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GAPDH/Siah1 CASCADE IS INVOLVED IN TRAUMATIC SPINAL CORD INJURY AND COULD BE ATTENUATED BY SIVELESTAT SODIUM

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Abstract—The glyceraldehyde-3-phosphate dehydrogenase (GAPDH)/Siah1 signaling pathway has been recognized as a sensor of nitric oxide (NO). It is associated with a variety of injurious conditions, suggesting its therapeutic potential for spinal cord injury (SCI). Sivelestat sodium (SIV), a neutrophil elastase (NE) inhibitor initially used to treat acute lung injury, has been known to protect against compression-induced and ischemic SCI. However, little is known about the relationship between the GAPDH/Siah1 cascade and SIV. Thus, we aimed to assess the role of GAPDH/Siah1 cascade in traumatic SCI and its possible link with SIV. Rats were assigned to four groups: sham group, SCI group, 5-mg/kg SIV group, and 10-mg/kg SIV. The traumatic SCI was induced by dropping a 10-g impactor from a height of 25 mm on the dorsal surface of T9 and T10. SIV was injected intraperitoneally immediately after surgery. Our results showed that the nuclear translocation of GAPDH was induced together with the nuclear translocation of Siah1 and the formation of the GAPDH/Siah1 complex in the spinal cord after traumatic SCI. However, the activation of the GAPDH/Siah1 cascade was attenuated by treatment with SIV. We also found that SIV suppressed apoptosis, NE and inducible nitric oxide synthase (iNOS) protein expressions, the number of NE and iNOS immunostained cells, the production of interleukin (IL)-1β and tumor necrosis factor-alpha (TNF-α), and the activation of nuclear factor kappa light-chain enhancer of activated B cells (NF-κB) signaling in the spinal cord. The behavioral tests showed that SIV promoted functional recovery after traumatic SCI as reflected in the sustained increase in the Basso–Beattie–Bresnahan (BBB) scores throughout the observation period. In conclusion, our results reveal GAPDH/Siah1 as a novel signaling pathway during the progression of SCI, which can be blocked by SIV. © 2016 Published by Elsevier Ltd on behalf of IBRO.

Key words: GAPDH, Siah1, sivelestat sodium, spinal cord injury.

INTRODUCTION

Traumatic spinal cord injury (SCI) results in irreversible neurological dysfunction, which in turn has a high health burden on the family and society (Hu et al., 2010; Simpson et al., 2012). During the progression of SCI, the primary injury, which is predominantly characterized by necrotic death, is followed by a secondary phase of injury resulting from massive apoptotic cell death and release of proinflammatory mediators, which may prevent functional recovery and ultimately lead to worsening of clinical outcomes (Hausmann, 2003; Brambilla et al., 2005). Therefore, prevention of persistent apoptosis and inflammations is considered crucial for functional recovery after SCI.

Originally, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was considered merely as a housekeeping enzyme constitutively expressed in the cytosol (Nicholls et al., 2012). However, emerging studies suggest that GAPDH could serve as regulatory molecules in a wide range of central nervous system disorders (Benhar and Stamler, 2005). Under pathological conditions, GAPDH can be S-nitrosylated by nitric oxide (NO) and then gain the ability to bind to Siah1, an E3 ubiquitin ligase, which in turn mediates the nuclear translocation of the GAPDH/Siah1 complex (Benhar and Stamler, 2005; Hara et al., 2005, 2006a). Upon translocation into the nucleus, the formed GAPDH/Siah1 complex would cause the degradation of substrates of Siah1, leading to the activation of apoptotic signaling cascades. Thus, GAPDH has been recognized as a sensor of NO, and the GAPDH/Siah1 signaling pathway is associated various diseases including Parkinson's disease (Huang et al., 2011), Alzheimer's disease (Sirover, 2013), cerebral ischemia–reperfusion injury (Li et al., 2012), and acute lung injury (Takaoka et al., 2014), strongly suggesting the key role of the GAPDH/Siah1 signaling pathway in injurious conditions. Thus, it can be speculated that GAPDH/Siah1 also acts as a mediator in apoptosis and the progression of secondary SCI. However, little is known about the

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Abbreviations: Bax, Bcl-2-associated X protein; BBB scores, Basso–Beattie–Bresnahan scores; Bcl-2, B-cell lymphoma 2; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HE, hematoxylin and eosin staining; IL-1β, interleukin-1 beta; iNOS, inducible nitric oxide synthase; IP, immunoprecipitation; NE, neutrophil elastase; NF-κB, nuclear factor kappa light-chain enhancer of activated B cells; NO, nitric oxide; SCI, spinal cord injury group; Siah1, E3 ubiquitin-protein ligase SIAH1; SIV, sivelestat sodium; TNF-α, tumor necrosis factor-alpha; TUNEL, Terminal deoxynucleotidyl transferase dUTP nick end labeling.

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potential of the GAPDH/Siah1 signaling pathway as a therapeutic strategy.

