Experimental Neurology 300 (2018) 30–40

Contents lists available at ScienceDirect

Experimental Neurology

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

Research Paper

Multiple mechanisms underlying neuroprotection by secretory phospholipase A2 preconditioning in a surgically induced brain injury rat model

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ARTICLE INFO

Keywords:
Surgical brain injury
sPLA2
Preconditioning
Neuroinflammation
Brain edema
Intra-operative hemorrhage

ABSTRACT

Background: Intra-operative bleeding, post-operative brain edema and neuroinflammation are major complications in patients with surgical brain injury (SBI). Phospholipase A2 (PLA2) is the upstream enzyme which initiates the PLA2, 5-lipoxygenase (5-LOX) and leukotriene B4 (LTB4) inflammatory pathway. We hypothesized PLA2preconditioning (PPC) prior to SBI can activate endogenous anti-inflammatory responses to protect against SBI. This study evaluated if PPC can ameliorate neurosurgical complications and elucidated PPC-mediated possible protective mechanisms in a rat SBI model.

Methods: Total 105 adult male Sprague Dawley rats were used for this study. SBI was induced by partial resection of the right frontal lobe. PLA2 or 0.9% NaCl was injected via rats' tail vein for 3 consecutive days prior to SBI. For mechanism study, a selective PLA2 inhibitor, Manoalide and 5-LOX inhibitor, Zileuton were injected intravenously with PPC to elucidate the role of PLA2 and 5-LOX in PPC-mediated anti-inflammatory effects. Brain water content (BWC) and lung water content, neurological tests, ELISA, western blot, immunohistochemistry, white blood cells (WBC) count, and spectrophotometric assay for intra-operative hemorrhage volume were evaluated.

Results: First, PPC reduced brain water content, intra-operative bleeding, and improved neurological function after SBI. Second, PPC decreased 5-LOX expression and brain leukocyte infiltration, while increasing glial fibrillary acidic protein (GFAP) expression in the peri-resection brain tissue after SBI. Third, PPC induced peripheral inflammation represented by mild pulmonary inflammation and increased peripheral blood WBC count and LTB4 level. Lastly, PPC increased blood glucose concentration and glucocorticoid levels after SBI. In addition, PPC mediated above-mentioned changes were partially reversed by administration of PLA2 inhibitor, Manoalide and 5-LOX inhibitor, Zileuton.

Conclusions: PPC conferred neuroprotection against SBI via multi-target involvement induced anti-inflammatory mechanisms.

1. Introduction

An estimated 800,000 cranial and spinal neurosurgeries are conducted every year in the United States (McGirt et al., 2015). Injury to brain tissue at the peri-resection site inevitably occurs during neurosurgical procedures, and this has been termed as surgical brain injury (SBI). Direct mechanical injury during surgical incisions and suction aspiration, as well as thermal injuries due to electronic drilling and electrocoagulation can lead to unavoidable trauma to the surrounding tissue. The traumatic nature of such injuries can lead to blood brain barrier (BBB) disruption, intra-operative bleeding, and direct cell death. In addition, primary and secondary neuroinflammation aggravate brain

Abbreviations: SBI, surgical brain injury; PLA2, phospholipase A2; PPC, PLA2 preconditioning; 5-LOX, 5-lipoxygenase; AA, arachidonic acid; LTB4, leukotriene-B4; BWC, brain water content; WBC, white blood cells; GFAP, glial fibrillary acidic protein; MPO, myeloperoxidase; NeuN, Neuronal Nuclei; Iba1, ionized calcium binding adaptor molecule 1

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http://dx.doi.org/10.1016/j.expneurol.2017.10.022
Received 8 August 2017; Received in revised form 12 October 2017; Accepted 21 October 2017
Available online 24 October 2017
0014-4886/

edema and worsen post-operative neurological function (Ghosh and Basu, 2012; Xu et al., 2015). Diuretics and steroids have conventionally been used to reduce post-operative complications in the clinics, but they are not always beneficial and sometimes may incur adverse effects (Un et al., 2013; Wakai et al., 2013). Neurosurgical procedures are often planned ahead of time; therefore, allow for preventative measures to be taken to reduce possible complications.

Preconditioning is described as a phenomenon in which brief exposure to noxious stimuli at small doses elicits endogenous protective response to induce tolerance against future massive or prolonged injurious insult (Abd-Elfattah and Wechsler, 1995; Przyklenk and Kloner, 1998). Various preconditioning strategies have been shown to be neuroprotective in brain injury models (Hickey et al., 2011; Pan et al., 2012). Attenuation of inflammation has been shown to be one of the mechanisms underlying preconditioning-induced neuroprotection against focal cerebral ischemia (Bowen et al., 2006). Phospholipase A2 (PLA2), 5-lipoxygenase (5-LOX) and leukotriene-B4 (LTB4) axis has been well documented to be a typical inflammatory pathway (Burnett and Levy, 2012; Hernandez et al., 2007; Xie et al., 2015). PLA2 is an important component of snake venoms responsible for the inflammatory effects of venoms (Tan et al., 2015; Vadas et al., 1989; Wei et al., 2009). Cobra venom-derived secretory PLA2 (sPLA2) catalyzes the hydrolysis of membrane phospholipids to produce the lipid mediator, arachidonic acid (AA), which is further oxygenated into LTB4 by the enzyme 5-LOX (Ghassemi and Rosenberg, 1992; Rosenberg et al., 1983; Yates et al., 1990). PLA2 isolated from *Naja sputatrix* venom was reported to induce pulmonary inflammation and edema when administered intravenously and intra-tracheally to rats (Cher et al., 2003). Previous studies show that PLA2 plays a role in the worsening of cerebral ischemic injury in rodent models (Bonventre et al., 1997; Clemens et al., 1996). This study was designed to investigate whether preconditioning with sPLA2 derived from *Naja mossambica mossambica* venom prior to SBI would provide neuroprotection against SBI induced complications via activation of the PLA2/5-LOX/LTB4 signaling pathway.

