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## ORIGINAL ARTICLE

# Effects of tamoxifen on traumatic brain injury-induced depression in male rats



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## KEYWORDS

depression-like behavior;  
extracellular signal-regulated kinases;  
forced swim test;  
selective estrogen receptor modulator;  
tamoxifen

**Abstract** *Background/Introduction:* Previous studies have investigated the neuroprotective effects of tamoxifen (TMX), but its antidepressant-like effects in traumatic brain injury (TBI) remain unclear.

*Purposes/Aims:* The present study was conducted to determine whether TMX can attenuate TBI-induced depression-like behavior and whether this effect involves the activation of extracellular signal-regulated kinase 1/2 (ERK1/2).

*Methods:* Anesthetized male Sprague–Dawley rats were divided into four groups: sham-operated controls, TBI controls, TBI + TMX treatment (1 mg/kg), and TMX (1 mg/kg) + ERK1/2 antagonist, SL327 (30 mg/kg). Depression-like behaviors were evaluated through forced swim tests on Day 4, Day 8, and Day 15. On Day 15 after TBI, phosphorylated ERK1/2 (p-ERK1/2) expression was investigated by Western blotting; neuronal apoptosis, p-ERK1/2, B-cell chronic lymphocytic leukemia/lymphoma 2 (BCL2), and brain-derived neurotrophic factor (BDNF) expression in neuronal cells were evaluated using double immunofluorescence.

*Results:* On Day 15 after TBI, TMX significantly reduced the duration of TBI-induced immobility compared with the TBI controls. The frequency of neuronal apoptosis and numbers of BCL2-positive, BDNF-positive, and p-ERK1/2-positive neuronal cells in hippocampal CA3 were significantly improved by TMX. However, these TMX effects were significantly blocked by SL327 administration.

*Conclusion:* Our results suggest that intraperitoneal injection of TMX may ameliorate TBI-induced depression-like behavior in rats by increasing neuronal p-ERK1/2 expression, which may be associated with neuronal Bcl2 and BDNF expression and decreased neuronal apoptosis.

Conflicts of interest: All authors report no biomedical financial interest or potential conflict of interest.

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This effect might represent a mechanism underlying the recovery from depression-like behavior.

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## 1. Introduction

Depression following a traumatic brain injury (TBI) is a severe complication. The incidence of posttraumatic depression is estimated to range from 10% to 77%, with variation according to the studied population, diagnostic criteria, and rating instruments used.<sup>1</sup> This post-TBI neuropsychiatric impairment contributes to disability after TBI and has become a chronic condition in an estimated 3.17 million Americans.<sup>2</sup> Therefore, developing novel therapeutic strategies and identifying new effective therapies are crucial.

The hippocampus is a crucial component of the limbic system, which is a site of the central nervous system that is involved in depressive behavior.<sup>3</sup> Previous studies have reported that hippocampal CA3 neurons appear to be particularly vulnerable to TBI,<sup>4,5</sup> which increases the likelihood that an insult to the hippocampus CA3 can induce hippocampal cell apoptosis and lead to depressive behavior.

The extracellular signal-regulated kinase (ERK1/2) pathways are associated with cell survival and apoptosis<sup>6</sup> and ERK1/2-mediated neuroprotective effects such as those induced through BCL2 and BDNF.<sup>7,8</sup> A previous study reported that the ERK1/2 signal transduction pathway in the hippocampus, but not that in the amygdala, may be involved in TBI-induced depression-like behavior in rats.<sup>9</sup> Intraperitoneal tamoxifen (TMX), a selective estrogen receptor modulator (SERM), may ameliorate TBI in rats by increasing neuronal ERK1/2 phosphorylation, which might lead to an increase in neuronal *Bcl2* expression and decrease in neuronal apoptosis after cortical ischemia.<sup>10</sup> Furthermore, Walf and Frye<sup>11</sup> demonstrated that subcutaneous or direct administration of estrogen into the hippocampus elicited antidepressant-like behavior compared with vehicle administration. TMX treatment in cultured hippocampal neurons increased the expression of the antiapoptotic protein, BCL2, an outcome linked to the neuroprotective effect of estrogen.<sup>12</sup> These phenomena increase the likelihood that TMX may have beneficial effects on TBI-induced apoptosis in hippocampal cells and subsequent antidepressant effects.

The neuroprotective effects of estrogen in TBI have been evaluated.<sup>13</sup> However, applying estrogen as a neuroprotective agent in humans may have several limitations such as causing estrogen-dependent peripheral tumors. In the current study, we selected TMX because, being a nonsteroidal SERM, its estrogen-like neuroprotective activity in the brain can act as an alternative agonist to estrogen<sup>14</sup>; it is brain–blood barrier permeable, achieving an increased concentration in the brain and serum upon administration<sup>15</sup>; and most importantly, the direct

mechanism of TMX in TBI-induced depression-like behavior remains unclear.