Neutrophils are the first inflammatory cells to arrive at the site of injury, which play an important role in the progression of secondary injury by releasing a series of mediators (Hausmann, 2003; Donnelly and Popovich, 2008). Among these mediators, neutrophil elastase (NE) is one of the most harmful cytotoxic mediators released from activated neutrophils. NE has been reported to damage endothelial cells and degrade connective tissue components, leading to critical tissue injury and an increase in vascular permeability (Smedly et al., 1986; Tonai et al., 2001). In addition, NE could also induce overproduction of NO (Hagiwara et al., 2009; Araki et al., 2011), which may confer the stressful signal to GAPDH, thereby activating the downstream GAPDH/Siah1 cascade as described previously. Sivelestat sodium (SIV), a NE inhibitor, has been used to treat acute lung injury or acute respiratory distress syndrome (ARDS) (Zeiher et al., 2002; Iwata et al., 2010). Other studies also indicate that SIV can be used to attenuate compression-induced or ischemic SCI (Tonai et al., 2001; Iwamoto et al., 2009). However, few studies have been performed on the relationship between the GAPDH/Siah1 cascade and SIV. Therefore, the aim of the study is to investigate whether SIV could alleviate traumatic SCI by inhibiting the GAPDH/Siah1 death cascade.

EXPERIMENTAL PROCEDURES

Animals

All experimental protocols and animal handling procedures were approved by the Ethics Committee for Animal Experimentation of the Fourth Military Medical University, and they were performed in accordance with the Guidelines for Animal Experimentation of the Fourth Military Medical University (Xi'an, China). Male Sprague–Dawley rats, weighing 280–320 g, were purchased from the Experimental Animal Center of the Fourth Military Medical University. They were kept in standard lab housing with a 12-h light/dark cycle at a temperature of 21 ± 2 °C and 60–70% humidity. The rats had access to standard diet and water *ad libitum*.

SCI

The rats were anesthetized with chloral hydrate (350 mg/kg, i.p.), and a laminectomy was performed at the T9–T10 level to expose the cord beneath without disrupting the dura. The spine was stabilized by clamping the spinous processes of T8 and T11, and the traumatic spinal cord contusions were induced by dropping a 10-g impactor (New York University weight-drop device) from a height of 25 mm on the exposed dorsal surface of the cord. The sham animals underwent a T10 laminectomy without weight-drop injury. After SCI, the rats were assisted manually to void the bladders by pressing on the abdomen above the bladder twice daily until sufficient recovery of autonomic bladder function.

Experimental groups

The rats were randomly assigned into four groups: (1) sham group (sham): rats were only subjected to a laminectomy at the T10 level; (2) SCI group (SCI): rats were subjected to spinal cord contusion at the T9–T10 level; (3) the 5-mg/kg SIV group (SIV 5 mg/kg): rats were subjected to spinal cord contusion at the T9–T10 level and injected intraperitoneally with 5 mg/kg of SIV (Cat# S7198, Sigma Chemical, St Louis, MO, USA) immediately after surgery; and (4) the 10-mg/kg SIV group (SIV 10 mg/kg): rats were subjected to spinal cord contusion at the T9–T10 level and injected intraperitoneally with 5 mg/kg of SIV immediately after surgery. SIV was prepared with saline, and the dosage was selected based on a previous study (Yang et al., 2012).

Behavioral tests

One day after the weight-drop injury and every week thereafter, hind-limb locomotor function was evaluated using Basso–Beattie–Bresnahan (BBB) scales by trained investigators who were blind to the experimental design (Basso et al., 1995; Faulkner et al., 2004; Erschbamer et al., 2007). Scores range from 0 (complete paralysis) to 21 (normal gait), which involve movement, weight support, and coordination. The BBB scores were analyzed statistically by repeated measures analysis of variance (ANOVA) followed by Tukey's multiple comparison test at each time point.

Western blot

At each specific time point, the rats were deeply anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and then euthanized with CO_2 asphyxiation. The total protein from the spinal cord tissue of the lesion epicenter was extracted by homogenization in ice-cold radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Nantong, China) with a complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA) and 1 mM phenylmethylsulfonyl fluoride (PMSF). Nucleic and cytoplasmic proteins were extracted with the Nuclear and Cytoplasmic Extraction Reagents kit (Pierce Biotechnology, Rockford, IL, USA) following the manufacturer's instructions. Equal amounts of the protein sample were resolved on 12% sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis (PAGE), transferred onto polyvinylidene difluoride (PVDF) membranes (Bio-Rad, Richmond, CA, USA), and detected with enhanced chemiluminescence (ECL) Western blotting detection reagents (Thermo Scientific, Rockford, IL, USA). The density of bands was analyzed with densitometry followed by quantification with the NIH image program (NIH Image Version 1.61). The following primary antibodies were used in the study: rabbit anti-active (cleaved) caspase-3 monoclonal antibody (Cell Signaling Technology, Beverly, MA, USA; Cat# 9664, 1:500), rabbit anti-total caspase-3 antibody (Cell Signaling Technology; Cat# 9662, 1:1000), mouse anti-

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