HMGB1 and Post-Aneurysmal Subarachnoid Hemorrhage

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Aneurysmal subarachnoid hemorrhage (aSAH) is characterized by a robust sterile inflammatory response immediately after the subarachnoidal bleed. Several damage-associated molecular pattern molecules (DAMPs) are liberated upon injury of the brain cells from different intracellular compartments and have the capability to activate immune cells through the ligation of their cognizant receptors (pattern recognition receptors (PRRs)). Among them, high mobility group box-1 (HMGB1), which acts normally as a transcription factor, when released extracellularly upregulates inflammation due to its interaction with TLR-2, TLR-4 and RAGE during early brain injury after aSAH. However, recent investigations show that different isoforms of HMGB1 exist and may dominate during different phases of the brain injury with different consequences. Surprisingly, the oxidized isoform of HMGB1 plays an anti-inflammatory and pro-resovling role contrary to well accepted pro-inflammatory role of HMGB1 after aSAH.

Keywords: SAH ; CVS ; serum biomarkers ; stroke ; inflammation ; cysteine ; pro-resolving ; anti-inflammatory ; DAMPs

1. Introduction

Subarachnoid hemorrhage (SAH) is a cerebrovascular disease that is characterized by bleeding into the subarachnoid space and sometimes may also accompany intraventricular or intracerebral bleeding [1]. The majority of SAH cases are a consequence of the rupture of an intracranial aneurysm [2][3]. Intracranial aneurysms often occur at the arterial bifurcation sites owing to chronic shear stress and chronic inflammation leading to the formation of weakened bulging lesions in the arterial brain vessels [4][5]. Almost 3–5% of the population harbors these intracranial aneurysms, with a slight prevalence in females [5]. Rupture of intracranial aneurysms introduces blood into the subarachnoid space at high pressure, leading to mechanical damage as well as interference with cerebral perfusion due to elevated intracranial pressure resulting in transient global ischemia. Moreover, the toxic effects of the blood and its derivatives cause stress and damage to the brain cells [1][6]. The bleeding aneurysms can be repaired through endovascular coiling or neurosurgical clipping; however, still, a large proportion of the patients deteriorate and attain poor clinical outcomes [7]. The events occurring over 72 h after aSAH cause early brain injury (EBI), which is characterized by highly upregulated local and systemic inflammation. Further, over the course of post-aSAH sequel, aSAH patients develop life-threatening complications. These include cerebral vasospasm (CVS), acute and chronic hydrocephalus, seizures, delayed cerebral ischemia (DCI), cortical spreading depression (CSD), and local/systemic infections [8]. These complications and ongoing inflammation probably contribute to poor clinical outcomes.

Over the recent years, an ever-expanding list of DAMPs have been discovered with varying natures, e.g., protein DAMPs such as high mobility group box-1 (HMGB1), peroxiredoxins, S100β; nucleic acid-based DAMPs such as nuclear and mitochondrial DNA, and RNA, ATP; extracellular matrix proteins and other components such as hyaluronan, tenascin C; monosodium urate crystals and cholesterol stones; hemoglobin and its derivatives, etc. These diverse molecules with different chemical natures bind to different PRRs belonging to different families and are expressed extracellularly on the cell surface as well as intracellularly on immune and other cells [7]. The accumulating evidence shows that several DAMPs molecules are upregulated after aSAH both at the CNS level and peripherally [7]. Along with these DAMPs, the expression of several PRRs is also upregulated after aSAH [7]. These DAMPs not only serve as the initiators of the acute inflammation, rather they are persistently upregulated to sustain the inflammation after aSAH and during post-aSAH complications [7].

High mobility group box 1 (HMGB1) is one of the non-histone DNA binding proteins that is ubiquitously expressed in the nucleus of all eukaryotic cells. Its name is derived from the fact that it showed high electrophoretic mobility on a polyacrylamide gel and was discovered in 1973 [9]. It is composed of 215 amino acids that consist of two boxes, i.e., HMG Box A (1–79 amino acids) and B (89–162 amino acids), and an acidic tail containing aspartic acid and glutamic acid

residues (186–215 amino acids) [9]. HMGB1 is involved in several vital functions within the nucleus, such as DNA repair, transcription, telomere maintenance, and genome stability [9]. The tail at the C-terminus is involved in the spatial arrangement of the Box A and Box B of HMGB1 and controls the DNA binding specificity of HMGB1 [10]. However, in contrast to these normal functions, HMGB1 can upregulate the inflammatory response extraordinarily due to its DAMP nature once released extracellularly upon cellular injury [11]. HMGB1 binds several pattern recognition receptors (PRRs). The most important of them are TLR-2, TLR-4, and RAGE (receptors for advanced glycation end products) on immune cells to upregulate inflammation [11].

