High mobility group box-1 protein as a therapeutic target in perinatal hypoxic-ischemic brain injury

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Perinatal hypoxic-ischemic (HI) brain injury is a leading cause of morbidity and long-standing disability in newborns (Millar et al., 2017). Improved neonatal intensive care has increased survival in infants with pregnancy and birth related complications. Nonetheless, many surviving neonates exhibit neurological abnormalities that can persist throughout life (Millar et al., 2017). Early neuroprotective strategies have the potential to improve neurological outcomes and attenuate developmental delay in neonates. However, hypothermia is the only currently approved intervention for HI encephalopathy in full-term infants, which is only partially protective (Millar et al., 2017). Findings in preterm and full-term infants suggest that elevations in pro-inflammatory cytokines are important in the pathogenesis of HI-related brain injury (Millar et al., 2017). The high mobility group box-1 (HMGB1), a representative damage associated-molecular pattern (DAMP) protein, has been reported to be implicated in a variety of brain related inflammatory diseases including traumatic brain injury, epilepsy, and stroke (Nishibori et al., 2019). Anti-HMGB1 therapies have gained increasing interest to treat inflammation related disorders in the brain (Nishibori et al., 2019). However, there is a paucity of information regarding the pathology of HMGB1 in HI-related brain injury during the perinatal period. The current perspective discusses the potential contributions of HMGB1 to HI-related brain injury during the perinatal period and also addresses the potential of HMGB1 as a therapeutic target of the brain injury. Furthermore, this perspective emphasizes the potential for combinational therapeutics for hypothermia with anti-HMGB1 monoclonal antibodies (mAb) in perinatal HI brain injury.

HMGB1 in adult hypoxic-ischemic brain injury: HMGB1 is a non-histone DNA-binding protein localized in the nuclear compartment that plays an important role in the regulation of transcriptional activity, maintenance of chromatin structure and DNA repair (Nishibori et al., 2019). Stress related events stimulate the translocation of HMGB1 from the nucleus to the cytoplasm and then, into the extracellular space. In the extracellular compartment, it functions as a DAMP molecule that binds to pattern recognition receptors including toll-like receptor-4 (TLR-4) and the receptor for advanced glycation end products (RAGE). After binding to these receptors, it stimulates the production of pro-inflammatory cytokines via the nuclear factor-kappa B (NF-κB) and mitogen-activated protein (MAP) kinase signaling pathways (Nishibori et al., 2019).

The progression of HI-related brain injury includes neuronal cellular death that releases danger signals and triggers innate immune responses in the brain (Li et al., 2017). DAMPs including HMGB1 initiate a downstream pro-inflammatory cascade by activating pattern recognition receptors such as TLRs and RAGE on the surface of microglia, astrocytes and brain endothelial cells (Li et al., 2017; Nishibori et al., 2019). Early pro-inflammatory mediators in this cascade include pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, which result in the initiation of post-ischemic neuroinflammation, disruption of the blood-brain barrier (BBB) and infiltration of peripheral leukocytes into the brain parenchyma (Li et al., 2017; Nishibori et al., 2019). Numerous studies have shown that HMGB1 translocation occurs at an early time point during the ischemic phase of brain injury (Nishibori et al., 2019). Zhang et al. delineated the time course of HMGB1 translocation and release from brain cells into the cerebrospinal fluid and the bloodstream in the study of a middle cerebral artery occlusion/reperfusion-induced ischemic injury in adult rats (Zhang et al., 2011; Nishibori et al., 2019). Similar observations have been observed in stroke patients with brain infarction (Zhang et al., 2011; Nishibori et al., 2019). Studies using western immunoblotting have demonstrated a dramatic decrease in HMGB1 in the core ischemic brain regions suggesting release of HMGB1 from severely injured areas (Zhang et al., 2011). In addition, the release of HMGB1 from injured areas is accompanied by alterations in the BBB including swelling of astrocytic endfeet surrounding capillaries, astrocyte detachment from the basal membrane from capillary vessels, and dissociation of the tight junctions between vascular endothelial cells resulting in increases in BBB permeability after ischemic injury (Zhang et al., 2011). Furthermore, treatment of ischemic injury with anti-HMGB1 antibodies prevented the increases of BBB permeability through the maintenance of its structure and clearance of circulating HMGB1 (Zhang et al., 2011).

