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Sestrin2 induced by hypoxia inducible factor1 alpha protects the blood-brain barrier via inhibiting VEGF after severe hypoxic-ischemic injury in neonatal rats

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ABSTRACT

Objective: Hypoxic ischemic (HI) encephalopathy remains the leading cause of perinatal brain injury resulting in long term disabilities. Stabilization of blood brain barrier (BBB) after HI is an important target, therefore, in this study we aim to determine the role of sestrin2, a stress inducible protein which is elevated after various insults, on BBB stabilization after moderate and severe HI injuries.
Methods: Rat pups underwent common carotid artery ligation followed by either 150 min (severe model) or 100 min (moderate model) of hypoxia. 1 h post HI, rats were intranasally administered with recombinant human sestrin2 (rh-sestrin2) and sacrificed for infarct area, brain water content, righting reflex and geotaxis reflex. Sestrin2 was silenced using siRNA and an activator/inhibitor of hypoxia inducible factor1α (HIF1α) was used to examine their roles on BBB permeability.
Results: Rats subjected to severe HI exhibited larger infarct area and higher sestrin2 expression compared to rats in the moderate HI group. rh-sestrin2 attenuated brain infarct and edema, while silencing sestrin2 reversed these protective effects after severe HI. HIF1α induced sestrin2 activation in severe HI but not in moderate HI groups. A HIF1a agonist was shown to increase permeability of the BBB via vascular endothelial growth factor (VEGF) after moderate HI. However, after severe HI, HIF1α activated both VEGF and sestrin2. But HIF1α dependent sestrin2 activation was the predominant pathway after severe HI which inhibited VEGF and attenuated BBB permeability.
Conclusions: rh-sestrin2 attenuated BBB permeability via upregulation of endogenous sestrin2 which was induced by HIF1α after severe HI. However, HIF1α's effects as a prodeath or prosurvival signal were influenced by the severity of HI injury.

1. Introduction

Hypoxic ischemic (HI) encephalopathy remains the major cause of perinatal brain injury, resulting in short and long term disabilities (Burnsed et al., 2015; Chicha et al., 2014). It affects 60% of preterm infants and 1–8 cases per 1000 births (Vannucci, 2000). In moderate to severe neonatal HI, the mortality rate is at 23%–27% prior to discharge, whereas the mortality at 18–22 months follow up is 37%–38% (Gluckman et al., 2005; Shankaran et al., 2005). The criteria used to distinguish between moderate and severe HI was based on level of consciousness (lethargic-moderate; coma-severe), spontaneous activity (decreased-moderate; none-severe), posture (complete extension-moderate; decerebrate-severe), tone (hypotonia-moderate; flaccid-severe), primitive reflexes (weak-moderate; absent-severe) and autonomic system (constricted pupils, bradycardia and periodic breathing – moderate; dilated pupils, variable heart rate and apnea – severe) (Shankaran et al., 2005).

HI is a result of reduced oxygen and blood supply to the brain, which increases blood brain barrier (BBB) permeability hence leading to edema (Bain et al., 2013). The BBB is a highly selective barrier, located at the endothelial cells, to blood borne substances which restricts their entry into the brain via tight and adherence junction proteins. The main tight junction proteins, such as occluding and claudins, are located close to the blood and act as an initial physical barrier to restrict the passage of solutes into the brain whereas the adherence molecules connect the actin cytoskeleton of neighboring cells and are found deeper in the endothelial cells (Yang and Rosenberg, 2011). Both tight and adherence junction proteins play a major role in the regulation of BBB permeability. Thus, impairment of these inter-endothelial junctions as a result of oxidative stress and inflammation, that occur after injury, results in an increase in BBB permeability which can lead to neurological diseases

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(Yang and Rosenberg, 2011). There are a number of studies that have shown that disturbances in BBB function can occur at an early (within 3 h) or late stage (after 24 h) after hypoxia in both adult and neonatal animal models of stroke, which can lead to neurological impairments (Li et al., 2015a; Moretti et al., 2015; Tu et al., 2011; Zhang et al., 2016). Several studies have shown that BBB permeability can begin as early as 2 h after injury, peaking at 6 h and remain elevated all the way up to 24 h after neonatal HI (Ek et al., 2015; Moretti et al., 2015). Thus, early onset, within 3 h of therapeutic window, of BBB permeability, exposes the neonatal brain to longer periods of blood-borne molecules which could result in greater infarct volumes (Moretti et al., 2015). Therefore, BBB stabilization at an early time point is a very important target in neonatal HI injury.

Sestrin2 or Hi 95, a conserved stress-inducible protein, was first discovered in 2001 and was shown to play a role in maintaining homeostasis, cellular repair and in eliminating toxic metabolites as a result of various insults (Budanov et al., 2002). More recent studies have confirmed that sestrin2 is a stress-inducible protein that responds to various insults such as hypoxia, energy deficiency and oxidative stress and has neuroprotective roles thus making it an attractive candidate for targeting after HI (Budanov et al., 2010; Lee et al., 2010; Lee et al., 2013).

