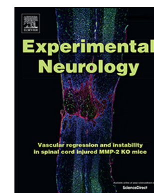
Experimental Neurology 311 (2019) 1–14

Contents lists available at ScienceDirect

Experimental Neurology

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



Research Paper

High-mobility group box-1 translocation and release after hypoxic ischemic brain injury in neonatal rats

Xiaodi Chen[a,1], Jiyong Zhang[a,1], Boram Kim[a], Siddhant Jaitpal[a], Steven S. Meng[a], Kwame Adjepong[a], Sayumi Imamura[a], Hidenori Wake[c], Masahiro Nishibori[c], Edward G. Stopa[b], Barbara S. Stonestreet[a,*]

[a] Department of Pediatrics, Women & Infants Hospital of Rhode Island, The Alpert Medical School of Brown University, Providence, RI, USA
[b] Department of Pathology and Neurosurgery, The Alpert Medical School of Brown University, Rhode Island Hospital, Providence, RI, USA
[c] Graduate School of Medicine, Dentistry, and Pharmaceutical Sciences, Okayama University, Okayama, Japan

A R T I C L E I N F O

Keywords:
Brain
Hypoxia
HMGB1
Ischemia
Neonate

A B S T R A C T

Inflammation contributes to neonatal brain injury. Pro-inflammatory cytokines represent key inflammatory meditators in neonatal hypoxic-ischemic (HI) brain injury. The high mobility group box-1 (HMGB1) protein is a nuclear protein with pro-inflammatory cytokine properties when it is translocated from the nucleus and released extracellularly after stroke in adult rodents. We have previously shown that HMGB1 is translocated from the nucleus to cytosolic compartment after ischemic brain injury in fetal sheep. In the current study, we utilized the Rice-Vannucci model to investigate the time course of HMGB1 translocation and release after HI injury in neonatal rats. HMGB1 was located in cellular nuclei of brains from sham control rats. Nuclear to cytoplasmic translocation of HMGB1 was detected in the ipsilateral-HI hemisphere as early as zero h after HI, and released extracellularly as early as 6 h after HI. Immunohistochemical double staining detected HMGB1 translocation mainly in neurons along with release from apoptotic cells after HI. Serum HMGB1 increased at 3 h and decreased by 24 h after HI. In addition, rat brains exposed to hypoxic injury alone also exhibited time dependent HMGB1 translocation at 3, 12 and 48 h after hypoxia. Consequently, HMGB1 responds similarly after HI injury in the brains of neonatal and adult subjects. We conclude that HMGB1 is sensitive early indicator of neonatal HI and hypoxic brain injury.

1. Introduction

Hypoxia-ischemia (HI) and hypoxic-ischemic encephalopathy (HIE) represent common causes of neurological injury in preterm and full term infants with birth related complications (Fatemi et al., 2009; Scafidi et al., 2009). Neonates exposed to HI injury can have poor neurological and behavioral outcomes including increased risk and incidences of learning deficits (Stephens et al., 2010). However, the underlying mechanisms by which HI and HIE result in brain injury have not been completely elucidated. Although recent studies have revealed numerous mechanisms predisposing to HI injury, these mechanisms are complex and new molecules are continually being identified. Hypothermia is the only approved intervention for HIE, which unfortunately is only partially protective (Perrone et al., 2012; Shankaran et al., 2010, 2012). Consequently, there is a critical need to identify additional mechanism(s) underlying HI in order to develop novel neuroprotective strategies.

HI is characterized by brain injury caused by hypoxia and/or reduced cerebral blood flow to the brain resulting in neuronal damage from intracellular accumulation of sodium, water, and calcium, intersynaptic glutamate accumulation, activation of nitric oxide, and synthesis of free radicals (Arundine and Tymianski, 2003; Back et al., 2007; Lipton et al., 1993). These cytotoxic processes result in various forms of cell death, such as cellular necrosis, apoptosis and autophagy (Balduini et al., 2009, 2012; Pulera et al., 1998). These events can induce a robust inflammatory reaction by activation of endogenous microglia (Weinstein et al., 2010). Inflammation has also been implicated in neuronal and white matter injury after neonatal HI (McAdams and Juul, 2012). Pro-inflammatory cytokines have been described as key contributors to inflammation after HI brain injury (Liu et al., 1994;

* Corresponding author at: Department of Pediatrics, Women & Infants Hospital of Rhode Island, The Alpert Medical School of Brown University, 101 Dudley Street, Providence, RI 02905-2499, USA.
E-mail address: bstonestreet@wihri.org (B.S. Stonestreet).
[1] Equal first author contributions.

https://doi.org/10.1016/j.expneurol.2018.09.007
Received 22 December 2017; Received in revised form 13 July 2018; Accepted 10 September 2018
Available online 12 September 2018
0014-4886/ © 2018 Elsevier Inc. All rights reserved.

