Experimental Neurology 307 (2018) 82–89

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Neurotrophin-3 provides neuroprotection via TrkC receptor dependent pErk5 activation in a rat surgical brain injury model

Onat Akyol[a], Prativa Sherchan[a], Gokce Yilmaz[a], Cesar Reis[a], Wingi Man Ho[a], Yuechun Wang[a], Lei Huang[b], Ihsan Solaroglu[c], John H. Zhang[a,b,d,*]

[a] Department of Physiology and Pharmacology, Loma Linda University, Loma Linda, CA 92354, USA
[b] Department of Neurosurgery, Loma Linda University, CA 92354, USA
[c] Koç University, School of Medicine, Department of Neurosurgery, Rumelifeneri Yolu, 34450 Sarıyer, Istanbul, Turkey
[d] Department of Anesthesiology, Loma Linda University, CA 92354, USA

ARTICLE INFO

Keywords:
Surgical brain injury
Brain edema
Neurotrophin 3
Tropomyosin related kinase receptor C
Extracellular signal related kinase 5

ABSTRACT

Background: Surgical brain injury (SBI) which occurs due to the inadvertent injury inflicted to surrounding brain tissue during neurosurgical procedures can potentiate blood brain barrier (BBB) permeability, brain edema and neurological deficits. This study investigated the role of neurotrophin 3 (NT-3) and tropomyosin related kinase receptor C (TrkC) against brain edema and neurological deficits in a rat SBI model.

Methods: SBI was induced in male Sprague Dawley rats by partial right frontal lobe resection. Temporal expression of endogenous NT-3 and TrkC was evaluated at 6, 12, 24 and 72 h after SBI. SBI rats received recombinant NT-3 which was directly applied to the brain surgical injury site using gelfoam. Brain edema and neurological function was evaluated at 24 and 72 h after SBI. Small interfering RNA (siRNA) for TrkC and Rap1 was administered via intracerebroventricular injection 24 h before SBI. BBB permeability assay and western blot was performed at 24 h after SBI.

Results: Endogenous NT-3 was decreased and TrkC expression increased after SBI. Topical administration of recombinant NT-3 reduced brain edema, BBB permeability and improved neurological function after SBI. Recombinant NT-3 administration increased the expression of phosphorylated Rap1 and Erk5. The protective effect of NT-3 was reversed with TrkC siRNA but not Rap1 siRNA.

Conclusions: Topical application of NT-3 reduced brain edema, BBB permeability and improved neurological function after SBI. The protective effect of NT-3 was possibly mediated via TrkC dependent activation of Erk5.

1. Introduction

Neurosurgery is one of the most practiced surgical procedures in daily hospital care either in emergency or elective situations. Neurosurgical procedures can cause inevitable injury to surrounding neural tissue due to trauma inflicted by the surgical technique itself. Additionally, the proximity of vulnerable anatomic regions in the central nervous system to the surgical site poses a risk of injury to the normal surrounding tissue during surgeries (Jadhav and Zhang, 2008). Especially after brain tumor surgeries, neural tissue close to the surgical removal area can have tissue edema which aggravates postoperative neurologic deficits depending on both the surgical location and severity of surgery. In a pre-clinical setting, the surgical brain injury (SBI) rat model, which involves a right partial frontal lobe resection has been established to demonstrate brain injury associated with neurosurgical procedures (Jadhav et al., 2007; Sulejczak et al., 2008).

Endogenous growth factors in recombinant form have been used as neuroprotective molecules in various brain injury and stroke models (Ren and Finklestein, 2005). Neurotrophins are endogenous polypeptide growth factors that play crucial roles during neuronal growth and differentiation primarily by acting on their specific tyrosine kinase receptors. Mitogen activated protein kinase (MAPK) cascade is the essential downstream pathway for neurotrophins functioning inside the cell (Cai et al., 2014). Neurotrophin-3 (NT-3) is one of the members of

Abbreviations: SBI, Surgical brain injury; BBB, Blood brain barrier; NT-3, Neurotrophin 3; TrkC, Tropomyosin related kinase receptor C; MAPK, Mitogen activated protein kinase; Erk5, Extracellular signal-regulated kinase 5; BDNF, Brain derived neurotrophic factor; ICV, Intracerebroventicular; GFAP, Glial Fibrillary Acidic Protein; vWF, von Willebrand Factor; NeuN, Neuronal Nuclei; TBI, Traumatic brain injury; GTPase, Guanine triphosphatase

* Corresponding author at: Departments of Anesthesiology and Physiology, Loma Linda University School of Medicine, Risley Hall, Room 219, 11041 Campus Street, Loma Linda, CA 92354, USA.
E-mail addresses: johnzhang3910@yahoo.com, jhzhang@llu.edu (J.H. Zhang).

https://doi.org/10.1016/j.expneurol.2018.06.002
Received 2 January 2018; Received in revised form 14 May 2018; Accepted 4 June 2018
Available online 05 June 2018
0014-4886/

the neurotrophin family which shows a higher expression profile throughout embryogenesis period and thereafter declines during adulthood. Topical application of NT-3 has previously been shown to have neuroprotective properties in cerebral ischemia and spinal cord injury animal models (Zhang et al., 1999; Sharma, 2007).

