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Extracellular signal–regulated kinase 1/2 is involved in a tamoxifen neuroprotective effect in a lateral fluid percussion injury rat model

Yin-Tzu Tsai, MD,[a,1] Che-Chuan Wang, MD,[b,c,f,1] Pak-On Leung, MD,[a] Kao-Chang Lin, MD,[d,e] Chung-Ching Chio, MD,[b] Chiao-Ya Hu, BD,[d] and Jinn-Rung Kuo, MD, PhD[b,d,e,*]

[a] Department of intensive Care Medicine, Chi-Mei Medical Center, Liouying, Tainan, Taiwan
[b] Department of Neurosurgery, Tainan, Taiwan
[c] Institute of Photonic Systems, National Chiao-Tung University, Tainan, Taiwan
[d] Department of Medical Research Chi-Mei Medical Center, Tainan, Taiwan
[e] Department of Biotechnology, Taiwan University of Science and Technology, Tainan, Taiwan
[f] Department of Child Care, Taiwan University of Science and Technology, Tainan, Taiwan

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ABSTRACT

Background: The aim of the present study was to determine whether tamoxifen (TMX) causes attenuation of traumatic brain injury (TBI) induced by fluid percussion injury.

Materials and methods: Immediately after the onset of fluid percussion TBI, anesthetized male Sprague-Dawley rats were divided into three major groups and intraperitoneally administered the vehicle solution (1 mL/kg), TMX (1 mg/kg), or TMX (1 mg/kg) plus the extracellular signal–regulated kinase 1/2 antagonist SL327 (30 mg/kg). Another group of rats were used as sham-operated controls. The functional outcomes, such as motor outcomes, were evaluated using an incline plane. The cellular infarction volume was evaluated by triphenyltetrazolium chloride staining. Neuronal loss, apoptosis, and p-ERK1/2 and Bcl2 expression in neuronal cortex cells were evaluated by immunofluorescence methods. All the parameters were assessed on day 4 after injury.

Results: Compared with the sham-operated controls, the TBI-induced motor deficits and cerebral infarction after TBI were significantly attenuated by TMX therapy. The TBI-induced neuronal loss and apoptosis were also significantly reduced by TMX therapy. The numbers of Bcl2- and phospho-ERK1/2-positive neuronal cells in the ischemic cortex after TBI were significantly increased by TMX therapy. These TMX effects were significantly blocked by SL327 administration.

Conclusions: Our results suggest that intravenous injection of TMX may ameliorate TBI in rats by increasing neuronal p-ERK1/2 expression, which might lead to an increase in neuronal Bcl2 expression and a decrease in neuronal apoptosis and cell infarction volume, and it might represent one mechanism by which functional recovery occurred. TMX may be a promising TBI treatment strategy.

* *Corresponding author*. Chi-Mei Medical Center, #901 Chung Hwa Road, Yung Kang City, Tainan, Taiwan. Tel.: +886 6 281 2811; fax: +886 6 282 8928.
E-mail address: kuojinnrung@gmail.com (J.-R. Kuo).

[1] The first two authors contributed equally to this work.

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1. Introduction

Traumatic brain injury (TBI) is a major global public health concern. Despite adequate treatment, traumatic head injury commonly causes neuronal loss and apoptosis, leading to long-term neurologic deficits [1–3]. In TBI, apoptosis has been demonstrated to commonly occur in the perilesioned area as a result of secondary brain insults in animal and human studies [4,5]. These events are referred to as the secondary injury mechanism. Therefore, preventing cell apoptosis post-TBI may be an important therapeutic strategy.

Estrogen, a ligand of estrogen receptor, activates signaling cascades in healthy neurons, enhances the biochemical, genomic, and morphologic mechanisms of memory and proactively induces mechanisms of protection against neurodegenerative insults [6]. Estrogen (E2) activation of extracellular signal–regulated kinase 1/2 (ERK1/2) may elicit mechanisms of neuroprotection, as treatment with mitogen-activated protein kinases inhibitors reduces the neuroprotective effects of estrogens [7,8]. However, the application of E2 as a neuroprotectant in humans presents numerous limitations, mainly because of the endocrine actions of the molecule on peripheral tissues, including estrogen-dependent tumors. The possibility of using selective estrogen receptor modulators (SERMs) to exert E2-like neuroprotective actions in the brain has emerged as an alternative to E2 [9].

