Biochemical and Biophysical Research Communications 472 (2016) 648–655

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Rosiglitazone attenuates inflammation and CA3 neuronal loss following traumatic brain injury in rats

Hao Liu [a, b], Marie E. Rose [a, b], Sherman Culver [a, c, d], Xiecheng Ma [a, c, d], C. Edward Dixon [a, c, d], Steven H. Graham [a, b, *]

[a] Geriatric Research Educational and Clinical Center, V.A. Pittsburgh Healthcare System, PA, USA
[b] Department of Neurology, University of Pittsburgh School of Medicine, PA, USA
[c] Department of Neurosurgery, University of Pittsburgh, PA 15216, USA
[d] Department of Critical Care Medicine, University of Pittsburgh, PA 15216, USA

ARTICLE INFO

Article history:
Received 23 February 2016
Accepted 3 March 2016
Available online 3 March 2016

Keywords:
Traumatic brain injury
PPARγ
Inflammation
Rosiglitazone
Behavioral
Cell survival

ABSTRACT

Rosiglitazone, a potent peroxisome proliferator-activated receptor (PPAR)-γ agonist, has been shown to confer neuroprotective effects in stroke and spinal cord injury, but its role in the traumatic brain injury (TBI) is still controversial. Using a controlled cortical impact model in rats, the current study was designed to determine the effects of rosiglitazone treatment (6 mg/kg at 5 min, 6 h and 24 h post injury) upon inflammation and histological outcome at 21 d after TBI. In addition, the effects of rosiglitazone upon inflammatory cytokine transcription, vestibulomotor behavior and spatial memory function were determined at earlier time points (24 h, 1–5 d, 14–20 d post injury, respectively). Compared with the vehicle-treated group, rosiglitazone treatment suppressed production of TNFα at 24 h after TBI, attenuated activation of microglia/macrophages and increased survival of CA3 neurons but had no effect on lesion volume at 21 d after TBI. Rosiglitazone-treated animals had improved performance on beam balance testing, but there was no difference in spatial memory function as determined by Morris water maze. In summary, this study indicates that rosiglitazone treatment in the first 24 h after TBI has limited anti-inflammatory and neuroprotective effects in rat traumatic injury. Further study using an alternative dosage paradigm and more sensitive behavioral testing may be warranted.

Published by Elsevier Inc.

1. Introduction

Within minutes of TBI, numerous inflammatory mechanisms are triggered that lead to activation of microglia, migration of inflammatory macrophages into brain and expression of a host of cytokines including tumor necrosis factor (TNF)α, interleukins (IL), matrix metallopeptidases (MMP), and iNOS [1–3]. These mechanisms may exacerbate acute injury but may also be important in repair and recovery of function after TBI [4]. Alteration of this immune response to favor later beneficial effects of inflammation after TBI may be a valuable strategy to improve functional recovery.

The nuclear PPARγ receptor regulates transcription of a number of genes that promote the resolution of inflammation and healing [5]. PPAR γ expression is upregulated after TBI and may play a role in recovery after TBI [6,7]. A number of specific synthetic PPARγ agonists have been developed that have potent effects upon lipid metabolism in adipocytes that makes them useful as anti-diabetic drugs. Rosiglitazone is an FDA-approved drug with few short-term side effects making it an excellent candidate for rapid translation to clinical trials. It has been shown to have potent protective effects in models of stroke [5,8,9], neurodegenerative diseases [10,11] and spinal cord injury [12,13], but less is known about the effect in TBI. Previous studies have shown that rosiglitazone reduces neuroinflammation, lesion volume, and oxidative stress and apoptotic markers acutely in mouse models of TBI [6,14]. Some studies have shown short-term improvements in motor behavior, but longer term behavioral effects of rosiglitazone treatment have not been reported [14].

The current study aims to determine the effects of rosiglitazone treatment upon microglial/macrophage activation, and histological outcome at 21 d after TBI in rats. In addition, the effect of

* Corresponding author. Geriatric Research Educational and Clinical Center, VA Pittsburgh Healthcare Center Research, Office Building #30, Mail Code 151 University Drive, Pittsburgh, PA 15240, USA.
E-mail address: Steven.Graham@va.gov (S.H. Graham).

http://dx.doi.org/10.1016/j.bbrc.2016.03.003
0006-291X/Published by Elsevier Inc.

rosiglitazone treatment upon vestibulomotor behavior and spatial memory function was evaluated. The effect of rosiglitazone treatment upon acute cytokine transcription was also examined.

2. Materials and methods

Animal studies were performed with the approval of the University of Pittsburgh Institutional Animal Care and Use Committee and conducted according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Animals were housed in a temperature and humidity controlled environment with 12 h light cycles and free access to food and water.

Chemicals: Unless otherwise noted, chemicals were purchased from Sigma Aldrich (St. Louis, MO).

