Neurobiology of Disease 117 (2018) 114–124

Contents lists available at ScienceDirect

Neurobiology of Disease

journal homepage: www.elsevier.com/locate/ynbdi

Bexarotene protects against neurotoxicity partially through a PPARγ-dependent mechanism in mice following traumatic brain injury

Junchi He, Han Liu, Jianjun Zhong, Zongduo Guo, Jingchuan Wu, Hongrong Zhang, Zhijian Huang, Li Jiang, Hui Li, Zhaosi Zhang, Liu Liu, Yue Wu, Lingjun Qi, Xiaochuan Sun*, Chongjie Cheng*

Department of Neurosurgery, the First Affiliated Hospital of Chongqing Medical University, No. 1 Youyi Road, Yuzhong District, Chongqing, China

ARTICLE INFO

Keywords:
Bexarotene
Traumatic brain injury
Neuron
Astrocyte
Microglia
Peroxisome proliferator-activated receptor gamma

ABSTRACT

Traumatic brain injury (TBI) causes a high rate of mortality and disability worldwide, and there exists almost none effective drugs to protect against TBI. Neurotoxicity occurring after TBI can be derived from microglia and astrocytes, and causes neuronal death and synapse loss. Bexarotene has been demonstrated to protect neurons in CNS diseases. In the present study, we aimed to investigate the potential role of bexarotene in protecting against neurotoxicity after TBI, as well as the underlying mechanism. The controlled cortical impact (CCI) model was established on adult C57BL/6 mice, followed by intraperitoneal administration of bexarotene for 14 consecutive days. We found that bexarotene improved sensorimotor function and cognitive recovery in CCI mice. In addition, bexarotene decreased neuronal death and synapse loss, as well as inhibited apoptotic cascade. Moreover, bexarotene treatment reduced M1 microglia polarization, microglia-derived pro-inflammatory cytokines, and the number of A1 astrocytes after CCI. These effects of bexarotene were partially abolished by T0070907, an antagonist of peroxisome proliferator–activated receptor gamma (PPARγ). Additionally, bexarotene enhanced nuclear translocation and transcriptional activity of PPARγ. These findings show that bexarotene inhibits neurotoxicity in mice after TBI, at least in part through a PPARγ-dependent mechanism.

1. Introduction

Traumatic brain injury (TBI) has so far been regarded as one of the most severe, disabling neurological disorder worldwide (Xiong et al. 2012). The primary damage occurred at the time of injury, while the secondary damage last for days, weeks, months or even years (Maas et al. 2017). There is increasing evidence showing that TBI may leads to a long-term risk for neurodegenerative processes and cognitive impairment, and is associated with persistent microglia activation and inflammatory state (Johnson et al. 2013; Ramlackhansingh et al. 2011). The neurotoxicity from glias has been shown to cause neuronal death and synapse loss (Liddelow et al. 2017; Wang et al. 2017). Though new therapeutic targets have been identified to treat TBI, clinical trials are still in their infancy.

Being a member of the nuclear hormone receptor superfamily, peroxisome proliferator–activated receptor gamma (PPARγ) exerts neuroprotective effects in CNS diseases, in term of attenuating neuroinflammation, decreasing excitotoxicity, and inhibiting neuronal death (Cai et al. 2017; Drew et al. 2015; Sauerbeck et al. 2011). PPARγ can bind to other nuclear receptors, like retinoid X receptor (RXR), in the form of heterodimer (Marciano et al. 2015). Agonists of either RXR or PPARγ can activate the RXR/PPARγ heterodimer to regulate the expressions of target genes (Bai et al. 2007; le Maire et al. 2009).

Bexarotene is a highly selective RXR agonist, previously approved by FDA as an antineoplastic agent (Cramer et al. 2012). Recent evidences showed that peripheral bexarotene treatment plays protective roles in CNS diseases (Cramer et al. 2012; Huuskonen et al. 2016; Riancho et al. 2015; Zhong et al. 2017a). However, it is unclear whether bexarotene has any effects on PPARγ and neurotoxicity after TBI. In this study, we aimed to investigate the potential role of bexarotene in modulating neurotoxicity after TBI, as well as the underlying mechanism.