Hypoxia inducible factor1α (HIF1α), a member of the hypoxia inducible factor (HIF) family encoded by the HIF1 gene, is a major transcriptional regulator of cellular responses to hypoxia. It has been shown to be a major player in brain development and in HI brain injury and it can exhibit both neuroprotective as well as neurotoxic properties (Fan et al., 2009). HIF1α has been linked with regulating the transcription of erythropoietin, which induces several pathways associated with neuroprotection; however, on the other hand, HIF1α also promotes the expression of vascular endothelial cell growth factor (VEGF), which is related to neovascularization, the formation of microvascular networks, in hypoxic–ischemic brain areas (Fan et al., 2009). HIF1α has also been shown to increase the expression of sestrin2, as seen in mouse epithelial tracheal cells exposed to oxidative stress (Olson et al., 2011). However, other cell types such as human glioblastoma cell and human lung carcinoma cell showed that sestrin2 activation kinetics was distinct from other HIF1α target genes. Sestrin2 is presumed to be activated upon prolonged hypoxia as a consequence of energy deprivation, but not hypoxia itself (Lee et al., 2013). This could be explained as hypoxia is capable of regulating the activation of pro- and anti-apoptotic genes through HIF1-dependent or independent pathways. Previous study demonstrated that suppression of HIF1α ameliorated neonatal brain injury via VEGF after HI (Chen et al., 2008). As the severity of brain injury in the neonatal HI model may vary considerably based on the amount of time the pups are exposed to hypoxia, therefore the signaling pathways involved may vary as well depending on whether the injury is severe or moderate. In our previous study we showed that sestrin2 has anti-apoptotic effects after neonatal HI, but the role of sestrin2 in BBB is still unclear. In this study, we aim to investigate the effects of sestrin2 on BBB stabilization in both severe and moderate HI models as well as examine the link between HIF1α and sestrin2.

2. Materials and methods

2.1. Animals

All procedures carried out on animals in the study have been approved by Loma Linda University Institutional Animal Care and Use Committee. Sprague Dawley rat mothers, with litters of 10–12 pups,

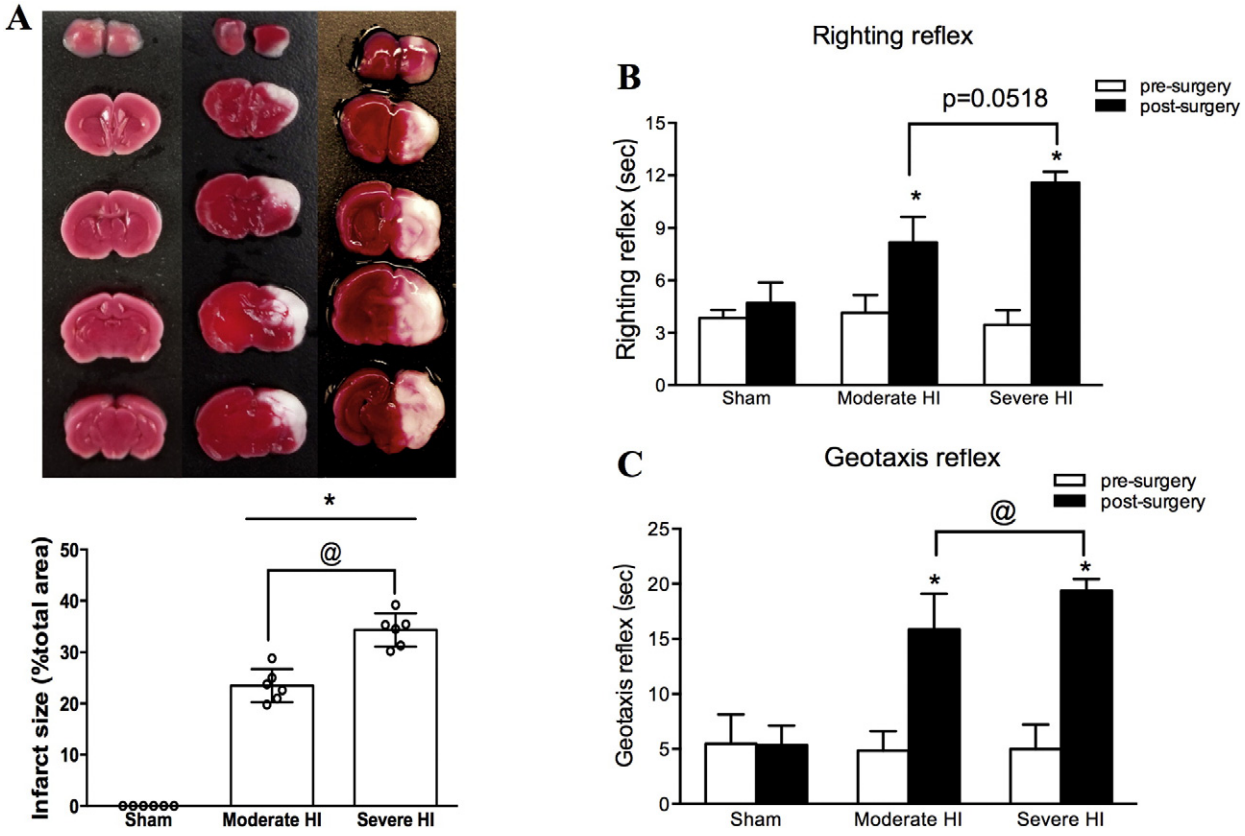

Fig. 1. Moderate and severe neonatal HI encephalopathy models. A: Brain infarct area was significantly increased in severe HI model when compared to moderate one at 24 h. B and C: At 24 h after HI injury, righting reflex and geotaxis reflex showed to be significantly impaired in both moderate and severe models. Rat pups in the severe HI group showed more significant neurological deficits than rat pups in the moderate HI group for geotaxis reflex test but not righting reflex test (*, $p < 0.05$ vs sham. @, $p < 0.05$. $n = 6$ in each group).