent in human breast cancer (William et al., 1998). Therefore, it is possible that the magnitude of adverse actions of TAM and TOR on memory function is proportional to their therapeutic potencies.

The mechanism of action of TAM and TOR on impairing learning and memory functions has not been clearly evaluated. Because both drugs are estrogen antagonists in the central nervous system (Haynes and Dowsett, 1999), it could be assumed that this activity is the main cause of their memory impairing action. A close interaction between estrogen and cholinergic function in the central nervous system has been reported (Henderson et al., 1994; Henderson, 1997). It has been inferred that estrogen affects learning memory behaviors by modulation of basal cholinergic function (Luine et al., 1980; Simpkins et al., 1997). For example, it is reported that steroidal sexual hormones can affect acetylcholinetransferase and acetylcholinesterase activities (Vazquez-Pereyra et al., 1995). The long-term memory is facilitated with estradiol in the test of one-trial passive avoidance conditioning in male Wistar rats (Vazquez-Pereyra et al., 1995).

In ovariectomized rats, high-affinity choline uptake is reduced in the hippocampus and in the frontal cortex by 24% and 34%, respectively. This decline in high-affinity choline uptake is associated with a significant decrease in

total avoidance in the tests of active avoidance. Estrogen administration can reverse the effects of ovariectomy on high-affinity choline uptake, active avoidance, and spatial memory behavior (Rajakumar et al., 1995). These data suggest that cholinergic neurons are estrogen-responsive and that continuous exposure to ovarian steroid is needed to maintain the normal memory function.

However, other mechanisms of action of TAM on memory function could not be excluded. It has been shown that TAM has a wide variety of pharmacological activities, such as the inhibition of protein kinase C (Grainger and Metcalfe, 1996; O'Brian et al., 1988), acting as a calmodulin antagonist (Allen et al., 1998; Furr and Jordan, 1984; Lam, 1984), blocking various chloride channels (Zhang et al., 1994), and acting as a histamine antagonist (Kroeger and Brandes, 1985). All these actions of TAM may directly or indirectly affect memory function.

The ovariectomized animals are often used in the pharmacological studies of antiestrogenic drugs to prevent the possible interference of estrous cycle of the animals on the experimental results (Carthew et al., 1999; Qu et al., 2000). However, in the present study, we demonstrated that in intact mice TAM and TOR showed significant and repeatable effects on memory function. In fact, patients who take such drugs as adjuvant therapy after surgery on breast cancer are not all postmenopausal women. Moreover, the similar kind of compounds as TAM or TOR will be developed undoubtedly in an attempt to meet a multitude of medical needs in both woman and man (Mitlak and Cohen, 1999). In the present study, it is also shown that both TAM and TOR affected memory function in male mice, suggesting that TAM and TOR affect memory function regardless of sex difference. Thus, the results of the present study may be reasonable to explain the adverse effects of impairing memory of TAM and TOR in clinical setting.

In summary, the results of the present study demonstrate the impairment in memory after intraperitoneal administration of TAM or TOR in mice. It is likely that this impairment is due to the estrogen antagonistic property of these drugs. The results suggest that it is worthwhile to pay more attention on memory function changes for the patient who takes TAM or TOR as adjuvant therapy for breast cancer.

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References

Allen MC, Newland C, Valverde MA, Hardy SP. Inhibition of ligand-gated cation-selective channels by tamoxifen. Eur J Pharmacol 1998;354:261–9.

Biegon A, Brewster M, Degani H, Pop E, Somjen D, Kaye AM. A permanently charged tamoxifen derivative displays anticancer activity and improved tissue selectivity in rodents. Cancer Res 1996;56:4328–31.

Brenner DE, Kukull WA, Stergachis A, van Belle G, Bowen JD, McCormick WC, Teri L, Larson EB. Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: a population-based case-control study. Am J Epidemiol 1994;140:262–7.

Brezden CB, Philips KA, Abdolell M, Bunstone T, Tannock IF. Cognitive function in breast cancer patients receiving adjuvant chemotherapy. J Chin Oncol 2000;18:2695–701.

Carr DB, Goate A, Phil D, Morris JC. Current concepts in the pathogenesis of Alzheimer's disease. Am J Med 1997;103(3A):3s–10s.

Carthew P, Edwards RE, Nolan BM. Uterotrophic effects of tamoxifen, toremifene, and raloxifene do not predict endometrial cell proliferation in the ovariectomized cd1 mouse. Toxicol Appl Pharmacol 1999;158:24–32.

Chugh Y, Saha N, Sankaranarayanan A, Sharma PL. Memory enhancing effects of granisetron (BRL 43694) in a passive avoidance task. Eur J Pharmacol 1991;203:121–3.

Cohen I, Beyth Y, Shapira J, Tepper R, Fishman A, Cordoba M, Bernheim J, Yigael D, Altaras MM. High frequency of adenomyosis in postmenopausal breast cancer patients treated with tamoxifen. Gynecol Obstet Invest 1997;44:200–5.

Early Breast Cancer Trialists Collaborative Group. Tamoxifen for early breast cancer: an overview of the randomised trials. Lancet 1998;351:1451–67.

Fisher B, Costantino JP, Wickerham DL, Redmond CK, Kavanah M, Cronin WM, Voqel V, Robidoux A, Dimitrov N, Atkins J, Daly M, Wieand S, Tan-Chiu E, Ford L, Wolmark N. Tamoxifen for prevention of breast cancer: receptor of the national surgical adjuvant breast and bowel project P-1 study. J Natl Cancer Inst 1998;90:1371–88.