The forced swim test (FST) is one of the most common animal models for assessing depression-like behavior. Longer periods of immobility during the test indicate more severe depression-like behaviors. Several antidepressants have been consistently shown to reduce the duration of immobility during testing by increasing active escape behaviors.<sup>16,17</sup>

In the current study, we hypothesized that TMX would have therapeutic effects on TBI-induced depression-like behaviors and that its beneficial effects may be associated with *ERK1/2* expression. To test this hypothesis, we applied SL327,<sup>18</sup> a brain-penetrating selective inhibitor of ERK1/2 that selectively inhibits phosphorylated ERK1/2 (p-ERK1/2), in the brain following systematic administration.<sup>19</sup> We investigated whether TMX in the presence or absence of SL327 activates neuronal ERK1/2, BCL2, and BDNF responses; reduces neuronal cell apoptosis; and ameliorates depression-like behaviors (evaluated by FST) after TBI in adult rats.

## 2. Methods

### 2.1. Animals

Adult male Sprague–Dawley rats weighing  $397 \pm 23$  g were used in this study. The animals were maintained under a 12-hour light/12-hour dark cycle and allowed free access to both food and water. Before the study was initiated, all protocols for the study followed the Animal Protection Act, Council of Agriculture, Executive Yuan, Taiwan and were approved by the Chi-Mei Medical Center's Animal Care and Use Committee (IACUC; Tainan, Taiwan) for all experimental procedures (IACUC Approval No: 100120711). The protocols also conformed to the National Institutes of Health guidelines (Publication No. 85-23, revised 1985) including minimizing discomfort to animals during surgery and the recovery period.

### 2.2. TBI

The rats were anesthetized by intraperitoneal administration of a mixture of ketamine [44 mg/kg, intramuscularly (i.m.); Nankuang Pharmaceutical, Tainan, Taiwan], atropine (0.062633 mg/kg, i.m.; Sintong Chemical Ind. Co., Taoyuan, Taiwan), and xylazine (6.77 mg/kg, i.m.; Bayer, Leverkusen, Germany). A craniectomy (radius = 2 mm) 4 mm from the bregma and 3 mm from sagittal sutures in the right parietal cortex was performed using a stereotaxic frame. After craniectomy and implantation of an injury

cannula, a fluid percussion injury (FPI) device (VCU Biomedical Engineering, Richmond, VA, USA) was connected to the rats through a Luer-lock fitting, and the brain was injured through percussion (2.0–2.2 atm, 25 milliseconds). The details are described in our previous study.<sup>10</sup> Transient hypertensive response, apnea, and seizure were observed immediately following the FPI, and these symptoms were used as the criteria for dividing the animals into the TBI and TBI + treatment groups.

### 2.3. Treatment intervention

The experimental design was established in three parts (Figure 1). In the first part of the study, the effects of TMX on TBI-induced immobility were investigated on Day 4, Day 8, and Day 15 after TBI by FST. The effects of TMX on *Erk1/2* expression in the hippocampus of the ipsilateral injury side were investigated on Day 15 after TBI by Western blotting. Moreover, whether SL327 would block the neuroprotective effect of TMX in the CA3 region of the hippocampus ipsilateral to the injury side was assessed by immunofluorescence staining. The effects of SL327 on depression-like behavior were also evaluated by FST.

The rats were randomly divided into the sham-treated, dimethyl sulfoxide vehicle-treated (4% intraperitoneal

injection; Merck, K42088831; vehicle), TBI control + vehicle-treated, TBI + TMX-treated (1 mg/kg intraperitoneal injection administered on 0 hours, 24 hours, and 48 hours after FPI; Sigma T5648, Axon, Groningen, The Netherlands, USA; SERM), and TBI + SL327-treated groups (30 mg/kg intraperitoneal injection administered 30 minutes before TBI; Axon, Axon 1122; ERK1/2 antagonist). The dosage, administration route, and time course of TMX injection were identical to those used in our previous study.<sup>10</sup> The first injection was administered immediately after injury, followed by the second and third injections after 24 hours and 48 hours, respectively. The intraperitoneal route of administration was considered convenient for the three consecutive daily injections in our model.