McAdams and Juul, 2012; Saito et al., 1996). Findings in preterm and full term infants also suggest that elevations in pro-inflammatory cytokines are important in the pathogenesis of HI brain injury (McAdams and Juul, 2012; Silverstein et al., 1997).

The high mobility group box-1 (HMGB1) protein is a ubiquitously abundant nuclear protein that functions as a structural co-factor for proper transcriptional regulation in the nucleus. HMGB1 binds to the minor groove with moderate affinity and folds DNA into a spiral (Ulloa and Messmer, 2006). In the developing brain under normal conditions, HMGB1 can also function as a neurite growth factor (Merenmies et al., 1991; Zhang et al., 2011). However, during injury, activated immune and damaged cells release HMGB1 into the extracellular space, where HMGB1 functions as a pro-inflammatory mediator and contributes to the pathogenesis of inflammation related brain injury (Wang et al., 1999). Numerous studies have demonstrated that ischemic brain injury results in HMGB1 translocation from the neuronal nucleus into the brain parenchyma in adult subjects (Kim et al., 2006; Muhammad et al., 2008; Zhang et al., 2011). The release of HMGB1 can occur as early as 2 h after ischemic reperfusion related brain injury in adult rats (Zhang et al., 2011). Extracellular HMGB1 stimulates glutamate release, inflammatory responses and blood-brain barrier injury in adult subjects (Zhang et al., 2011). Most importantly, several *in vivo* studies have shown that anti-HMGB1 treatment of ischemic injury attenuates brain inflammation and suppresses cell death in the early phase of ischemic injury (Liu et al., 2007; Muhammad et al., 2008; Yang et al., 2010; Zhang et al., 2011). However, there is very limited information regarding its characteristics after HI or hypoxia in the immature brain.

We have previously reported that HMGB1 is translocated from the nucleus to the cytosolic compartment in the cerebral cortex after ischemic brain injury in the fetal brain (Zhang et al., 2016). In our previous work, HMGB1 was localized primarily to the cellular nuclei and partially to the cytoplasmic compartment in the cerebral cortex of sham operated control fetal sheep. Ischemia increased the amount of neuronal cells that demonstrated cytoplasmic HMGB1 staining (Zhang et al., 2016). In addition, Western immunoblots revealed that nuclear HMGB1 expression decreased and cytosolic HMGB1 increased in the brains of the fetal sheep exposed to ischemia compared with the sham control treatment (Zhang et al., 2016). Our findings in the fetal sheep brain are consistent with previous findings in adult rodents after ischemic injury, and suggest that alterations in HMGB1 expression and localization after ischemia in fetal brain could contribute to ischemia-related inflammation. Other studies are also consistent with our findings suggesting cytosolic localization of HMGB1 in neurons and astrocytes in the normal fetal sheep brain (Frasch and Nygard, 2017). However, systemic acidosis induced by multiple umbilical cord occlusions caused subtle and brain region-specific reverse shifts in neuronal HMGB1 patterns in cortical gray matter (Frasch and Nygard, 2017; Frasch et al., 2016) Differences among the studies summarized above are most likely a result of differences in the animal models and methodology (Frasch and Nygard, 2017; Frasch et al., 2016). Nonetheless, these studies have not examined potential sequential changes in HMGB1 in the immature brain after hypoxic ischemic injury.

In the current study, we utilized the well characterized Rice-Vannucci model of neonatal HI (Rice 3rd et al., 1981) to determine the time course of the HMGB1 translocation and release in neonatal rat brain. In addition, we identified cell specific translocation and release of HMGB1 and the relationship between HMGB1 release and cell death, along with the time course of alterations in HMGB1 in serum and brain by Western immunoblot and ELISA after neonatal HI brain injury. Additionally, we examined the effects of hypoxia without ischemia on HMGB1 expression in neonatal rat brain.