2. Materials and methods

2.1. Controlled cortical impact model in mice

A total of 217 male C57BL/6 mice (8–12 weeks old), weighing 18–22 g, were housed on a 12-h light/dark cycle with food and water available *ad libitum*, and anesthetized using 3% isoflurane in 67% N_2O/

* Corresponding authors.
E-mail addresses: sunxch1445@qq.com (X. Sun), 358187887@qq.com (C. Cheng).

https://doi.org/10.1016/j.nbd.2018.06.003
Received 9 October 2017; Received in revised form 5 May 2018; Accepted 6 June 2018
Available online 07 June 2018

30% O_2 until they were unresponsive to the tail pinch test. Then 1.5% isoflurane was used for anesthesia maintenance. TBI was induced in mice with a controlled cortical impact (CCI) device (Precision Systems and Instrumentation) as described previously (Huang et al. 2016). A 5-mm diameter craniotomy was performed in the right bone at 2.0 mm posterior to bregma and 1.0 mm lateral to the midline using a drill. A 3.0 mm-diameter impactor tip compressed the brain to a depth of 1.5 mm below the dura at a speed of 4.0 m/s with a dwell time of 200 ms to mimic a moderate TBI. Body temperature of these mice was maintained at 37 °C during the whole procedure. All animal experiments in this study were approved by Experimental Animal Center of Chongqing Medical University, in Chongqing, China and adhered to the Animal Research: Reporting *In Vivo* Experiments (ARRIVE) ethical guidelines.

2.2. Drug administration

Bexarotene was purchased from MedChem Express (USA) and dissolved as previously described (Zhong et al. 2017a). A potent and selective PPARγ antagonist T0070907, with the molecular weight of 277.67 Da, was widely used in CNS diseases (including CCI) for PPARγ inhibition, in the manner of peripheral administration (Pan et al. 2015; Sauerbeck et al. 2011; Thal et al. 2011; Villapol et al. 2015). T0070907 has been reported to function in neurons, microglia, astrocytes, leukocytes (Pan et al. 2015; Raman et al. 2012; Shibata et al. 2009; Zhao et al. 2017). In the present study, T0070907 (MedChem Express, USA) was firstly dissolved in DMSO (50 mM) and further dissolved in phosphate buffered saline (PBS) (1 mM). Bexarotene solution (5 mg/kg) or the equal volume of vehicle (prepared as bexarotene) was administered intraperitoneally 2 h after CCI for the first time, followed by daily injection for 14 consecutive days. T0070907 solution (2 mg/kg) was administrated intraperitoneally 1 h after CCI, followed by daily injection for 14 consecutive days.

2.3. Western blot

On the 1st, 3rd, 7th, and 14th days after CCI, mice were deeply anesthetized and perfused with PBS. Ipsilateral brains were rapidly dissected out. Primary antibodies used included rabbit anti- complement 3 (C3) (1:1000, Abcam, UK), rabbit anti-myxovirus resistance 1 (MX1) (1:200, Proteintech, China), rabbit anti-cleaved caspase-3 (1:2000, Abcam, UK), rabbit anti-Bcl-2 (1:500, Wanleibio, China), rabbit anti-Bcl-2 associated X protein (Bax) (1:1000, Abcam, UK), rabbit anti- synaptophysin (1:20,000, Abcam, UK), rabbit anti- post synaptic density protein 95 (PSD95) (1:1000, Abcam, UK), rabbit anti-RXRα (1:400, Proteintech, China), and rabbit anti-PPARγ (1:1000, Proteintech, China) antibody. Besides, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:2000, Proteintech, China), β-actin (1:2000, Proteintech, China) and histone 3 (1:5000, Proteintech, China) were used as loading controls for whole cell, cytoplasmic proteins and nuclear proteins, respectively. Western blot was performed following a standard technique as previously described (Teng et al. 2016). Blots were detected by enhanced chemiluminescence and quantified using Image J software (NIH, USA).