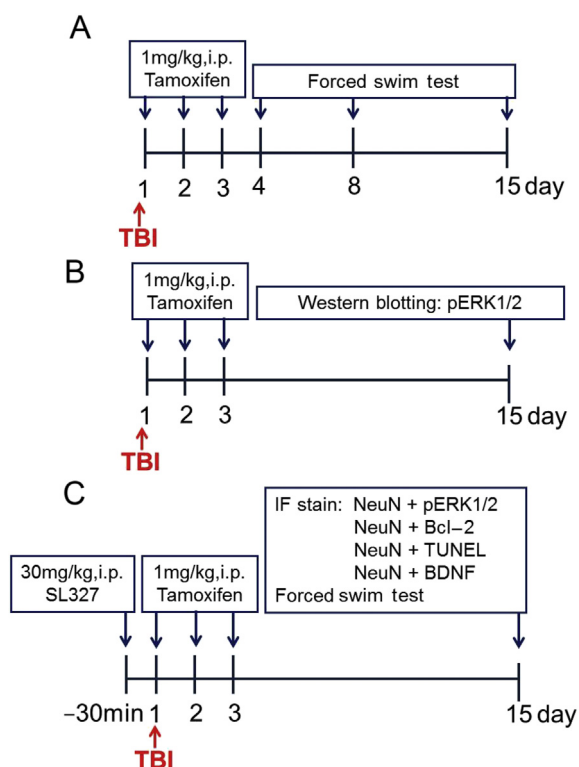
All tests were performed by investigators blinded to the study groups, which were revealed only at the end of the analysis. The animals used for the histological or behavioral studies were provided food and water *ad libitum* throughout the study. The rats were sacrificed with an overdose of urethane at the end of the experiments.

### 2.4. FST

All FST studies were conducted on Day 4, Day 8, and Day 15. The rats were placed in clear Plexiglas cylinders [65 cm (height) × 25 cm (diameter)] filled up to 48 cm of height with water at 25°C. After 15 minutes of forced swimming, the rats were removed from the water, dried with towels, and placed in a warm enclosure for 30 minutes. The cylinders were emptied and cleaned between two consecutive tests. The rats were retested for 5 minutes under identical conditions 24 hours after the forced swimming. In accordance with the method described by Basso et al,<sup>16</sup> the FST data presented in this manuscript were collected during the retest sessions, which were videotaped from the side of the cylinders.

### 2.5. Western blot analysis

Brain tissue from the hippocampus ipsilateral to the injury side was homogenized in the T-PER reagent at the ratio of 1:20 w/v and centrifuged (14,000 rpm) to obtain tissue debris. The protein concentration was measured using a bicinchoninic assay modified for microplate use. The homogenates (150 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and incubated with a polyclonal antibody specific for p-ERK1/p-ERK2 (StressGen Biotechnologies Corp., cell signaling technology, Beverly, MA, USA). The detailed information on the antibody is described herein. Mouse Phospho-p44/42 MAPK (*Erk1/2*; 42 kDa, 44 kDa, 1:1000, Cell Signaling Technology, #5726), detected with Rabbit Polyclonal Secondary Antibody to Mouse immunoglobulin IgG H&L (HRP; 1:10,000; Abcam, ab6728, Abcam Cambridge, FL, UK); Rabbit Polyclonal Anti-Erk1 + Erk2 Antibody (44,42 kDa, 1:1000, Abcam, ab4819), detected with Goat Polyclonal Secondary Antibody to Rabbit IgG H&L (HRP; 1:10000, Abcam, ab6721); and mouse monoclonal beta-actin (43 kDa, 1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA; sc-81178), detected with Rabbit Polyclonal Secondary Antibody to Mouse IgG H&L (HRP; 1:10,000;



**Figure 1** Overall experimental design. (A) The effects of TMX on FPI-induced immobility were assessed using the FST. (E) The effects of TMX on *Erk1/2* expression in the hippocampus on Day 15 were assessed by Western blotting. (C) Immunofluorescence staining was used to determine whether SL327 (an ERK1/2 antagonist) blocks the neuroprotective effect of TMX. ERK1/2 = extracellular signal-regulated kinase 1/2; FPI = fluid percussion injury; FST = forced swim test; TMX = tamoxifen.

Abcam, ab6728). Samples were quantified relative to the standard, and the data are expressed as percentages of the corresponding control group. The p-ERK1/2 levels were measured on Day 15.

## 2.6. Determination of p-Erk1/2, Bcl2, and BDNF expressions in neuronal cells in the hippocampus using an immunofluorescence assay.

On Day 15, adjacent 6- $\mu$ m sections corresponding to the coronal coordinates 4.80–6.04 mm posterior to the bregma were incubated with primary antibodies in phosphate buffered saline (PBS) containing 1% normal goat serum overnight at 4°C. After being washed in PBS, the sections were incubated with secondary antibodies for 1 hour at room temperature. The antibodies used in this study were monoclonal mouse antineuronal-specific nuclear protein (NeuN, 1:800 dilution; Abcam, ab104224) antibody, detected with Alexa-Fluor 568-conjugated antimouse IgG antibody (1:400 dilution; Invitrogen, A11031, Invitrogen, Eugene, Oregon, USA); rabbit anti-BCL2 protein (1:400 dilution; Abcam; ab136285) antibody, detected with Alexa-Fluor 488-conjugated antirabbit IgG antibody (1:400 dilution; Invitrogen, A11034); rabbit anti-p-ERK protein (1:200 dilution; Abcam, ab4370) antibody, detected with Alexa-Fluor 488-conjugated antirabbit IgG antibody (1:400 dilution; Invitrogen, A11034); and rabbit anti-BDNF protein (1:200 dilution; Santa Cruz Biotechnology; sc-20981) antibody, detected with Alexa-Fluor 488-conjugated antirabbit IgG antibody (1:400 dilution; Invitrogen; A11034).

