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Recombinant Slit2 attenuates neuroinflammation after surgical brain injury by inhibiting peripheral immune cell infiltration via Robo1-srGAP1 pathway in a rat model☆

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ABSTRACT

Background and purpose: Peripheral immune cell infiltration to the brain tissue at the perisurgical site can promote neuroinflammation after surgical brain injury (SBI). Slit2, an extracellular matrix protein, has been reported to reduce leukocyte migration. This study evaluated the effect of recombinant Slit2 and the role of its receptor roundabout1 (Robo1) and its downstream mediator Slit-Robo GTPase activating protein 1 (srGAP1)-Cdc42 on peripheral immune cell infiltration after SBI in a rat model.

Methods: One hundred and fifty-three adult male Sprague–Dawley rats (280–350 g) were used. Partial resection of right frontal lobe was performed to induce SBI. Slit2 siRNA was administered by intracerebroventricular injection 24 h before SBI. Recombinant Slit2 was injected intraperitoneally 1 h before SBI. Recombinant Robo1 used as a decoy receptor was co-administered with recombinant Slit2. srGAP1 siRNA was administered by intracerebroventricular injection 24 h before SBI. Post-assessments included brain water content measurement, neurological tests, ELISA, Western blot, immunohistochemistry, and Cdc42 activity assay.

Results: Endogenous Slit2 was increased after SBI. Robo1 was expressed by peripheral immune cells. Endogenous Slit2 knockdown worsened brain edema after SBI. Recombinant Slit2 administration reduced brain edema, neurological deficits, and pro-inflammatory cytokines after SBI. Recombinant Slit2 reduced peripheral immune cell markers cluster of differentiation 45 (CD45) and myeloperoxidase (MPO), as well as Cdc42 activity in the perisurgical brain tissue which was reversed by recombinant Robo1 co-administration and srGAP1 siRNA.

Conclusions: Recombinant Slit2 improved outcomes by reducing neuroinflammation after SBI, possibly by decreasing peripheral immune cell infiltration to the perisurgical site through Robo1-srGAP1 mediated inhibition of Cdc42 activity. These results suggest that Slit2 may be beneficial to reduce SBI-induced neuroinflammation.

1. Introduction

Surgical brain injury (SBI) is the inadvertent injury to brain tissue at the perisurgical site which occurs due to neurosurgical maneuvers such as incision, retraction, and electrocoagulation that can aggravate post-operative brain edema and neurological deficits (Jadhav et al., 2007a, Huang et al., 2014). Major complications are encountered in 13–27% patients after intracranial surgeries (Bruder, 2002). Measures to reduce neurosurgical complications are limited (Jadhav et al., 2007a). Furthermore, routine therapy used against SBI including hyperosmolar agents and steroids can have unwanted adverse effects (Li et al., 2014, Xu et al., 2014). Therapeutic strategies that augment endogenous protective mechanisms would be a safe approach to reduce post-operative complications in neurosurgical patients.

Neuroinflammation is a major pathophysiological consequence after SBI (Yamaguchi et al., 2007, Hyong et al., 2008) which contributes to brain edema that can worsen post-operative neurological function (Ayer et al., 2012). Following brain injury, resident immune cells get

Abbreviations: SBI, surgical brain injury; Robo1, roundabout1; CD45, cluster of differentiation 45; MPO, myeloperoxidase; srGAP1, Slit-Robo GTPase activating protein 1; ELISA, enzyme linked immunosorbent assay; ICV, intracerebroventricular; PBS, phosphate buffered saline; NeuN, neuronal nuclei; GFAP, glial fibrillary acidic protein; PAK, p21 activated kinase; PBD, p21 binding domain.

☆ Conflicts of interest: none.

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activated and release cytokines and chemokines that promote migration of peripheral immune cells to injury site in the brain (Wang and Dore, 2007, Rhodes, 2011, Ma et al., 2014). Immune cell infiltration and inflammatory mediators were increased in adjacent brain tissue at the perisurgical site following experimental resection (Jadhav et al., 2007a, Hyong et al., 2008). Infiltrated immune cells release inflammatory mediators that further promote neuroinflammation (Petty and Lo, 2002, Yilmaz et al., 2006, Lo, 2009).

Slit2 is a secreted extracellular matrix protein (Ballard and Hinck, 2012) expressed endogenously in the brain by neurons and astrocytes (Hagino et al., 2003, Prasad et al., 2007). Slit2 was recently identified as an inhibitor of leukocyte chemotaxis (Wu et al., 2001, Ballard and Hinck, 2012) and was shown to be protective in experimental models of systemic inflammation (Kanellis et al., 2004, Tole et al., 2009, London et al., 2010). Slit2 reduced migration of leukocytes to the cortical venules after global cerebral ischemia in mice (Altay et al., 2007). The anti-migratory function of Slit2 is mediated by binding to its receptor roundabout1 (Robo1) (Wong et al., 2002, Ballard and Hinck, 2012). Furthermore, it has been established that Slit-Robo GTPase Activating Protein 1 (srGAP1) a downstream effector of the receptor Robo1 (Wong et al., 2002) can inhibit Cdc42, which is a critical mediator for cell migration (Bishop and Hall, 2000, Yiin et al., 2009). Robo1 has been shown to be expressed on the surface of peripheral immune cells (Wu et al., 2001, Guan and Rao, 2003, Prasad et al., 2007), which we propose induces the signal transduction pathway that mediates the anti-migratory effect of Slit2 and thereby reduces brain infiltration of peripheral immune cells.