HMGB1 in hypoxic-ischemic brain injury during the perinatal period: Although there are several differences between HI-related brain injury during the neonatal period and ischemic brain damage after stroke in adults (Millar et al., 2017), disruption of the BBB is also observed in the early hours after HI-related brain injury during the perinatal period. This disruption is associated with a response in the basal lamina, which is comprised of astrocytes, pericytes, and immune cells, all of which could affect BBB function to further exacerbate parenchymal brain injury (Disdier and Stonestreet, 2020). The immediate innate immune response occurs in the early period after the HI-related insults in the neonatal brain. Damage to neurons directly results in the diffuse activation of microglia and astrocytes, which have direct effects on neuronal apoptosis by releasing pro-inflammatory cytokines, such as TNF-α, IL-1β, and IL-6 and reactive oxygen species (ROS) (Li et al., 2017; Millar et al., 2017). Moreover, the release of matrix metalloproteinases (MMPs) and inflammatory mediators (chemokines and pro-inflammatory cytokines) from activated microglia and astrocytes augments the recruitment of peripheral leukocytes, which migrate into the brain parenchyma and release neurotoxic substances, such as pro-inflammatory cytokines, nitric oxide, and ROS, to further predispose to neuronal death (Li et al., 2017). Therefore, BBB disruption predisposes to neuronal damage and could be a potential target for treatment. In this regard, HMGB1 could contribute to HI-related brain injury in the newborn, similar to the findings after stroke in adults. Although numerous studies have demonstrated that ischemic brain injury results in HMGB1 translocation and release from the neuronal nucleus into the brain parenchyma in adult subjects, there is limited information regarding the characteristics of HMGB1 in HI-related brain injury during the perinatal period. Recently, we have shown that HMGB1 is translocated from the nucleus to the cytosolic compartment after ischemic brain injury in the cerebral cortex of the ovine fetal brain and neonatal rat brain (Zhang et al., 2016; Chen et al., 2019). Glial fibrillary acidic protein-positive cells exhibited negative staining of HMGB1 48 hours after HI injury, suggesting that HMGB1 could have been translocated and released from astrocytes at the later phase after HI injury (Chen et al., 2019). These studies suggest that the translocation of HMGB1 may enable the action of this DAMP protein as a pro-inflammatory cytokine to accentuate HI-related injury in the developing brain. Sun et al. (2019) demonstrated that HMGB1 was upregulated in activated microglia after exposure of neonatal subjects to HI and that HMGB1 inhibition alleviated HI-related brain injury. Furthermore, treatment with an HMGB1-specific inhibitor glycyrrhizin reversed HI-related loss of neurons and myelin in the hippocampus and reduced neurobehavioral impairment in a dose-dependent manner. These findings can be interpreted to suggest that HMGB1 could significantly contribute to the neuropathology of hippocampus-related dysfunction and behavioral outcomes after exposure to HI in neonates (Le et al., 2020). Considering these reports, HMGB1 could be a potential therapeutic target of perinatal HI-related brain injury.

Effect of therapeutic hypothermia on HMGB1 in hypoxic-ischemic brain injury: Therapeutic hypothermia in full-term infants is the only approved treatment for perinatal HI encephalopathy (Millar et al., 2017). Lee et al. (2016) demonstrated that therapeutic hypothermia reduces the production of inflammatory cytokines and helps salvage peri-infarct regions from the propagation of ischemic injury by inhibiting HMGB1 in adult rats. This study suggests that one of the therapeutic effects of hypothermia could be attenuation in the expression of HMGB1 and that reductions in HMGB1 could lengthen the therapeutic window for the treatment of stroke (Lee et al., 2016). In neonatal subjects, Nakamura et al. (2013) showed that therapeutic hypothermia resulted in decreased blood concentrations of HMGB1 in human newborns. Although there are currently no studies that have examined the effects of therapeutic hypothermia on HMGB1 release in the brain after exposure to HI-related brain injury during the perinatal period, damage to neurons by the sudden onset of hypoxia-ischemia elicits a rapid release of HMGB1 into the bloodstream after the injury (Chen et al., 2019). Consequently, HMGB1 could be considered a potential target not only in the adults but also in neonates exposed to HI-related brain injury.