The sections were washed in PBS, incubated with fluorescein isothiocyanate-conjugated antirabbit IgG in 1% normal goat serum/PBS/0.25% Triton-x100 for 60 minutes, and mounted with antifade-mounting medium. The numbers of labeled cells were calculated in two coronal sections from each rat and are expressed as the mean number of cells per section. To obtain negative coronal sections, all procedures were performed using the same protocol without the primary antibodies.

## 2.7. Assay of neuronal apoptosis in the hippocampus by immunofluorescence staining

On Day 15, the apoptotic cells were identified by staining with terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL)<sup>20</sup> following previously described procedures.<sup>10</sup>

## 2.8. Statistical analysis

All results are expressed as the mean  $\pm$  standard error of the mean. A two-way analysis of variance for repeated measurements (in the same rats) was used for the factorial experiments, and Dunnett's test was used for the *post hoc* multiple comparisons of the means. A *p* value of <0.05 was considered to indicate a significant difference.

## 3. Results

### 3.1. TMX significantly reduced the duration of TBI-induced immobility 15 days after TBI

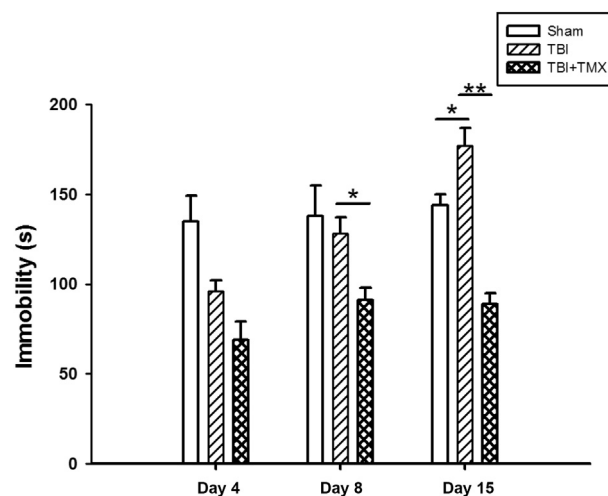
On Day 1, the rats were subjected to lateral FPI via a rapid injection of a small volume of saline into the closed cranial cavity. In the FST, the TBI rats displayed despair behavior, as indicated by an increase in the duration of immobility 15 days after TBI ( $p < 0.05$ ,  $n = 6$  in each group). TBI-induced immobility was significantly lowered when TMX was administered on Day 8 and Day 15 after TBI. These results suggested that TMX induced antidepressant-like behavior, particularly on Day 15 after TBI (Figure 2).

### 3.2. TMX significantly enhanced the TBI-induced reduction in total p-Erk expression in the region of the hippocampus ipsilateral to the injury side

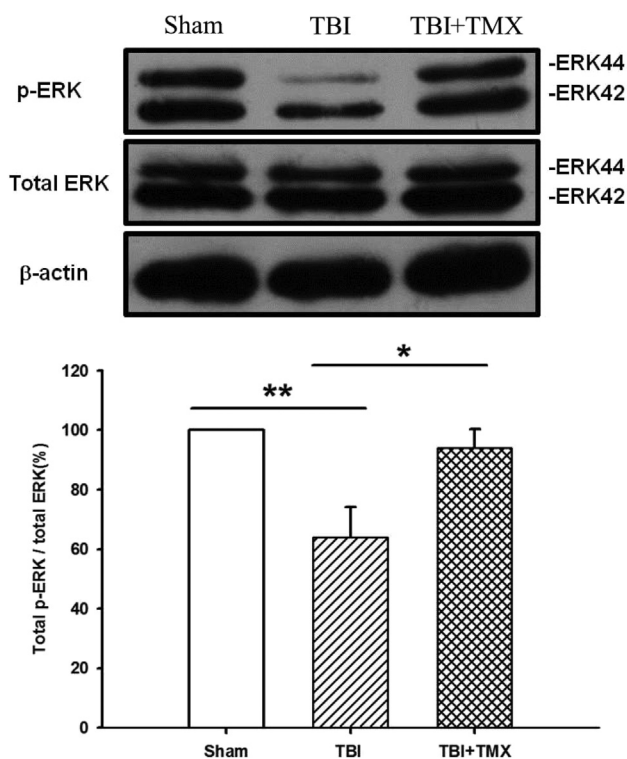
To determine whether ERK1/2 is involved in the antidepressant effects of TMX, the total expression of ERK1/2 and p-ERK1/2 in the hippocampus was investigated by Western blotting on Day 15 after TBI. The results reveal that the TBI-induced reduction in the ratio of total p-ERK1/2 to total ERK was significantly increased by TMX ( $p < 0.01$ ,  $n = 6$  in each group). These results support the hypothesis that ERK1/2 is involved in the antidepressant effects induced by TMX (Figure 3).