Neurotrophin-3 binds to tropomyosin related kinase receptor C (TrkC), a receptor with tyrosine kinase activity, to exert its effects. Rap1 is a small G protein that mediates neurotrophin signaling involved in neuronal growth and polarization, and it has been shown to function as an upstream of MAPK cascade with the binding of NT-3 to its receptor TrkC (York et al., 1998; Je et al., 2006). Extracellular signal-regulated kinase 5 (ERK5) is a member of MAPKs family with slight structural differences from other classical MAPKs and has a role in cell survival and neuronal differentiation (Nishimoto et al., 2005; Nishimoto and Nishida, 2006). Previous study indicated the involvement of endothelial Rap1-Erk5 interaction during angiogenesis (Doebele et al., 2009) and brain derived neurotrophic factor (BDNF) activated Erk5 via RAP1 mediated signaling in cortical neurons (Wang et al., 2006). However, there is no research on the association of NT-3 and Rap1-Erk5 in brain injury models including SBI.

The objective of the current study was to evaluate the blood brain barrier (BBB) protective effects of NT-3 and to evaluate the contribution of its receptor TrkC and Rap1-Erk5 pathway in the protective effects of NT-3 in a SBI rat model.

2. Materials and methods

2.1. Animals and surgical brain injury model

All procedures in this study were approved by the Institutional Animal Care and Use Committee at Loma Linda University and followed instructions of NIH Guide for Care and Use of Laboratory Animals. Adult male Sprague Dawley rats (weight 280–300 g) were housed in the animal facility for a minimum of 3 days before surgery with a 12 h light/dark cycle and ad libitum access to food and water. A total of 162 male Sprague Dawley rats were used. Animal groups and numbers used per group/per outcome are shown in Table 1.

The rodent model of SBI was made as established previously (Hyong et al., 2008). Isoflurane 4% was used as for anesthesia induction in an induction chamber and isoflurane 2.5% delivered via nasal mask was used for anesthetic maintenance. Rats were placed prone in a stereotactic frame under surgical microscope. A midline skin incision was made and the periosteum was reflected to expose the skull and bregma was identified. A craniotomy 5 × 5 mm square was made using a microdrill on the right frontal skull. The dura was incised after bone removal and the underlying right frontal lobe was exposed and a partial right frontal lobe resection was performed at a distance of 2 mm lateral to the sagittal suture and 1 mm proximal to the coronal suture. The resection cavity was packed and normal saline irrigation was performed to obtain hemostasis. The incision was closed with sutures and subcutaneous injection of 0.03 mg/kg buprenorphine was administered for postoperative pain. Sham animal underwent identical surgical procedure with craniotomy removal of the bone flap but without partial right frontal lobe resection. Vital parameters were monitored during surgery and recovery. Animals were sacrificed at different time points as indicated in the experiments.

2.2. Animal treatments and experimental groups

2.2.1. Experiment 1

The time course expression of endogenous NT-3 and TrkC expression was evaluated in whole brain samples at 6, 12, 24 and 72 h after SBI. Rats were randomly divided into five groups sham, SBI-6 h, SBI-12 h, SBI-24 h and SBI-72 h and whole brain samples were used for western blot. The time course expression of TrkC expression in the right frontal lobe was characterized at 6, 12, 24 and 72 h after SBI. Rats were randomly divided into five groups sham, SBI-6 h, SBI-12 h, SBI-24 h and SBI and right frontal lobe samples were used for western blot.

2.2.2. Experiment 2

The cell types expressing TrkC was identified at 24 h after SBI. The TrkC receptor was co-localized with astrocytes, vascular endothelial cell and neurons at the right frontal lobe perisurgical site using immunofluorescence staining.

2.2.3. Experiment 3

The effect of topical application of recombinant NT-3 in SBI outcomes was evaluated. Recombinant NT-3 was directly delivered to the

Table 1
Animal groups and numbers used per outcome in the study.

Experimental groups	Animals used					Mortality	Total
	BWC (24 h)	BWC (72 h)	Evans blue assay	Western blot	IHC		
Experiment 1							
Sham				3			3
SBI				16		1	17
Experiment 2							
Sham							
SBI					2		2
Experiment 3							
Sham	8	6	6				20
SBI + Vehicle	8	7	7			2	24
SBI + NT-3 (5 μg)	8					1	9
SBI + NT-3 (10 μg)	8	6	7			2	23
Experiment 4							
Sham				6			6
SBI + Vehicle				8		2	10
SBI + NT-3 (10 μg)				10			10
SBI + NT-3 + Scramble siRNA				9		1	10
SBI + NT-3 + Rap1 siRNA				10			10
SBI + NT-3 + TrkC siRNA				8		2	10
SBI + Scramble siRNA				4			4
SBI + TrkC siRNA				4			4
Total	32	19	20	78	2	11	162

SBI, Surgical Brain Injury; NT-3, Neurotrophin 3; TrkC, Tropomyosin related kinase receptor C; BWC, Brain Water Content; IHC, Immunohistochemistry.