HMGB1 as a therapeutic target in perinatal hypoxic-ischemic brain injury: Hypothermia alone is not adequate to prevent all HI-related brain injury or abnormalities during the perinatal period, emphasizing the need for adjunctive therapeutic strategies for use in conjunction with hypothermia (Millar et al., 2017). Davidson et al. (2018) demonstrated a close association between rewarming at 48 hours, subsequent deterioration in the electroencephalogram power along with increases in cortical inflammation, as a result of proliferation in cortical microglia. These findings suggest that inflammation can be activated during rewarming in premature fetal sheep (Davidson et al., 2018). Rewarming after 72 hours of hypothermia was superior to rewarming after 48 hours with regard to improved neuronal survival (Davidson et al., 2018).

Xu et al. (2020) demonstrated that chronic cold exposure results in stress to mice with disruption of homeostasis in the hippocampus, upregulation of inflammatory responses and neuronal injury. Persistent cold exposure activates microglia and acetylated HMGB1, which augmentes neuroinflammation through the HMGB1/TLR4/NF-κB signaling pathways (Xu et al., 2020). The findings in these reports can be interpreted to suggest that inflammation after the rewarming may result from release of HMGB1 from injured cells. In this regard, HMGB1 could be a reasonable biomarker during treatment with hypothermia for HI encephalopathy in the newborn. Further study is required to examine the duration of hypothermia and the elevations in HMGB1 after therapeutic hypothermia, and its effect on neurological sequela. Moreover, these reports suggest that anti-HMGB1 therapies could potentially be an adjunctive therapeutic strategy to hypothermia (Zhang et al., 2016; Chen et al., 2019; Xu et al., 2020). There are several drugs that are able to neutralize the activity of HMGB1 or inhibit its release in models of stroke (Nishibori et al., 2019). Anti-HMGB1 mAb antibodies are a promising intravenous drugs that can protect against BBB disruption in adult subjects after ischemia (Zhang et al., 2011). The anti-HMGB1 mAb not only suppresses the inflammatory responses in the brain and protects the BBB integrity, but also attenuates the translocation and release of HMGB1 from neurons, suggesting the existence of a positive feedback loop between HMGB1 mobilization and brain inflammatory responses (Nishibori et al., 2019). HMGB1 translocation occurs at a very early stage after HI-related brain injury in neonatal subjects (Zhang et al., 2016; Chen et al., 2019). Therefore, early interventions with anti-HMGB1 therapies might be necessary to improve outcomes by attenuating HMGB1 stimulation of inflammation. Nonetheless, futher experiments would be required to evaluate the possibility of anti-HMGB1 therapies as an adjunctive treatment to therapeutic hypothermia in order to further attenuate HI-related brain injury during the perinatal period. In addition, an anti-HMGB1 therapeutic strategy might also be benificial to treat premature subjects with HI-related brain injury. The potential mechanism(s) of HMGB1 derived inflammation and potential anti-HMGB1 therapeutic treatment strategies in perinatal HI brain injury are schematically illustrated in **Figure 1**.