The role of Slit2 after SBI is unknown, and the mechanism by which Slit2 elicits neuroprotection has not been evaluated. The objective of this study was to evaluate the effects of recombinant Slit2 as a novel therapeutic strategy to reduce neuroinflammation after SBI in a rat model.

2. Materials and methods

2.1. Animals

All procedures were approved by the Institutional Animal Care and Use Committee at Loma Linda University and complied with NIH Guide for the Care and Use of Laboratory Animals. Adult male Sprague–Dawley rats (280–350 g) were housed in humidity and temperature controlled environment with 12 h light/dark cycle. One hundred and fifty-three rats were subjected to either Sham surgery (n = 26) or SBI surgery (n = 127) by inducing partial resection of the right frontal lobe.

2.2. Experimental design

2.2.1. Experiment 1

The time course expression and localization of endogenous Slit2 and Robo1 were characterized at 24 h, 72 h, and day 7 after SBI. Rats (n = 24) were divided into 4 groups: Sham, SBI 24 h, SBI 72 h, and SBI day 7. Brain samples from the residual right frontal lobe were collected for enzyme linked immunosorbent assay (ELISA), Western blot and for immunohistochemistry.

2.2.2. Experiment 2

The role of endogenous Slit2 after SBI was evaluated. Rats (n = 37) were divided into 4 groups: Sham, SBI, SBI + Slit2 siRNA, SBI +Scramble siRNA. Endogenous Slit2 knockdown was performed by intracerebroventricular (ICV) injection of Slit2 siRNA (Life Technologies, Grand Island, NY, USA) 24 h before SBI. Brain water content, neurological function and Western blot were evaluated at 72 h after surgery.

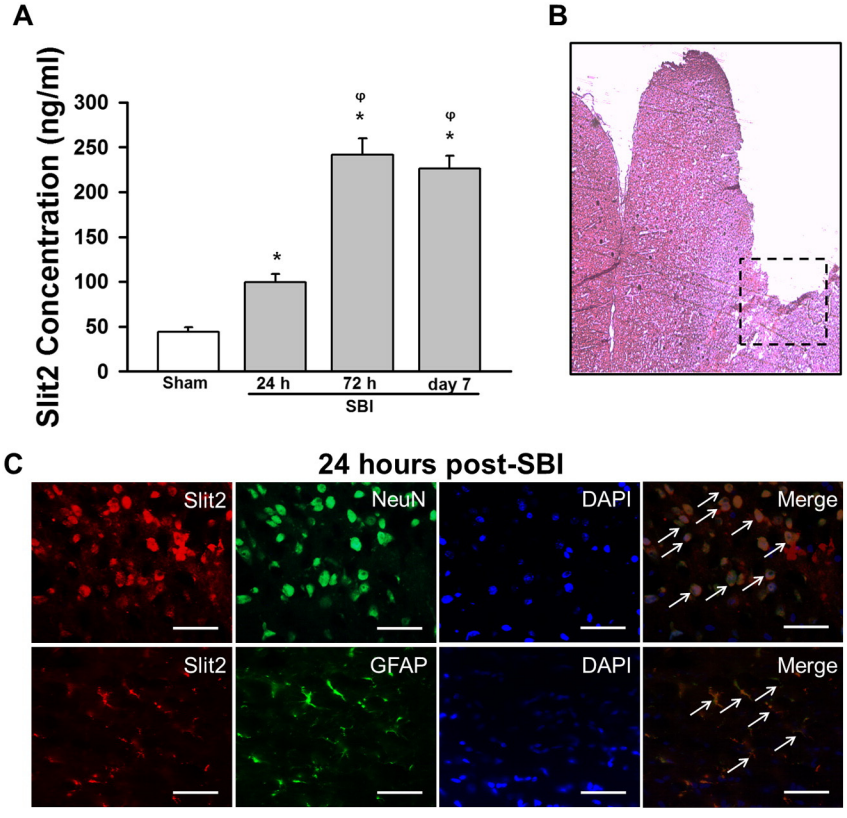

Fig. 1. Temporal expression and localization of endogenous Slit2 in the brain after SBI. (A) ELISA showed that Slit2 concentration increased at 24 h, 72 h and 7 days after SBI with a peak at 72 h. Data are expressed as mean ± SEM. N = 4 to 6 per group. ANOVA, SNK. *p < 0.05 compared to Sham, $^{\varphi}$p < 0.05 compared to 24 h SBI. (B) Histology section showing perisurgical site in the inset used for immunofluorescence staining. (C) Representative microphotographs of immunofluorescence staining showing co-localization of Slit2 (Texas Red/red), with neuronal nuclei (NeuN) or glial fibrillary acidic protein (GFAP) (FITC/green) and DAPI at 24 h after SBI. Arrows indicate merged cells. Scale bar = 50 μm.