Experimental Neurology 261 (2014) 396–403

Contents lists available at ScienceDirect

Experimental Neurology

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

Regular Article

Cannabinoid receptor type 2 agonist attenuates apoptosis by activation of phosphorylated CREB–Bcl-2 pathway after subarachnoid hemorrhage in rats

Mutsumi Fujii [a], Prativa Sherchan [a], Yoshiteru Soejima [a], Yu Hasegawa [a], Jerry Flores [a], Desislava Doycheva [a], John H. Zhang [a,b,c,*]

[a] Department of Physiology, Loma Linda University, Loma Linda, CA, USA
[b] Department of Neurosurgery, Loma Linda University, Loma Linda, CA, USA
[c] Department of Anesthesiology, Loma Linda University, Loma Linda, CA, USA

ARTICLE INFO

Article history:
Received 8 May 2014
Revised 27 June 2014
Accepted 10 July 2014
Available online 22 July 2014

Keywords:
Cannabinoid receptor type 2
JWH133
Subarachnoid hemorrhage
Apoptosis
Brain edema
CREB

ABSTRACT

Early brain injury (EBI) which comprises of vasogenic edema and apoptotic cell death is an important component of subarachnoid hemorrhage (SAH) pathophysiology. This study evaluated whether cannabinoid receptor type 2 (CB2R) agonist, JWH133, attenuates EBI after SAH and whether CB2R stimulation reduces pro-apoptotic caspase-3 via up-regulation of cAMP response element-binding protein (CREB)-Bcl-2 signaling pathway. Male Sprague–Dawley rats (n = 123) were subjected to SAH by endovascular perforation. Rats received vehicle or JWH133 at 1 h after SAH. Neurological deficits and brain water content were evaluated at 24 h after SAH. Western blot was performed to quantify phosphorylated CREB (pCREB), Bcl-2, and cleaved caspase-3 levels. Neuronal cell death was evaluated with terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end-labeling staining. Additionally, CREB siRNA was administered to manipulate the proposed pathway. JWH133 (1.0 mg/kg) improved neurological deficits and reduced brain water content in left hemisphere 24 h after SAH. JWH133 significantly increased activated CREB (pCREB) and Bcl-2 levels and significantly decreased cleaved caspase-3 levels in left hemisphere 24 h after SAH. CREB siRNA reversed the effects of treatment. TUNEL positive neurons in the cortex were reduced with JWH133 treatment. Thus, CB2R stimulation attenuated EBI after SAH possibly through activation of pCREB–Bcl-2 pathway.

Introduction

Subarachnoid hemorrhage (SAH), with a case fatality rate of around 50%, occurs in approximately 85% cases due to rupture of an intracranial aneurysm (van Gijn et al., 2007). Aneurysmal rupture initiates global ischemia due to elevated intracranial pressure and reduced cerebral blood flow which leads to early brain injury (EBI). EBI is compounded by inflammation, oxidative stress, and ionic disturbances, which leads to blood–brain barrier (BBB) disruption and subsequent vasogenic edema as well as neuronal apoptosis resulting in neurological deterioration after SAH (Fujii et al., 2013). Therefore, therapies that decrease BBB disruption and apoptosis may provide beneficial effects for EBI following SAH (Park et al., 2004; Zhang et al., 2012).

Recent studies have demonstrated neuroprotective properties of drugs based on marijuana-derived cannabinoids (Cohen-Yeshurun et al., 2011). Cannabinoid receptors include the cannabinoid receptor type 1 (CB1R) and cannabinoid receptor type 2 (CB2R), which are G protein-coupled receptors expressed by neurons, vascular endothelial cells, and immune cells (Glass and Northup, 1999; Ramirez et al., 2012). While CB1R activation accounts for the psychoactive effects of cannabinoids (Glass and Northup, 1999), CB2R agonist showed neuroprotective effects against experimental ischemic stroke (Murikinati et al., 2010; Zarruk et al., 2012), encephalitis (Ramirez et al., 2012), traumatic brain injury (Amenta et al., 2012), and in a model of remote