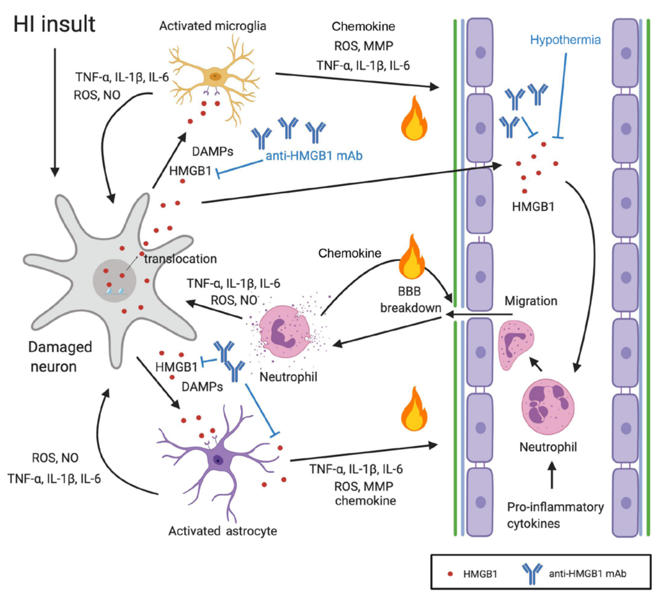

Figure 1 | Potential mechanism(s) of HMGB1 derived inflammation in perinatal HI brain injury and its treatment.
HMGB1 translocates from nucleus to cytoplasm in damaged neurons and is released to extracellular environment as DAMPs in a very early phase of perinatal HI insult. They bind to receptors including RAGE or TLR4 on microglia and astrocytes and activate them. Activated microglia and astrocytes release pro-inflammatory cytokines, chemokines, ROS species and MMPs, resulting in endothelial activation, BBB breakdown and neutrophil attraction. Neutrophils in brain blood vessels are activated and migrate from vessels to brain parenchyma through the damaged BBB. The activated neuroglial cells and migrated neutrophils release pro-inflammatory cytokines, ROS, and NO species, which further enhance neuronal cell death. Anti-HMGB1 monoclonal antibodies (mAb) could neutralize the HMGB1 in the bloodstream and brain parenchyma, inhibiting the neuronal inflammation. Therapeutic hypothermia has been shown to inhibit the HMGB1 in the blood flow after perinatal HI-related insults. This figure is created with BioRender.com. BBB: Blood-brain barrier; DAMPS: damage associated-molecular pattern; HI: hypoxic-ischemic; HMGB1: high mobility group box-1; IL-1β: interleukin-1β; IL-6: interleukin-6; MMPs: matrix metalloproteinases; NO: nitric oxide; RAGE: receptor for advanced glycation end products; ROS: reactive oxygen species; TNF-α: tumor necrosis factor-alpha.

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References

Chen X, Zhang J, Kim B, Jaitpal S, Meng SS, Adjepong K, Imamura S, Wake H, Nishibori M, Stopa EG, Stonestreet BS (2019) High-mobility group box-1 translocation and release after hypoxic ischemic brain injury in neonatal rats. Exp Neurol 311:1-14.

Davidson JO, Draghi V, Whitham S, Dhillon SK, Wassink G, Bennet L, Gunn AJ (2018) How long is sufficient for optimal neuroprotection with cerebral cooling after ischemia in fetal sheep? J Cereb Blood Flow Metab 38:1047-1059.

Disdier C, Stonestreet BS (2020) Hypoxic-ischemic-related cerebrovascular changes and potential therapeutic strategies in the neonatal brain. J Neurosci Res 98:1468-1484.

Le K, Wu S, Chibaatar E, Ali AI, Guo Y (2020) Alarmin HMGB1 plays a detrimental role in hippocampal dysfunction caused by hypoxia-ischemia insult in neonatal mice: evidence from the application of the hmgb1 inhibitor glycyrrhizin. ACS Chem Neurosci 11:979-993.

Lee JH, Yoon EJ, Seo J, Kavoussi A, Chung YE, Chung SP, Park I, Kim CH, You JS (2016) Hypothermia inhibits the propagation of acute ischemic injury by inhibiting HMGB1. Mol Brain 9:81.

Li B, Concepcion K, Meng X, Zhang L (2017) Brain-immune interactions in perinatal hypoxic-ischemic brain injury. Prog Neurobiol 159:50-68.

Millar LJ, Shi L, Hoerder-Suabedissen A, Molnar Z (2017) Neonatal hypoxia ischaemia: mechanisms, models, and therapeutic challenges. Front Cell Neurosci 11:78.

Nakamura T, Yamada S, Yoshioka T (2013) Brain hypothermic therapy dramatically decreases elevated blood concentrations of high mobility group box 1 in neonates with hypoxic-ischemic encephalopathy. Dis Markers 35:327-330.

Nishibori M, Mori S, Takahashi HK (2019) Anti-HMGB1 monoclonal antibody therapy for a wide range of CNS and PNS diseases. J Pharmacol Sci 140:94-101.

Sun Y, Hei M, Fang Z, Tang Z, Wang B, Hu N (2019) High-mobility group box 1 contributes to cerebral cortex injury in a neonatal hypoxic-ischemic rat model by regulating the phenotypic polarization of microglia. Front Cell Neurosci 13:506.

Xu B, Lang LM, Lian S, Guo JR, Wang JF, Liu J, Yang HM, Li SZ (2020) Neuroinflammation induced by secretion of acetylated HMGB1 from activated microglia in hippocampi of mice following chronic cold exposure. Brain Res 1726:146495.

Zhang J, Takahashi HK, Liu K, Wake H, Liu R, Maruo T, Date I, Yoshino T, Ohtsuka A, Mori S, Nishibori M (2011) Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats. Stroke 42:1420-1428.

Zhang J, Klufas D, Manalo K, Adjepong K, Davidson JO, Wassink G, Bennet L, Gunn AJ, Stopa EG, Liu K, Nishibori M, Stonestreet BS (2016) HMGB1 Translocation after ischemia in the ovine fetal brain. J Neuropathol Exp Neurol 75:527-538.

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