### 3.3. TMX significantly attenuated TBI-induced neuronal apoptosis in the CA3 region of the hippocampus ipsilateral to the injury side

The TUNEL + Neu-N staining assay performed on Day 15 ( $n = 6$  in each group) revealed that the number of neuronal



**Figure 2** Effects of TMX (1 mg/kg, i.p.) on the TBI-induced rats assessed by FST on Day 4, Day 8, and Day 15. \*  $p < 0.05$ , \*\*  $p < 0.01$  ( $n = 8$  in each group). FST = forced swim test; i.p. = intraperitoneally; TBI = traumatic brain injury; TMX = tamoxifen.



**Figure 3** Analysis of the effects of TMX on *Erk1/2* expression in the hippocampus on Day 15 by Western blotting. Total *p-Erk1/2* expression in the hippocampus was significantly reduced after TBI and reversed by TMX treatment. \*  $p < 0.05$ , \*\*  $p < 0.01$  ( $n = 6$  in each group). TBI = traumatic brain injury; TMX = tamoxifen.

apoptotic cells in the hippocampi of the vehicle-treated rats was significantly increased compared with that in the sham group ( $p < 0.01$ ). The TBI-induced increase in the number of neuronal apoptotic cells was significantly enhanced after TMX treatment ( $p < 0.05$ ). However, the beneficial effects of TMX were significantly reversed by SL327 treatment ( $p < 0.05$ ; Figure 4).

### 3.4. TMX significantly increased neuronal p-ERK1/2, Bcl2, and BDNF expression in the CA3 region of the hippocampus ipsilateral to the injury side

On Day 15, the numbers of p-ERK1/2-positive (Figure 5A), BCL2-positive (Figure 5B), and BDNF-positive (Figure 5C) neurons in the hippocampi of the vehicle-treated rats were significantly reduced compared with those in the sham group ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ , respectively;  $n = 6$  in each group). The TBI-induced decrease in the numbers of p-ERK1/2-positive, BCL2-positive, and BDNF-positive neuronal cells were significantly improved after TMX treatment. However, the numbers of both Neu-N and either p-ERK1/2 or BCL2 double-positive cells were significantly reduced in the TMX + SL327-treated group ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.05$ , respectively;  $n = 6$  in each group).

### 3.5. SL327 significantly reversed the beneficial effects of TMX on immobility on Day 15 after TBI

The FST revealed that the TBI-induced immobility was significantly reduced by TMX on Day 15 after TBI ( $177.6 \pm 10.8$  seconds vs.  $89.9 \pm 6.5$  seconds,  $p < 0.01$ ). However, the beneficial effects of TMX were reversed in the rats pretreated with SL327 ( $89.9 \pm 6.5$  seconds vs.  $162.9 \pm 2.9$  seconds,  $p < 0.001$ ).

## 4. Discussion

### 4.1. Novelty of the current study

TMX was administered at a dosage of 1 mg/kg for 3 consecutive days to counteract neuronal apoptosis in the CA3 region of the hippocampus and the depression-like behavior induced by TBI in the rats through ERK1/2-mediated antidepressant effects. Based on our research, this is the first study to demonstrate the antidepressant-like effects of TMX injection in traumatic central nervous system injury and possible involvement of ERK1/2 activation in this TMX-induced effect. These data may serve as a foundation for future studies on the importance of SERM therapy in TBI-induced depression-like behavior. Our results suggest that using TMX to stimulate ERK1/2 may be a novel strategy for treating TBI-induced depression-like behavior in the future.

