NEUROSCIENCE
INTERNATIONAL BRAIN IBRO RESEARCH ORGANIZATION

RESEARCH ARTICLE

W. Li et al. / Neuroscience xxx (2018) xxx–xxx

Lithium Posttreatment Alleviates Blood–Brain Barrier Injury After Intracerebral Hemorrhage in Rats

Weishan Li, Rui Li, Sha Zhao, Cheng Jiang, Zhen Liu and Xiaobo Tang*

College of Pharmacy, Harbin Medical University, Department of Biopharmaceutical Sciences (State-Province Key Laboratories of Biomedicine-Pharmaceutics of China), China

Abstract—Vasogenic cerebral edema formation after blood–brain barrier (BBB) damage aggravates the devastating consequences of intracerebral hemorrhage (ICH). The present study aims to probe into a therapeutic method on BBB preservation after ICH with a glycogen synthase kinase-3β (GSK-3β) inhibitor, lithium. Intrastriatal infusion of semicoagulated autologous whole blood or sham surgery was performed on male Sprague-Dawley (SD) rats (n = 208). Experimental animals received administration of 4,6-disubstitutedpyrrolo-pyrimidine (TWS119), lithium alone or in combination with a phosphatidylinositol 3-kinase inhibitor, wortmannin, after ICH. Behavioral tests, brain edema, and BBB permeability were determined at 24 and 72 h after surgery. Expressions of Akt, GSK-3β, β-catenin, claudin-1 and claudin-3 were evaluated via Western blots. Our results showed lithium alone posttreatment activated GSK-3β, therefore increasing active β-catenin and claudin-1 and claudin-3 expressions, which were accompanied with improved BBB integrity and ameliorated sensorimotor deficits and brain edema in ICH animals. We concluded that lithium alone reduced BBB damage after ICH, likely through regulating Akt/GSK-3β pathway and stabilizing β-catenin.

Key words: intracerebral hemorrhage, blood–brain barrier, lithium, glycogen synthase kinase-3β, β-catenin.

INTRODUCTION

Intracerebral hemorrhage (ICH) is the most severe subtype of stroke with a high mortality rate and limited effective treatment (Adeoye and Broderick, 2010; Hemphill et al., 2015). The formation of edema is a critical step for its devastating nature of the disease (Gebel et al., 2002; Rosenberg, 2012). Blood–brain barrier (BBB) disruption results in vasogenic brain edema after ICH (Keep et al., 2008). The main constituent of BBB is tight junctions (TJs) including claudins, occludin, and junctional adhesion molecules (Keep et al., 2008; Abbott et al., 2010). Moreover, decreased expression of these transmembrane proteins indicates reduced barrier integrity and increased paracellular permeability (Liebner et al., 2008; Tran et al., 2016).

The adherens junction component (AJ) (Lampugnani et al., 1995), β-catenin, is not only required for maintaining BBB integrity, but also for regulating transcription of claudin-1 and claudin-3 when combines with lymphoid enhancer factor/T-cell factor (Shiou et al., 2007; Liebner et al., 2008). Inactive glycogen synthase kinase 3β (GSK-3β), which is phosphorylated in serine residues, has been shown to confer cytoprotective effects following ICH and is involved in the degradation of β-catenin (Krafft et al., 2013).

Our previous study has indicated that lithium, through blocking GSK-3β pathway, reduced cell apoptosis and inflammation in rats subjected to experimental hemorrhagic stroke (Zheng et al., 2017). However, the treatment effect of lithium on BBB integrity still remains unclear after ICH. In this study, we aim to investigate whether lithium, a GSK-3β inhibitor, when administered after induction of experimental ICH, will increase active β-catenin and subsequently increase claudin-1 and claudin-3 expressions resulting in maintaining/stabilizing BB integrity in rats.

EXPERIMENTAL PROCEDURES

Animal and drug treatments

Animal (n = 208) interventions for these experiments complied with guidance of the Experimental Animal Ethic Committee of Harbin Medical University, China (Animal Experimental Ethical Inspection Protocol No.

*Corresponding author. Address: College of Pharmacy, Harbin Medical University, Department of Biopharmaceutical Sciences State (Province Key Laboratories of Biomedicine-Pharmaceutics of China), P.O. Box 19, 157 Baojian Road, Nangang District, Harbin, Heilongjiang 150081, China. Fax: +86-451-86684073.
E-mail address: ty6163@aliyun.com (X. Tang).

Abbreviations: BBB, blood–brain barrier; EB, Evans Blue; GSK-3β, glycogen synthase kinase-3β; ICH, intracerebral hemorrhage; p-Akt, phospho-Akt; p-GSK-3β, phospho-glycogen synthase kinase-3β; p-β-catenin, phospho-β-catenin; TWS119, 4,6-disubstitutedpyrrolo-pyrimidine.