2.4. Immunohistochemistry

On the 14th day after CCI, mice were deeply anesthetized and perfused with PBS and 4% paraformaldehyde for perfusion-fixation. Isolated brains were post-fixed in 4% paraformaldehyde for 72 h, embedded in OCT compound for frozen section, and cut into 20 μm-thick coronal sections, and then immunolabeled with fluorescence according to standard indirect techniques as previously described (Han et al. 2015). Primary antibodies used include mouse anti-glial fibrillary acidic protein (GFAP) (1:500, Proteintech, China), rabbit anti-MX1 (1:50, Proteintech, China), goat anti-ionized calcium-binding adaptor molecule 1 (Iba1) (1:200, Abcam, USA), rabbit anti-cluster of differentiation 16 (CD16) (1:200, Abcam, UK), rabbit anti-cleaved caspase-3 (1:150, Abcam, USA), mouse anti-neuronal nuclei (NeuN) (1:200, Novus, USA), rabbit anti-synaptophysin (1:100, Abcam, UK), and rabbit anti-PSD95 (1:50, Abcam, UK). The number of GFAP positive cells, $MX1^+/GFAP^+$ cells, $CD16^+/Iba1^+$ cells, cleaved caspase-3 positive cells, and NeuN positive cells in ipsilateral cortex was counted respectively in five randomly selected fields (400×) for each section using Image J software. Three coronal sections of one brain were imaged.

2.5. Quantitative real-time polymerase chain reaction (qRT-PCR)

qRT-PCT was performed as described previously (Zhong et al. 2017b). The total RNA was extracted using the Trizol reagent (Invitrogen) and quantified by spectrophotometry. cDNA was prepared using a cDNA Synthesis SuperMix (Biotool, Houston, TX, USA) according to manufacturer's instructions. RT-PCR was performed using quantitative PCR (ABI 7500, Thermo Fisher Scientific, Waltham, MA, USA) in the presence of a fluorescent dye (SYBR Green; Biotool, Houston, TX, USA). The Ct value was normalized to GAPDH of the same sample. The relative expression levels were quantified using the $2^{-\Delta\Delta Ct}$ method. The forward and reverse primer sequences for each gene (Sangon Biotech, shanghai, China) are as follows:

CD16: F: TTT GGA CAC CCA GAT GTT TCA G,
R: GTC TTC CTT GAG CAC CTG GAT C;
CD32: F: AAT CCT GCC GTT CCT ACT GAT C,
R: GTG TCA CCG TGT CTT CCT TGA G.
iNOS: F: CAG CTG GGC TGT ACA AAC CTT,
R: CAT TGG AAG TGA AGC GTT TCG;
TNF-α: F: CAA GGG ACA AGG CTG CCC CG,
R: GCA GGG GCT CTT GAC GGC AG;
IL-1α: F: ACG TCA AGC AAC GGG AAG AT,
R: AAG GTG CTG ATC TGG GTT GG;
C1qα: F: GAA AGG CAA TCC AGG CAA TA,
R: ATG GAC TCT CCT GGT TGG TG;

2.6. H&E staining

On the 21st day after CCI, mice were deeply anesthetized and perfused transcardially with 0.1 PBS, pH 7.4, followed by 4% paraformaldehyde in PBS. The brains were then removed, post-fixed in 4% paraformaldehyde at 4 °C for 48 h, processed into paraffin blocks, and dissected into sections of 10 μm every 20 μm. Each section then was deparaffinized, hydrated, washed, and stained with Hematoxylin-eosin (H&E) staining. The lesion area was calculated as: the area of the ipsilateral hemisphere subtracted from the area of the contralateral hemisphere. The lesion volume was presented as a volume percentage of the lesion compared to the contralateral hemisphere.

2.7. Isolation of microglia from mouse brain

On the 3rd, 7th, and 14th days after CCI, ipsilateral brains were harvested from mice perfused with ice-cold PBS, minced into 2–4 mm pieces using scissors, added with trypsin, and incubated at 37 °C for 20 min. To eliminate clumps and debris, cells were dispersed by gentle pipetting and filter through a 70 μm cell strainer and the cell suspension was collected and centrifuged for 7 min (300 *g*/min) at 18 °C. Then the cell pellet was resuspended with PBS. Cell count and viability analysis were performed. Cells were stained with anti-mouse CD11b-APC (eBioscience, San Diego, CA, USA) at 4 °C for 30 min, washed with magnetic buffer, and incubated with 50 μl of magnetic anti-APC particles (BD biosciences, USA) at room temperature for 30 min. Then The mixture was added with magnetic buffer, placed onto the BD Imagnet™, and incubated for 8 min. After the supernatant was removed, the rest positive fraction was used for following Western blot and qRT-PCR.