### 4.2. Optimal time course of TMX administration

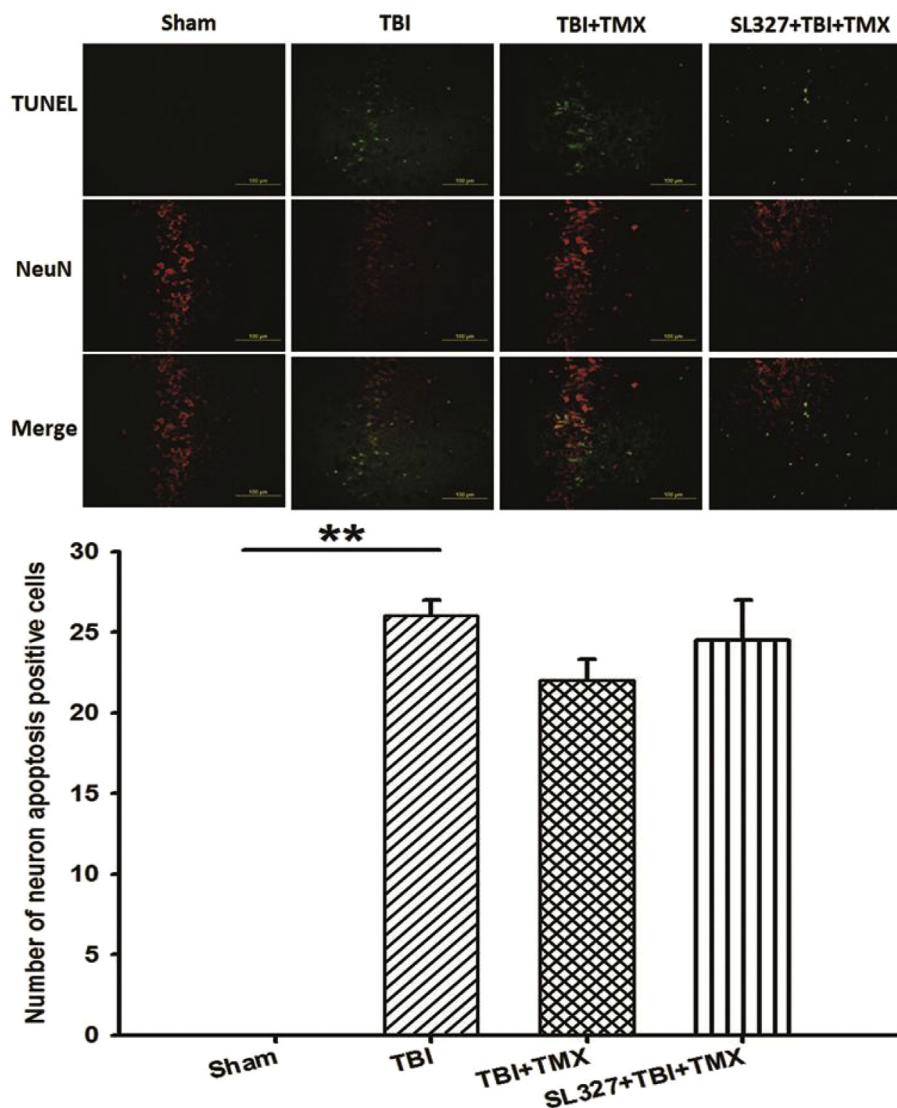
Sato et al<sup>5</sup> observed the maximum levels of degenerating neurons in the cortex and hippocampus 1 day and 3 days after TBI, respectively. Pierce et al<sup>21</sup> also indicated that TBI-induced functional and pathological dysfunction persisted from 72 hours to 1 year after lateral FPI.<sup>17</sup> Following our previous study, in which we observed a significant increase in TBI-induced cell apoptosis 4 days following TBI compared with the sham group, we injected TMX for 3 consecutive days after TBI in the current study.<sup>10,22</sup>

### 4.3. Optimal time points for observing depression-like behavior

The FST is a widely used and sensitive assay with good predictive validity for depressants. The current study demonstrated that the duration of immobility decreased on Day 4 and increased significantly on Day 15 ( $p < 0.05$ ) in the TBI group, improving after TMX treatment on Day 15 ( $p < 0.001$ ). We believe that the decrease in immobility time on Day 4 might be associated with the struggling effects after insult. Therefore, based on our FST measurements, we selected Day 15 after TBI to investigate cell morphology and biochemistry. We suggest that Day 15 after TBI could be the optimal time for observing depression-like behavior.

### 4.4. Phosphorylation of ERK1/2 in the hippocampus following TBI may be attenuated

The temporal profile of p-ERK1/2 in the hippocampus of the rat brain from 5 minutes to 72 hours following TBI, which



**Figure 4** Effects of TMX on TBI-induced neuronal apoptosis on Day 15 following TBI. The top panels depict representative Neu-N-positive and TUNEL-positive staining for one sham rat, one TBI rat, one TBI + TMX-treated rat, and one TBI + TMX + SL327-treated rat. \*  $p < 0.05$ , \*\*  $p < 0.01$  ( $n = 6$  in each group). TBI = traumatic brain injury; TMX = tamoxifen; TUNEL = terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling.

presents an initial increase and a subsequent progressive decrease, has been previously evaluated.<sup>23</sup> Our previous study concluded that TBI led to a significant decrease in p-ERK1/2 in the hippocampus 72 hours after TBI.<sup>10</sup>