https://doi.org/10.1016/j.neuroscience.2018.05.001
0306-4522/

2016101). The amount of animals and animals suffering were minimized as much as possible. Adult male Sprague–Dawley (SD) rats used in the experiments needed to gain between 230 g and 280 g of weight, which were purchased from Experimental Animal Center of the Second Affiliated Hospital of Harbin Medical University (Harbin, China). They were then kept under 12-h light/dark cycles at a 20–23 °C environment with adequate food and water. All experimental rats were subjected to ICH (n = 168) or sham surgery (n = 40). Experimental ICH rats received intraperitoneal injections of either a GSK-3β inhibitor 4,6-disubstitutedpyrrolo-pyrimidine (TWS119) (MedChem Express; 30 mg/kg, 2 h post-ICH) (Wang et al., 2016), lithium (Sigma–Aldrich; 60 mg/kg; 2 h post-ICH) (Feng et al., 2008; Zheng et al., 2017), lithium plus a PI3K inhibitor wortmannin (Sigma–Aldrich; 15 μg/kg; 30 min post-ICH) or wortmannin alone (Duris et al., 2011). Rats in ICH and sham groups were injected with an equal volume of 0.9% saline. All drugs were administered according to protocols reported previously (Duris et al., 2011; Zheng et al., 2017). All assessments were performed in a double blinded way among experimenters, as previously reported (Hua et al., 2002; Li et al., 2015a; Zheng et al., 2017).

ICH induction

The method of injecting semi-coagulated autologous whole blood into striatum was used to induce experimental ICH rats, as has been described in our previous studies (Liu et al., 2015;Xu et al., 2017). SD rats were placed in a stereotaxic apparatus (Shanghai Ruanlong Science and Technology Development Co., Ltd., Shanghai, China), a burr hole (1 mm of diameter) was drilled in the skull after incising the scalp 0.7 mm anterior, 3 mm lateral to bregma. Then 80 μL semi-coagulated autologous whole blood was injected into the right striatum (5 mm in depth from bregma) with an electronic microinjector pump through the burr hole during 8 min (Shanghai Alcott Biotech Co., Ltd., Shanghai, China). Rectal temperature of rats was maintained at 37 ± 0.5 °C using a rectal isothermal heating unit. The injection needle was slowly removed after placement for 40 min; and the wound was sutured. Sham surgery was induced by needle insertion only.

Behavioral assessment and analysis

The forelimb placing test and corner turn test were used to evaluate sensorimotor deficits in rats at 24 and 72 h after surgery (n = 10 per group) as previously reported (Hua et al., 2002; Li et al., 2015a; Zheng et al., 2017). For the forelimb placing test, torsos of rats were held allowing free hanging and brushing the respective vibrissae on the corner edge of a countertop by moving the animal up and down. Normal rats put the contralateral forelimb of intact hemisphere quickly onto the corner of the countertop. According to the degree of injury, the rat's ability to place its contralateral forelimb of the injured hemisphere might be impaired. Each rat was tested ten times for the forelimb contralateral to the injury, and the percentage of trials in which the rat placed the forelimb was calculated. In corner turn test, rats were placed into a 30-degree-angled corner. When vibrissae of rats touched with wall, rats would be likely to exit the corner via conditioned reflex. Rats turned to the left or to the right to exit the corner depending on extent of contralateral hemisphere injury. The choice for each trial was documented 10–15 times. And only the turns involving full rearing along either wall were recorded within 30 s.

Brain water content

Brain edema was evaluated in rats at 24 and 72 h after surgery (n = 5 to 6 per group) via the wet weight/dry weight method (Ma et al., 2011; Li et al., 2015b). Animals were anesthetized and decapitated. Brains were quickly harvested and divided into two hemispheres along with midline. The cerebellum was measured as an internal control. Samples were then dried at 100 °C for 24 h before measuring the dry weight. Wet and dry weights of all samples were determined using an electronic analytical balance. The results of brain water content (%) were expressed as [(wet weight − dry weight)/wet weight × 100%].

Evans Blue (EB) staining

The integrity of BBB was evaluated using EB staining (n = 6 per group) (Yang et al., 2015). At 24 and 72 h after ICH induction, experimental rats were injected with EB dye (Sigma–Aldrich, 2% in 0.9% saline; 4 ml/kg) which would bind to albumin (Li et al., 2015b). Two hours later, the anesthetized rat was perfused with phosphate-buffered saline to remove the intravascular EB (Wang et al., 2015a). The samples were removed and stored at −80 °C for later use (Yang et al., 2015). The brain was weighed and homogenized with 2 ml of 50% trichloroacetic acid solution. After the lysates were centrifuged at 14,000g for 30 min, the supernatants were diluted 1:3 with ethanol (Zeynalov et al., 2015). EB dye extravasation was measured using a spectrophotometer (excitation 620 nm, emission 680 nm). And EB standard was also measured with a spectrophotometer (excitation 620 nm, emission 680 nm) for calculating a standard curve. The result was graphed using a standard curve and demonstrated as μg/g of tissue.

Western blot analysis

Rats were decapitated at 24 h after ICH induction. Brain tissues were immediately extracted (n = 6 per group) and sliced into 6-mm-thick coronal brain sections, and processed as described (Shan et al., 2012). Protein extraction from tissue was obtained using RIPA lysis buffer (Beyotime, Shanghai, China) with further centrifugation at 15,000g for 15 min. The samples containing 50 μg of proteins were separated in 6–15% sodium dodecyl sulfate–polyacrylamide gradient gels (SDS–PAGE). Then protein samples were subjected to electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked with blocking buffer for 1 h and incubated with monoclonal antibody overnight at 4 °C. We used anti-Akt (1:500; Bioworld, USA), anti-phospho-Akt