Abbreviations: EBI, early brain injury; SAH, subarachnoid hemorrhage; CB2R, cannabinoid receptor type 2; CREB, cAMP response element-binding protein; siRNA, small interfering ribonucleic acid; pCREB, phosphorylated CREB; BBB, blood–brain barrier; CB1R, cannabinoid receptor type 1; ERK, extracellular signal-regulated kinase; AMPK, AMP-activated protein kinase; BWC, brain water content; TUNEL, terminal deoxynucleotidyl transferase-mediated uridine 5′-triphosphate-biotin nick end-labeling; ANOVA, analysis of variance; SD, standard deviation; cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PI3K, phosphatidylinositol 3-kinase; WB, western blot analysis; IHC, immunohistochemistry; ICV, intracerebroventricular infusion.

* Corresponding author at: Department of Neurosurgery, Loma Linda University Medical Center, 11234 Anderson Street, Loma Linda, CA 92354, USA. Fax: +1 909 558 0119.
E-mail address: johnzhang3910@yahoo.com (J.H. Zhang).

http://dx.doi.org/10.1016/j.expneurol.2014.07.005
0014-4886/

cell death through its anti-inflammatory and anti-apoptotic properties (Viscomi et al., 2009).

Previous studies show that CB2R activation enhances the phosphorylation of extracellular signal-regulated kinase (ERK) 1/2 (Cohen-Yeshurun et al., 2011; Ofek et al., 2011) and AMP-activated protein kinase (AMPK) (Choi et al., 2013). Once phosphorylated, ERK and AMPK can in turn phosphorylate and thereby activate cAMP response element-binding protein (CREB) (Choi et al., 2013; Ofek et al., 2011). CREB is a transcription factor that promotes cell survival by up-regulating Bcl-2 expression and inhibiting caspase-3 activation (Meller et al., 2005; Tokudome et al., 2004; Wilson et al., 1996). Although apoptosis plays an important role in the pathophysiology of EBI after SAH, the specific anti-apoptotic role of CB2R after SAH has not been explored.

In this study we tested two hypotheses: (A) CB2R agonist, JWH133, attenuates EBI and improves neurological function after SAH. (B) CB2R stimulation activates CREB–Bcl-2 signaling pathway, which decreases pro-apoptotic caspase activation.

Materials and methods

Animals and pharmacological interventions

This experiment was a controlled in vivo laboratory study conducted in an animal research laboratory. All protocols were approved by the Institutional Animal Care and Use Committee at Loma Linda University in accordance with the National Institute of Health guidelines. Adult male Sprague–Dawley rats (Harlan, IN) weighing 226–338 g were housed with a 12/12 hour light/dark cycle in a temperature and humidity controlled environment.

SAH rats received vehicle (0.2 ml of ethanol with 1.8 ml of 0.9% saline) or the selective CB2R agonist JWH133 (0.3, 1.0, or 3.0 mg/kg, Tocris Bioscience, MN; *Ki* value = 3.4 nM), which was dissolved in the vehicle, by intraperitoneal injection at 1 h after SAH. The dose of JWH133 was selected based on previous publication (Murikinati et al., 2010). To ensure that the anti-apoptotic effects of CB2R activation were mediated by phosphorylation of CREB, we injected CREB small interfering ribonucleic acid (siRNA) via intracerebroventricular route to deactivate CREB 24 h before SAH-induction surgery. SAH rats were injected with control siRNA or CREB siRNA and were divided into cont-JWH and siRNA-JWH groups.

We used 123 animals in this study. The rats were randomly assigned to the following groups: SAH + vehicle in physiological parameter study (n = 5), SAH + JWH133 (1.0) in physiological parameter study (n = 5), sham-operated (sham group: n = 15), SAH + vehicle (vehicle group: n = 23), SAH + JWH133 (0.3) (low-JWH group: n = 10), SAH + JWH133 (1.0) (JWH group: n = 25), SAH + JWH133 (3.0) (high-JWH group: n = 10), control siRNA with SAH + JWH133 (1.0) (cont-JWH group: n = 7), and CREB siRNA with SAH + JWH133 (1.0) (siRNA-JWH group: n = 7). Sixteen rats died within 1 h after SAH during which time the rats had not received either the drug or the vehicle yet.