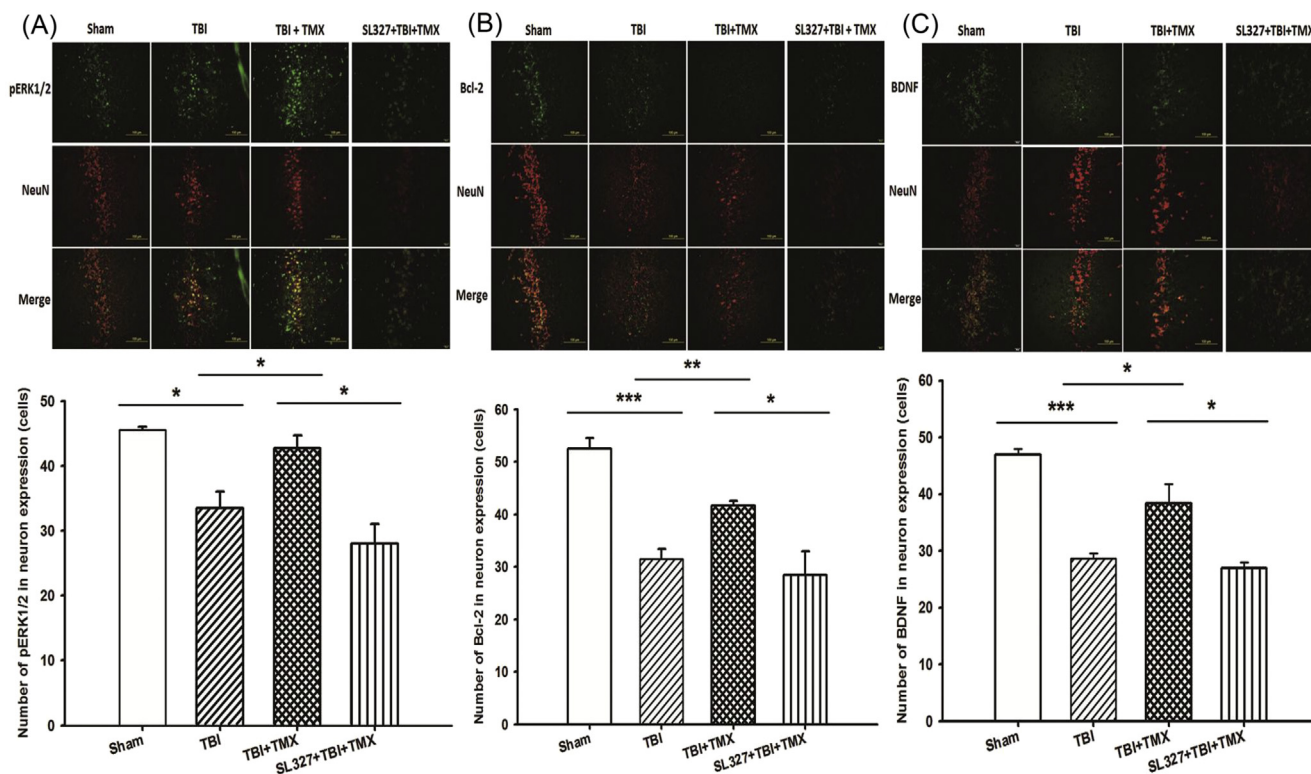
The Western blotting analysis revealed that the TBI-induced decrease in the ratio of total p-ERK1/2 to total ERK was significantly improved by TMX on Day 15 after TBI. These findings suggest that ERK1/2 may be closely involved in signal transduction in the rat hippocampus after TBI.

#### 4.5. Potential involvement of ERK1/2 pathway in the neuroprotective effects of TMX in the CA3 region of the hippocampus after TBI

Cell apoptosis is a well-known secondary insult after TBI.<sup>24</sup> This complication has been observed in the perilesioned areas in animal and human studies.<sup>25</sup> Consistent with

previous studies that have reported selective invulnerability to TBI induced by FPI in CA3 neurons in the hippocampus,<sup>4,5</sup> the current study demonstrates that the numbers of neurons lost and Neu-N-positive and TUNEL-positive cells observed at the primary injury site in the CA3 region of the hippocampus were significantly increased on Day 15 after TBI but were reduced after TMX treatment. These results are consistent with those observed in studies on spinal cord injury.<sup>26</sup> Our results suggest that the antidepressant effects of TMX may be at least partly due to the attenuation of apoptotic activity in the brain tissue after TBI.

The RSK-ERK-Creb-BCL2 signaling pathway was proposed to promote cell survival through transcriptional upregulation of antiapoptotic BCL2 proteins.<sup>7</sup> Using the brain-penetrating ERK1/2 selective inhibitor, SL327, our study demonstrates that TMX-induced antiapoptosis in



**Figure 5** (A) Effects of TMX (1 mg/kg, i.p.) on TBI-induced neuronal p-ERK1/2 staining in the hippocampus on Day 15 following FPI. The top panels depict representative Neu-N-positive and p-ERK1/2-positive staining for one sham rat, one TBI rat, one TBI + TMX-treated rat, and one TBI + TMX + SL327-treated rat. (B) Effects of TMX on *Bcl2* expression in neuronal cells in the hippocampus (BCL2 + Neu-N staining assay) on Day 15. The top panels depict representative Neu-N-positive and BCL2-positive staining for one sham rat, one TBI rat, one TBI + TMX-treated rat, and one TBI + TMX + SL327-treated rat. (C) Effects of TMX on *BDNF* expression in neuronal cells in the hippocampus (BDNF + Neu-N staining assay) on Day 15. The top panels depict representative Neu-N-positive and BDNF-positive staining for one sham rat, one TBI rat, and one TBI + TMX-treated rat. \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  ( $n = 6$  in each group). BDNF = brain-derived neurotrophic factor; FPI = fluid percussion injury; i.p. = intraperitoneally; p-ERK1/2 = phosphorylated ERK1/2; TBI = traumatic brain injury; TMX = tamoxifen.