2.1. Traumatic brain injury

The controlled cortical impact model of TBI used in this study was performed as previously described [15,16]. Rats were anesthetized in 5% isoflurane in 60% nitrous oxide, balance oxygen, then endotracheally intubated, and maintained on a small-animal ventilator (Harvard Apparatus, Holliston, MA) at 1–2% isoflurane in the same carrier gas mixture. After craniectomy, the impactor tip (6 mm) was zeroed to the surface of the brain prior to injury (impact speed: 4 m/sec, injury depth: 2.7 mm, dwell time: 50 msec). Following TBI, anesthesia was discontinued, the incision was closed and the animals were ventilated with 100% oxygen until spontaneous return of respiration. Sham surgery was identical to that described above without trauma. Rats were administered 6 mg/kg rosiglitazone (Cayman Chemical, Ann Arbor, MI) diluted in DMSO and PBS (1:3) via intraperitoneal injection (IP) at 5 min, 6 h and 24 h post TBI or vehicle only (DMSO/PBS 1:3). n = 15 per group.

2.2. Vestibular motor function

Beam Balance and Beam Walking tests were performed post day injury 1–5 with training prior to injury on Day 0. Gross vestibulomotor function was assessed using a beam-balance task on days 0–5 post TBI [17]. The animal was placed on a suspended, narrow wooden beam (1.5 cm wide) 30 inches above a padded surface and latency on the beam was measured, up to 60 s. Three trials per animal per day were performed with a 30 s intertrial rest period. Finer components of vestibulomotor function and coordination were assessed using a modified beam-walking task. On the day prior to injury the rats were trained to escape a bright light and loud white noise by traversing a narrow wooden beam (2.5 × 100 cm), and entering a darkened goal box at the opposite end. Four pegs (3 mm diameter and 4 cm high) were equally spaced along the center of the beam to increase the difficulty of the task. Performance was assessed by measuring the latency to traverse the beam. If the rats did not cross the beam in 60 s or fell off, the light and noise were stopped and the rat was placed in the goal box.

2.3. Spatial learning

Spatial learning was assessed by Morris water maze performance as previously described [18]. A large circular tank 180 cm in diameter and 45 cm high was filled with water maintained at 26 ± 1 °C to a height of 30 cm containing a transparent circular platform (10 cm in diameter and 29 cm high) located in a fixed position in the tank 45 cm from the tank wall and 1 cm below the water surface. Extra-maze visual cues aid the rat in locating the escape platform. Animals underwent cognitive performance evaluation by placing them in the MWM on post-injury day (PID) 14 without prior training or exposure to the MWW, for five consecutive days (4 trials/day) with a hidden goal platform. Rats were randomly placed in the water against and facing the tank wall, and then released to swim freely about the tank to find the hidden platform, up to 120 s. If the rat was unable to locate the platform within the allotted time, it was manually directed to the platform. After a 4 min intertrial interval, the animal was returned to the maze for the next trial. On PID 19, the rats underwent a probe trial where the platform was removed from the maze and time spent in the "target quadrant" (the quadrant where the platform was previously located) was measured using a video-tracking system (AnyMaze, Stoelting, Inc. Wood Dale, IL) for 120 s. This was then compared with the time spent in the remaining three quadrants. A visible platform task was performed on PID 19–20 to evaluate nonspecific visual deficits. The platform was raised to 1 in. above the water level and the rats were released from each of four randomized starting positions to locate, swim to, and mount the visible platform. Latency in finding the platform was measured.

2.4. Cell survival measurement

Rats were anesthetized with 4% isoflurane in N_2O/O_2 (2:1) and transcardially perfused with 200 mL heparinized saline and 200 mL 10% buffered formalin 21 days post TBI. Whole brains were embedded in paraffin and cut in 7 μm thick sections every 1 mm. Sections were stained with hematoxylin and eosin and hippocampi were examined using 20× brightfield magnification. Morphologically normal neurons (those with visible nucleoli) were counted in ipsilateral and contralateral CA1, CA3 and CA4 in slices located in the center of the contusion (3.60 mm from Bregma). Data are expressed as percent contralateral.

2.5. Assessment of lesion volume

Brain lesion volumes were calculated by measuring ipsilateral and contralateral hemispheric areas at each slice then multiplying slice area X slice interval thickness and adding together all slices [18]. Ipsilateral lesion volumes are expressed as percent contralateral and are calculated as follows: (contralateral-ipsilateral)/contralateral × 100.

2.6. IBA-1 immunostaining assessment

IBA-1 immunofluorescent staining was performed as previously described [19]. Brain sections taken at the center of the lesion were immunostained with anti-IBA-1 antibody (1:250, WAKO Chemicals, Richmond, VA) then incubated with AlexaFluor 488-conjugated secondary antibody (Life Technologies, Grand Island, NY). Brains sections were photographed using an Olympus BX51 microscope and Stereo Investigator software. IBA-1-positive cells were counted in 400 × 800 pixel fields located in CA1 and CA3 hippocampus, dorsal thalamus, and peri-contusional cortex using ImageJ software (U. S. National Institutes of Health, Bethesda, MD). Brain sections incubated without application of primary antibody served as controls.

2.7. RNA extraction and Quantitative real-time Polymerase chain reaction (qPCR) analysis

A separate cohort of rats underwent TBI and drug administration as described above (n = 6 per group) and were sacrificed 24 h after injury. Total brain tissue RNA and protein was extracted using a PARIS kit (Invitrogen, Grand Island, NY). Extracted RNA was treated with DNase to remove contaminating DNA. RNA concentrations were measured, and first-strand cDNA was synthesized using 5 μg of total RNA with a SuperScript III first-strand cDNA