A great body of evidence highlights the inflammatory role of HMGB1 in several different types of diseases (**Table 1**). HMGB1 has been shown to be implicated in the pathophysiology of sepsis, meningitis, pneumonia, systemic vasculitis, atherosclerosis, abdominal aortic aneurysms, cardiac, kidney, respiratory and gastrointestinal diseases, hepatic damage, autoimmune disorders, metabolic disorders, and several types of neoplastic disorders such as renal cell carcinoma, lung cancer, pancreatic cancer, gastric cancer, colorectal cancer, hepatocellular carcinoma, etc. [12].

Table 1. List of different diseases in which HMGB1 levels are altered.

Sr. No.	Disease Condition	Study Type	No. of Patients	Sample Measured	Time Duration of Measurement	HMGB1 Status	Other Biomarkers	Reference
1.	Sepsis and septic shock	Observational cohort study	Derivation cohort: controls = 46, sepsis = 58, septic shock = 84 validation cohort: sepsis = 24, septic shock = 53	Plasma	One time within 48 h	↑sed	RIPK3, MLKL	[13]
2.	Coronary artery disease (CAD)	Observational study	CAD = 98, controls = 30	Serum	Day after admission	↑sed	High sensitivity (hs) CRP, cardiac troponin I	[14]
3.	ST segment elevation myocardial infarction (STEMI) and mortality	Observational study	STEMI patients = 141, healthy controls (HCS) = 42	Plasma	At admission	↑sed after STEMI and doubled in patients who died	Troponin I, creatine kinase myocardium	[15]
4.	Community-acquired pneumonia (CAP)	Subjects drawn from larger genetic and inflammatory markers of sepsis (GenIMS) study	CAP patients = 122, healthy controls = 38	Plasma	After enrolment, daily for 1st week, then weekly until discharge	↑sed	IL-6, IL-10, TNF-α	[16]
5.	Chronic kidney disease (CKD)	Cross-sectional study	CKD = 177, healthy controls = 48	Serum	After overnight fast	↑sed	hs-CRP, TNF-α, IL-6, Hb, HbA_{1c}	[17]
6.	Juvenile idiopathic arthritis (JIA)	Prospective longitudinal study	JIA children = 64, reactive arthritis = 9, HC = 15	Serum	1st visit and at 1st, 3rd, and 6th month follow-up	↑sed	CRP, neutrophils,	

2. Anti-HMGB1 Therapies and Subarachnoid Hemorrhage

Glycyrrhizic acid is an inhibitor of HMGB1, and its application in an in-vitro model of SAH has been shown to antagonize the inflammatory effects of HMGB1. During this research, glycyrrhizic acid application prevented the upregulated release of IL-1β from mixed glial cells subjected to the medium obtained after the application of hemoglobin (Hb) to neuronal

culture in-vitro [18]. Several other natural products such as purpurogallin (a natural phenol) and 4′-*O*-β-d-glucosyl-5-*O*-methylvisamminol (4OGOMV), rhinacanthin-C (an extract from *Rhinacanthus nasutus*), resveratrol, glycyrrhizin, and berberine have been shown to downregulate the expression of HMGB1 in different models of SAH [19][20]. All these potential treatments afforded several beneficial effects due to the antagonism of HMGB1, such as the downregulation of proinflammatory cytokine expression, downregulation of the intracellular inflammatory pathways, reduced brain edema and neuronal apoptosis, reversal of the cerebral vasospasm, and improvement in the neurological function and outcome [7][19]. Besides these natural products, anti-HMGB1 monoclonal antibody administration ameliorates the HMGB1-induced inflammation in a model of SAH, further inhibits the development of CVS, and protects from brain injury [21]. Interestingly, the administration of GSK'872, an inhibitor of RIPK-3, prevented the cytosolic translocation and expression of HMGB1 and necroptosis after experimental SAH and reduced BBB disruption, and improved neurological outcomes [22]. Even exosomes derived from the mesenchymal stem cells and the use of soluble decoy form of RAGE have been shown to protect against the damage due to HMGB1-induced inflammation after experimental SAH [23][24]. These lines of evidence clearly demonstrate an early inflammatory role of the HMGB1 in post-SAH sequelae and the therapeutic value of early antagonism of HMGB1.