SAH rat model

SAH was induced by the endovascular perforation method as previously described (Bederson et al., 1995; Suzuki et al., 2010). Briefly, rats were anesthetized, intubated and kept on artificial ventilation during surgery with 3% isoflurane in 70%/30% medical-air/oxygen. Normothermia was maintained by a heating lamp. A sharpened 4-0 nylon suture was introduced into the left internal carotid artery until resistance was felt (approximately 18 mm from the common carotid bifurcation). The suture was advanced to perforate the bifurcation of the anterior cerebral artery and middle cerebral artery until resistance was overcome after which the suture was immediately withdrawn. In sham-operated animals, the suture was inserted into the left internal carotid artery and then removed without perforating the artery. The skin incision was closed, and rats were individually housed in cages with temperature maintained at 98 °F by a heating pad until recovery. Buprenorphine (0.01 mg/kg) was administered subcutaneously for post-operative analgesia.

Physiological parameters

The right femoral artery was cannulated for continuous measurement of mean arterial blood pressure, heart rate and for blood sampling as previously described (Fujii et al., 2012). Arterial blood gases, pH and serum glucose were measured 15 min before, immediately after, and every 30 or 60 min after SAH. The monitoring was continued for 120 min after SAH induction.

SAH grade

The SAH severity was evaluated in a blinded manner as previously described (Sugawara et al., 2008). Briefly, picture of the base of the brain was taken and divided into six segments. Each segment was sub-scored (0 to 3) depending on the amount of blood in the subarachnoid space and a total score was calculated as the sum of all sub-scores. The score ranged from 0 (no SAH) to 18 (severe SAH). Animals with SAH score of 7 or less were excluded from the study as previously described (Sugawara et al., 2008).

Neurological score

Neurological deficits were evaluated blindly using modified Garcia test (Garcia et al., 1995; Sugawara et al., 2008). The test consisted of six parameters which included spontaneous activity; symmetry in the movement of all four limbs; forepaw outstretching; climbing; body proprioception; and response to vibrissae stimulation. The total score ranged from 2 to 18 (Fujii et al., 2012). Neurological score was evaluated at 24 h after sham or SAH-induction surgery. Additionally, neurological test was performed before the SAH-induction surgery and at 24 h after the surgery in rats allocated to intracerebroventricular injection groups (cont-JWH and siRNA-JWH group).

Brain water content measurement

Brain water content was measured 24 h after SAH. Rats were sacrificed under lethal isoflurane anesthesia, and brains were quickly removed and then separated into left hemisphere, right hemisphere, cerebellum, and brain stem to measure brain edema as previously described (Suzuki et al., 2010). Each part was weighed immediately after removal (wet weight) and after drying at 100 °C for 72 h (dry weight). The percentage of brain water content (BWC) was calculated as [(wet weight − dry weight) / wet weight] × 100%.

Intracerebroventricular infusion

Under 3% isoflurane anesthesia, the needle of a 10-μL Hamilton syringe (Microliter No. 701; Hamilton Company, NV) was inserted through a burr hole on the skull into the left lateral ventricle according to the following coordinates relative to bregma: 1.5 mm posterior, 0.8 mm lateral, and 4.2 mm below the horizontal plane as previously described with modification (Peters et al., 2009; Suzuki et al., 2010). CREB-1 siRNA or an irrelevant control siRNA, 500 pmol each in 1 μL siRNA dilution buffer (Santa Cruz Biotechnology, CA) was injected by a microinfusion pump (Harvard Apparatus, MA) at a rate 0.5 μL/min at 24 h before SAH-induction. The needle was removed 20 min after the end of infusion, and the burr hole was plugged with bone wax. All the rats received JWH133 (1.0 mg/kg) intraperitoneally 1 h after SAH.