Tamoxifen (TMX) is a triphenylethylene derivative, nonsteroidal first-generation SERM [10]. TMX is brain-blood barrier permeable, and its concentration has been reported to be much higher in the brain than in the serum [11,12]. Its metabolite, 4-hydroxy-tamoxifen, has a shorter half-life but binds to estrogen receptor with a binding affinity 20–30 times greater than that of TMX and equivalent to that of E2 [13,14]. TMX has been demonstrated to be a neuroprotectant for spinal cord injury [14], cerebral ischemia injury [15–17], irradiation-induced brain injury [18], and methamphetamine-induced toxicity [19]. These results imply that TMX may have estrogenic neuroprotective actions similar to those of E2 in the brain.

Furthermore, administration of TMX to ovariectomized rats increases the expression of Bcl2 and decreases the expression of Bax in the hippocampus [20]. Treatment with TMX in cultured hippocampal neurons increases the expression of the antiapoptotic protein Bcl2, an outcome that has been linked to estrogen's neuroprotective effects [8]. These findings introduce the possibility that TMX may have beneficial effects on TBI-induced cortex cell apoptosis and subsequent neuronal protective effects.

Currently, whether TMX has a similar protective effect on TBI remains to be investigated. In the present study, we chose TMX specifically because it is a SERM, it is blood-brain barrier permeable, and it has clear pharmacokinetic activity in the central nervous system (CNS). TMX has been found to be neuroprotective in both transient and permanent experimental ischemic stroke and spinal cord injury. However, it remains unknown whether this agent displays a similar beneficial effect after TBI and what its underlying mechanisms are. In the present study, we have applied SL327 [21], a brain-penetrating selective inhibitor of ERK kinase, which was demonstrated to be able to selectively inhibit ERK activation in the brain following systematic administration [22], to investigate the role of TMX in neuroprotection after TBI using a fluid percussion cerebral injury model in rats.

In this article, we investigated whether TMX would activate a pERK1/2 and Bcl2 response, reduce neuronal cell apoptosis, decrease neuronal loss, and ameliorate impaired motor function after adult rat TBI. The results provide evidence that TMX might constitute an effective therapeutic neuroprotectant for TBI.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 290 ± 16 g were used in the experiments. The animals were kept under a 12/12-h light/dark cycle and allowed free access to food and water. The Chi Mei Medical Centre Animal Care and Use Committee approved all the experimental procedures, which conformed to the National Institute of Health guidelines, including minimizing discomfort to the animals during surgery and during the recovery period. At the end of the experiments, 72 h after TBI, the experimental rats were killed with an overdose of urethane for special stain.

2.2. Traumatic brain injury

The animals were anesthetized with an intraperitoneal (i.p.) administration of a mixture of ketamine (44 mg/kg, intramuscularly [i.m.]; Nankuang Pharmaceutical, Taiwan), atropine (0.062633 mg/kg, i.m.; Sintong Chemical Ind Co, Taiwan), and xylazine (6.77 mg/kg, i.m.; Bayer, Leverkusen, Germany). A craniectomy (2 mm in radius) 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, the fluid percussion device (VCU Biomedical Engineering, Richmond, VA) was connected to the animal via a Luer-loc fitting, and the brain was injured with a 2.0–2.2 atm, 25 ms percussion. This injury produces moderately severe brain trauma, as originally described by Mclntosh *et al.* [23]. A transient hypertensive response, apnea, and seizure were observed immediately following the fluid percussion injury (FPI) and were used as the criteria for separating the animals into TBI or TBI + treatment groups.

2.3. Treatment intervention

The rats were randomly divided into four major groups: sham-operated ($n = 6$), treated with dimethyl sulfoxide (DMSO) vehicle (4%, i.p., K42088831, vehicle; Merck, Darmstadt, Germany); TBI control + vehicle-treated; TBI + TMX-treated (1 mg/kg, T5648, SERM; Sigma-Aldrich, Shanghai, China) ($n = 6$), and TBI + SL327 (30 mg/kg; Axon 1122, ERK1/2 antagonist; Axon, Groningen, Netherlands) + TMX-treated ($n = 6$). The dosage and time course injection of TMX were