3. Pro-Resolving and Protective Effects of HMGB1 and Subarachnoid Hemorrhage

Neurovascular remodeling continues days to weeks after stroke and involves the cross-talk of neurons, glia, endothelial cells, and extracellular matrix components [25]. Reactive astrocytes release numerous growth factors such as nerve growth factor (NGF), vascular endothelial growth factor (VEGF), brain-derived neurotrophic factor (BDNF), and other factors after stroke, which participate in brain tissue remodeling, peri-infarct angiogenesis and repair of the neurovascular units to improve the neurological functional outcomes after brain injury [26][27][28]. As already mentioned above, HMGB1 unveils its second face during the late phase of the stroke involving brain tissue remodeling and repair. HMGB1 secreted by the astrocytes during the late phase of stroke promotes the function of the endothelial progenitor cells, which support the repair of the neurovascular unit and angiogenesis near the infarct territories [26]. Moreover, HMGB1 has already been shown to promote endothelial activation and sprouting [29]. Further, HMGB1 promotes neurogenesis after intracerebral hemorrhage (ICH) in a RAGE-dependent manner [30].

Intriguingly, a study investigating the effects of HMGB1 during the late phase of SAH unveiled the neuroprotective and pro-resolving effects of HMGB1 as opposed to conventional proinflammatory DAMPs-based effects during the early brain injury after SAH [28]. Tian and co-authors utilized ethyl pyruvate and glycyrrhizic acid to inhibit HMGB1, and FPS-ZM1 to inhibit the expression of RAGE. Consequently, authors were able to delineate the reduced expression of HMGB1 and RAGE upon inhibitors administration; however, it was associated with reduced levels of the different neurogenic growth factors such as NGF, VEGF, and BDNF and also reduction in the cortical neurogenesis as reflected by a decrease in BrDU and DXC-positive neurons [28]. It is interesting to note that different isoforms of HMGB1 exist with distinct redox states of the three cysteine residues (C23, C45, and C106) in the box B of the HMGB1 molecule, and these isoforms have been characterized to function differently over the course of the disease [31]. The cysteine residues in reduced and thiolated form have chemotactic properties, whereas disulfide-linked C23-S–S-C45 possess proinflammatory cytokine-like activity, and the fully oxidized and sulfonated form is inert and found during the resolution of inflammation [31]. Therefore, in the study of Tian and colleagues, two modified forms of HMGB1 at C106 were used, i.e., one isoform that was oxidized at the C106 position and the other in which C106 was in the reduced state [28]. So, when the reduced form of HMGB1, which retains the cytokine-inducing capability, was used, it aggravated the brain damage. However, utilization of the oxidized form of HMGB1 with no cytokine stimulating potential led to a decrease in the production of TNF-α and promoted the recovery of the brain through the upregulation of the neurotrophic factors [28]. Furthermore, these effects were also abolished upon antagonism of RAGE, suggesting that the oxidized form of HMGB1 mediates neuroprotective, anti-inflammatory, and pro-resolving effects through RAGE [28].

SAH is characterized by a robust oxidative stress response as evidenced by the progressive decline in glutathione and other antioxidant agents along with an early increase in lipid peroxidation products, which decline with the passage of time in the peripheral circulation of aSAH patients [32]. Hemoglobin and its degradation products liberated from the extravasated SAH blood upregulates the generation of reactive oxygen species (ROS), leading to oxidation of cellular components, and ROS also triggers several subcellular inflammatory pathways [33]. Microglia, the resident immune cells of the CNS, polarize to M1 proinflammatory phenotype during an early phase of SAH and then dynamically shift into M2 anti-inflammatory and tissue reparative phenotype [34]. Interestingly, M1-microglia upregulate the generation of ROS and induce the activation of NF-κB-mediated proinflammatory genes expression early in the inflammatory response. Later on, microglia polarize to an M2 phenotype that is characterized by the upregulation of antioxidant machinery during the late phase of an inflammatory response and supports tissue repair and resolution of inflammation [35]. Interestingly, it is known

that under mild oxidative conditions, C23 and C45 are cross linked through disulfide bridge and exert a potent pro-inflammatory response [36]. Consequently, it has been shown that this disulfide form of HMGB1 is associated with priming and neuroinflammatory response in microglia [37]. Intriguingly, the disulfide isoform of HMGB1 has also been shown to reduce the proliferation of oligodendrocyte precursor cells [38]. However, the redox-dependent dual roles of HMGB1 in connection to the different polarized states of microglia during early and delayed brain injury after SAH are still to be investigated.

The above-mentioned evidence clearly shows the second face of HMGB1, which is evident during the late phase of SAH. Consequently, it is intimidating to further explore the effects of different anti-HMGB1 therapies during the delayed brain injury phase after aSAH. Furthermore, there is also a need to validate the dynamics of different isoforms of HMGB1 over the phases of EBI and DBI and their contribution to different post-aSAH complications and neuroinflammation. Interestingly, it opens new avenues for the development of isoform-selective anti-HMGB1 therapies that may be utilized to curb the damaging effects of inflammation and boost the reparative anti-inflammatory effects.

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