hippocampal CA3 neurons is associated with p-ERK1/2 activation and that neuronal *Bcl2* expression and the observed beneficial effects are attenuated by pretreatment with SL327. Increased levels of p-ERK1/2 and BCL2 in the injured hippocampus, which decrease neuronal apoptosis, are possible mechanisms underlying the recovery from depression-like behavior.

BDNF is associated with neuronal survival and produces antidepressant effects in psychiatric disorders through the AMPA–ERK–BDNF signaling pathway.<sup>8</sup> Decreased *BDNF* expression is associated with depression.<sup>27</sup> The direct administration of exogenous BDNF produces antidepressant-like effects in the FST and learned helplessness paradigms in rats.<sup>28</sup> The current study shows that the TBI-induced decrease in the number of BDNF-positive neuronal cells was significantly increased by TMX treatment. The study further shows that *BDNF* expression was reduced by the ERK1/2 inhibitor. Our results support the crucial role of ERK1/2 in BDNF production,<sup>8</sup> which is a possible mechanism underlying its antidepressant-like effect after TBI.

The current study evaluated the effect of TMX on hippocampal neurons only. Future studies should consider clarifying whether *p-ERK1/2* expression in astrocytes,

microglia, and oligodendroglia can induce cell apoptosis or survival with TMX treatment after TBI.

#### 4.6. Controversial role of ERK1/2 in depression

In the current study, the activation of the ERK1/2 pathway produced antidepressant-like effects in a TBI-induced depression-like rat model. This result accords with that reported by Duman et al,<sup>27</sup> who demonstrated that using SL327 produced a depression-like response in rats, and a decrease in p-ERK1/2 temporally coincided with depressive-like behavior in a rat model with a chronic exposure to an oral corticosteroid.<sup>28</sup> However, Fumagalli et al<sup>29</sup> reported that a reduction in ERK1/2 function may be associated with the therapeutic effects of fluoxetine in a rat model with chronic fluoxetine administration. Furthermore, Freitas et al<sup>30</sup> demonstrated that the olfactory bulbectomy animal model of depression caused an increase in p-ERK1, an effect that was abolished by fluoxetine treatment. These results are inconsistent possibly because the effects of p-ERK1/2 depend on the stimuli and cell types involved and because the balance between the intensity and duration of proapoptotic versus antiapoptotic signals

transmitted by ERK1/2 determines whether a cell survives or undergoes apoptosis.<sup>6</sup>

#### 4.7. Controversial effects of TMX on depression

Despite the positive effects of TMX on depression-like behavior in our study, it remains unclear whether it will have any effect on depression under different conditions. In a study on a female rat model, Azizi-Malekabadi et al<sup>31</sup> suggested that the effects of TMX on depression differ depending on the presence of ovarian hormones. In a survey of breast cancer patients, Thompson et al<sup>32</sup> showed that TMX increased the incidence of depressive symptoms, whereas Lee et al<sup>33</sup> reported no relationship between TMX use and depression. These inconsistent results may indicate that TMX has both agonistic and antagonistic effects on estrogen receptors.<sup>34</sup> Therefore, we considered estrogen receptor expression with or without TMX in our TBI male rat model. This approach will facilitate future investigations.

#### 4.8. Limitations of the current study

Several limitations of the current study should be considered. First, only male rats were investigated. Future studies should evaluate whether TMX protects female rats from TBI-induced depression-like behavior. Second, because of equipment limitations, FST was the only method used to evaluate depression-like behavior. Third, based on our previous study on 2,3,5-Triphenyltetrazolium hydrochloride (TTC) staining,<sup>10</sup> damage to the contralateral injury side was not evident after FPI in our model. Therefore, we could not determine the contralateral hippocampal changes after TBI. Because depression-like behavior is a whole-brain phenomenon, future studies should evaluate whether damage to the hippocampus contralateral to the injury occurs. Finally, because estrogen is associated with depression, studies on the effects of TMX on estrogen receptor expression are warranted. Therefore, several prospective studies are required.

#### 4.9. Conclusions

An intraperitoneal injection of TMX administered at a dosage of 1 mg/kg for 3 consecutive days, during the acute stage attenuated TBI in rats by promoting neuronal *p-Erk1/2*, *Bcl2*, and *BDNF* expression and reducing neuronal apoptosis, resulting in antidepressant-like behavior. Our results suggest that the ERK1/2 pathway may play a role in mediating the behavioral effect of TMX. We also suggest that TMX may be a promising treatment strategy for TBI-induced depression-like behavior.

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