2. Materials and methods

This study was conducted after approval by the Institutional Animal Care and Use Committees of the Alpert Medical School of Brown University and Women & Infants Hospital of Rhode Island and in accordance with the National Institutes of Health Guidelines for the use of experimental animals.

2.1. Animal preparation, study groups, and experimental study design

The subjects were neonatal rats born to time-mated dams (Charles River Laboratories; Wilmington, MA) in the Animal Care Facility at Brown University. Pregnant Wistar rats on embryonic day 15 (E15) or E16 were shipped and then housed in a 12-hour light/dark cycled facility with *ad libitum* access to food and water in the Animal Care Facility at Brown University. After the delivery date was confirmed for each pregnant dam, the date upon which the rat pups were born was designated as postnatal day 0 (P0). Pups from different litters born on the same day were then culled and balanced with regard to sex differences so that each dam had no > 10 pups. On P7, the pups were randomly assigned to sham operated control, sham operated hypoxia alone, and HI exposed groups. HI was induced in the pups by carotid artery ligation on the right side along with exposure to 8% oxygen for 2 h using previously described methods (Rice 3rd et al., 1981).

Anesthesia was induced in the pups with 3–4% isoflurane and maintained with 1–2% isoflurane during the procedure. Body temperature was maintained at 36 °C during surgery with an isothermal heating pad. A skin incision was made at the midline of the neck overlying the trachea using sterile surgical scissors. The right common carotid artery (RCCA) was separated from the trachea and the surrounding nerves and double ligated using 5–0 silk sutures. The incision was closed and sterilized with betadine and alcohol. Sham treated subjects were exposed to the same procedure except the RCCA was not ligated. The pups were sutured and labeled with neonatal tattooing system (Neo-9, Animal Identification & Marking Systems, Inc., Hornell, NY, USA). The pups were returned to their dams for 1.5–3 h for feeding and recovery from surgery. They were then placed in a hypoxia chamber with 8% humidified oxygen and balanced nitrogen for 2 h with a constant temperature of 36 °C. Sham control subjects were exposed to room air for 2 h. The sham treated subjects that were exposed to 8% oxygen for 2 h are hereafter designated as the hypoxia alone group. The rats in each study group were spread among different litters to account for the potential inter-litter variability (Rice 3rd et al., 1981). Animals in the HI or hypoxia alone groups were sacrificed at various time points after exposure to HI or hypoxia.

2.2. Brain collection and immunohistochemical staining

Brains were obtained from the neonatal rat pups that were exposed to carotid artery ligation and hypoxia (HI), or sham treatment with and without hypoxia. Brains were collected, paraffin-embedded, and sectioned for immunostaining from the sham operated control ($n = 25$) and from neonatal rats at zero (n = 8), 3 (n = 8), 6 (n = 7), 12 (n = 14), 24 (n = 15), and 48 h (n = 14) after exposure to HI. Brains were also collected and similarly treated for immunostaining from the sham operated control group (n = 9) and from neonatal rats at zero (n = 9), 3 (n = 9), 12 (n = 8), 48 h (n = 9) after exposure to hypoxia alone. For the purpose of this report, zero, 3, and 6 h were considered as the early phases of recovery from HI or hypoxia alone, and 24 and 48 h were considered as the later phases of recovery from HI or hypoxia alone.

The pups were first sedated with an intraperitoneal injection of a mixture of ketamine (74 mg/kg) and xylazine (4 mg/kg). The injection was followed by a quick pinch on the hind leg of the animal to ensure adequate sedation. Blood samples were collected from the left ventricle, and brains were perfused with cold saline and 4% paraformaldehyde (PFA) at a flow rate of 3 ml/min. Brains were removed and post fixed in PFA for 24 h. The paraffin-embedded brain tissues were sectioned in coronal planes at 6 μm thicknesses. One coronal section containing the dorsal hippocampus per brain (bregma −3.12 ± 0.6 mm) was utilized

Download English Version:

https://daneshyari.com/en/article/10215431

Download Persian Version:

https://daneshyari.com/article/10215431

Daneshyari.com