Furr BJ, Jordan VC. The pharmacology and clinical uses of tamoxifen. Pharmacol Ther 1984;25:127–205.

Grainger DJ, Metcalfe JC. Tamoxifen: teaching an old drug new tricks? Nat Med 1996;2:381–5.

Haynes B, Dowsett M. Clinical pharmacology of selective estrogen receptor modulators. Drugs Aging 1999;14:323–36.

Henderson VW. Estrogen, cognition, and a woman's risk of Alzheimer's disease. Am J Med 1997;103(3A):11s–8s.

Henderson VW, Paganini-Hill A, Emanuel CK, Dunn ME, Buckwalter JG. Estrogen replacement therapy in older women: comparisons between Alzheimer's disease and nondemented control subjects. Arch Neurol 1994;51:896–900.

Kroeger EA, Brandes LJ. Evidence that tamoxifen is a histamine antagonist. Biochem Biophys Res Commun 1985;131:750–5.

Lam HY. Tamoxifen is a calmodulin antagonist in the activation of cAMP phosphodiesterase. Biochem Biophys Res Commun 1984;118:27–32.

Li Z, Guo YY, Wu CF, Li X, Wang JH. Protective effects of pseudoginsenoside-F_{11} on scopolamine-induced memory impairment in mice and rats. J Pharm Pharmacol 1999;51:435–40.

Love RR, Wiebe DA, Newcomb PA, Cameron L, Leventhal H, Jordan VC, Feyzi J, DeMets DL. Effect of tamoxifen on cardiovascular risk factors in postmenopausal woman. Ann Intern Med 1991;115:860–4.

Luine V, Park D, Joh T, Reis D, McEwen B. Immunochemical demonstration of increased choline acetyltransferase concentration in rat preoptic area after estradiol administration. Brain Res 1980;191:273–7.

Mitlak BH, Cohen FJ. Selective estrogen receptor modulators: a look ahead. Drugs 1999;57:653–63.

Mortel KF, Meyer JS. Lack of postmenopausal estrogen replacement therapy and the risk of dementia. J Neuropsychiatry Clin Neurosci 1995;7:334–7.

O'Brian CA, Ward NE, Anderson BW. Role of specific interactions between protein kinase C and triphenylethylenes in inhibition of the enzyme. J Natl Cancer Inst 1988;80:1628–33.

Pyrhonen S, Valavaara R, Modig H, Pawlicki M, Pienkowski T, Gundersen S, Bauer J, Westman G, Lundgren S, Blanco G, Mella O, Nilsson I, Hietanen T, Hindy I, Vuorinen J, Hajba A. Comparison of toremifene

and tamoxifen in post-menopausal patients with advanced breast cancer: a randomized double-blind, the 'Nordic' phase study. Br J Cancer 1997;76:270–7.

Qu Q, Zheng H, Dahllund J, Laine A, Cockcroft N, Peng Z, Koseinen M, Hemminki K, Kangas L, Vaananen K, Harkonen P. Selective estrogenic effects of a novel triphenylethylene compound, FC1271a, on bone, cholesterol level, and reproductive tissues in intact and ovariectomized rats. Endocrinology 2000;141:809–20.

Rajakumar G, de Fiebre N, Taube J, Simpkins JW. Estradiol reduces cognitive decline caused by severe hypoglycemia. Neurosci Abstr 1995;21:164.

Schagen SB, van Dam FS, Muller MJ, Boogerd W, Lindeboom J, Bruning PF. Cognitive deficits after postoperative adjuvant chemotherapy for breast carcinoma. Cancer 1999;85:640–50.

Simpkins JW, Green PS, Gridley KE, Singh M, de Fiebre NC, Rajakumar G. Role of estrogen replacement therapy in memory enhancement and the prevention of neuronal loss associated with Alzheimer's disease. Am J Med 1997;103(3A):19s–25s.

Sudha S, Lakshmana MK, Pradhan N. Chronic phenytoin induced impairment of learning and memory with associated changes in brain acetylcholine esterase activity and monoamine levels. Pharmacol, Biochem Behav 1995;52:119–24.

van Dam FS, Schagen SB, Muller MJ, Boogerd W, vd Wall E, Droogleever Fortuyn ME, Rodenhuis S. Impairment of cognitive function in women receiving adjuvant treatment for high-risk breast cancer: high-dose versus standard-dose chemotherapy. J Natl Cancer Inst 1998;90:210–8.

van den Koedijk CD, Blankenstein MA, Thijssen JH. Speculation on the mechanism of action of triphenylethylene antiestrogens. Biochem Pharmacol 1994;47:1927–37.

Vazquez-Pereyra F, Rivas-Arancibia S, Loaeza-Del Castillo A, Schneider-Rivas S. Modulation of short term and long term memory by steroid sexual hormones. Life Sci 1995;56:PL255–60.

Williams GM, Ross PM, Jeffrey AM, Karlsson S. Genotoxicity studies with the antiestrogen toremifene. Drug Chem Toxicol 1998;21:449–76.

Zhang JJ, Jacob TJ, Valverde MA, Hardy SP, Mintenig GM, Sepulveda FV, Gill DR, Hyde SC, Trezise AE, Higgins CF. Tamoxifen blocks chloride channels — a possible mechanism for cataract formation. J Clin Invest 1994;94:1690–7.