2. Materials and methods

2.1. Animals

All animal protocols followed NIH Guide for the Care and Use of Laboratory Animals and were approved by Institutional Animal Care and Use Committee at Loma Linda University. All experiments complied with ARRIVE guidelines. Adult male Sprague Dawley rats, 260 g to 300 g (n = 105) were used for the study. All animals used in the study were randomly assigned to various groups used for all the experiments. Rats (n = 20) were used for Sham and (n = 85) were used for SBI groups. None of the Sham rats died. A total 13 of the 85 SBI rats died (15.3%) within 24 h after SBI due to excessive intra-operative bleeding or brain edema. The animal number used per group and mortality in each group is listed in Table 1. Animals were housed in humidity and temperature controlled environment with a 12 h light/dark cycle and free access to food.

2.2. Preconditioning regime and experimental groups

The Experimental design for the study and animal numbers used per assay are shown in Fig. 1.

Experiment 1 was performed to evaluate the effects of PLA2 preconditioning (PPC) on SBI-induced brain edema and neurobehavioral deficits at 24 h and 72 h after SBI. SBI rats were subjected to either PPC or 0.9% NaCl preconditioning (SPC) as controls. sPLA2 derived from *Naja mossambica mossambica* venom (Sigma Aldrich, St. Louis, MO) was injected into the tail vein of rats in PPC + SBI group, once each day for 3 consecutive days prior to SBI surgery. The dose (125 mg/kg body weight) and intravenous injection route for sPLA2 administration were

Table 1
The table shows animal number and mortality in each group. Outcomes for Experiment 1 were evaluated at 24 h and 72 h after surgery. Outcomes for Experiments 2 and 3 were evaluated at 24 h after surgery. *Samples for SPC + SBI group were shared with Experiment 2. Vehicle refers to ethanol. SPC, saline preconditioning; SBI, surgical brain injury; PPC, PLA2 preconditioning.

Groups	Total	Mortality (%)
Experiment 1		
24 h outcomes		
Sham	6	0/6 (0%)
SPC + SBI	7	1/7 (14.2%)
PPC + SBI	7	1/7 (14.2%)
72 h outcomes		
Sham	6	0/6 (0%)
SPC + SBI	7	1/7 (14.2%)
PPC + SBI	7	1/7(14.2%)
Experiment 2		
Sham	8	0/8 (0%)
SPC + SBI	10	2/10 (20%)
PPC + SBI	10	2/10 (20%)
Experiment 3		
SPC + SBI	*	*
PPC + SBI	9	1/9 (11.1%)
Vehicle + PPC + SBI	10	2/10 (20%)
Manoalide + PPC + SBI	9	1/9 (11.1%)
Zileuton + PPC + SBI	9	1/9 (11.1%)
Total animals	105	13/85 (15.3%)

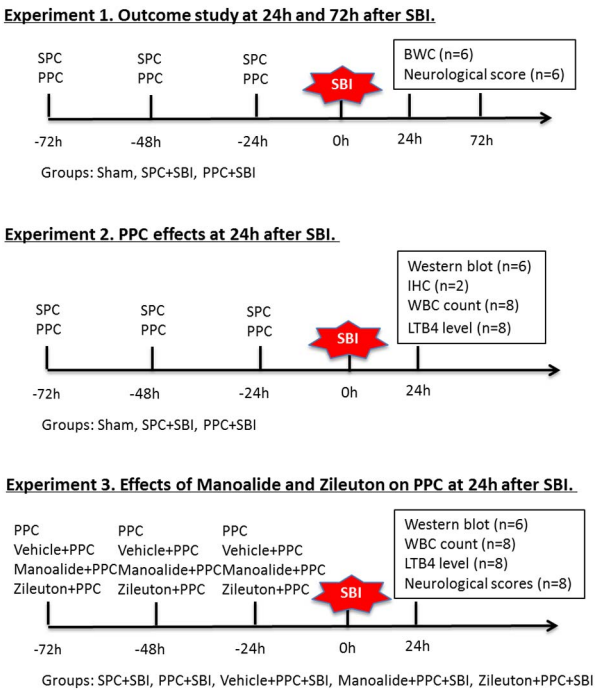

Fig. 1. Experimental design and animal groups. Experiment 1: neurological tests were evaluated at 24 h and 72 h after surgery following which the animals were euthanized to collect brain samples for brain water content (BWC) measurement (n = 6/group/time point). Experiment 2: animals were euthanized at 24 h after surgery. Peripheral blood sample was collected during sacrifice for WBC count and LTB4 assay (n = 8/group), and brain samples were harvested from the same animals for western blot (n = 6/group) and immunohistochemistry (n = 2/group). Experiment 3: neurological tests were evaluated at 24 h following which animals were euthanized. Peripheral blood sample was collected during sacrifice for WBC count and LTB4 assay (n = 8/group), after which brain samples were harvested from the same animals for western blot (n = 6/group). Samples for SPC + SBI were shared with Experiment 2. Vehicle refers to ethanol. SBI, surgical brain injury; SPC, saline preconditioning, PPC, PLA2 preconditioning; BWC, brain water content, IHC, immunohistochemistry; WBC, white blood cells; LTB4, leukotriene B4.