HMGB1

Subjects: Immunology | Pathology

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Aneurysmal subarachnoid hemorrhage (aSAH) is a complex and potentially deadly disease. Despite successful obliteration of aneurysm from the circulation, the clinical outcome of aSAH patients is often poor. The reasons for poor outcomes are numerous, including cerebral vasospasm (CVS), post-hemorrhagic hydrocephalus, systemic infections and delayed cerebral ischemia. Although CVS with subsequent cerebral ischemia is one of the main contributors to brain damage after aSAH, little is known about the underlying molecular mechanisms of brain damage. Damaged central nervous system cells release damage-associated molecular pattern molecules (DAMPs) that are important for initiating, driving and sustaining the inflammatory response following an aSAH. The evidence suggested that HMGB1contributes to brain damage during early brain injury and also to the development of CVS during the late phase. Different pharmacological interventions employing natural compounds with HMGB1-antagonizing activity, antibody targeting of HMGB1 or scavenging HMGB1 by soluble receptors for advanced glycation end products (sRAGE), have been shown to dampen the inflammation mediated brain damage and protect against CVS. The experimental data suggest that HMGB1 inhibition is a promising strategy to reduce aSAH-related brain damage and CVS. Clinical studies are needed to validate these findings that may lead to the development of potential treatment options that are much needed in aSAH.

HMGB1, SAH, CVS, EBI, Inflammation

1. Introduction

HMGB1 is a highly conserved non-histone nuclear protein and an important prototypical protein DAMP that is released both in the CSF and the systemic circulation in clinical and experimental SAH. The interaction between HMGB1 and pattern recognition receptors leads to the activation of downstream signaling pathways (including NF-κB pathways) and consequently to the expression of multiple pro-inflammatory genes.

The treatment of CVS via smooth muscle relaxants, such as calcium antagonists, endothelin receptor antagonists, and Rho-kinase inhibitors, does not significantly improve clinical outcomes. The lack of beneficial effects of smooth muscle relaxants is due to the complex pathophysiology of SAH, where the causal relation to the development of a delayed CVS is poorly understood. Both brain and vascular inflammation are closely related to the development of EBI and a delayed CVS [1]. Recent experimental studies have shown that HMGB1 is released from vascular smooth muscle cells and intracranial vessel walls, which may be the source of circulating HMGB1 as suggested in some clinical studies [2]. A causal relationship between the HMGB1 release and CVS has been established with in vitro experiments showing the reversal of a spastic vascular phenotype after treatment with anti-HMGB1 antibodies

[3]. The release of HMGB1 may induce the expression of pro-inflammatory cytokines and vasoconstriction-inducing receptors, including PAR-1, TXA2 receptor, AT1 receptor and the ET_A receptor via interactions with pattern recognition receptors [3]. As an upstream event, the HMGB1 release during an EBI could theoretically be an excellent target for the treatment of both the EBI and the delayed CVS. Indeed, targeting HMGB1 with monoclonal antibodies or with pharmacological agents have reversed the delayed CVS (Figure 1) in animal SAH models [3].

2. Effects

HMGB1 effects could be mediated by multiple receptors, including TLR-4, TLR-2 and RAGE. Interestingly, these receptors have been shown to be involved in the inflammatory response after the SAH [4][5][6]. For instance, the HMGB1 ligation of the TLR-4 has been shown to activate MMP-9 (Matrix metalloproteinase-9), which contributes to early brain injury after an experimental SAH [7]. Nevertheless, further investigations are needed to establish the exact receptor pathway that is involved in the induction of delayed CVS. Intriguingly, on one hand the HMGB1 ligation of RAGE on monocytes/macrophages has been shown to enhance the ischemic brain damage, and on the other hand, HMGB1-signaling via RAGE drives an IL-10 release from M2-like macrophages (the anti-inflammatory phenotype of macrophages). In line with this notion, serum HMGB1 levels measured within 24 h after the aSAH showed a correlation with a latter increase in serum IL-10 levels measured on day 7 after the aSAH [8]. It is well known that anti-inflammatory mechanisms also upregulate in parallel to pro-inflammatory mechanisms to limit the damage, however, it would be interesting to evaluate further how these pro-inflammatory mechanisms are dominated by anti-inflammatory mechanisms, and how they contribute towards different post-aSAH complications and clinical outcomes, as several lines of evidence also report immunodepression after aSAH [8][9].

Anti-HMGB1 antibody treatment blocked the expression of pro-inflammatory cytokines (including IL-6, TNF, and TLR-4, iNOS) and vasoconstriction-inducing receptors, and reversed the contractile phenotype of the basilar artery and improved neurological outcomes [3]. Furthermore, HMGB1 has been implicated in vascular smooth muscle cell phenotype switching and vascular remodeling, which may underlie the thickened vascular walls along with the reduced intraluminal diameter [10]. These changes ultimately lead to cerebral ischemia and neurological deficits [10]. These results suggest that targeting HMGB1 may be a better option to treat delayed CVS than simply with smooth muscle relaxants as previously done. Another possible mechanism of inflammation-mediated delayed CVS is the expression of COX-2 in the vasculature. COX-2 is a target gene of NF-κB that can be activated by HMGB1 via pattern recognition receptors. Thus, a continuous mobilization of HMGB1 from cerebral vessels, starting early after the aSAH, leads to the expression of pro-inflammatory cytokines and receptors that in turn mediate the CVS. Anti-HMGB1 antibodies are perhaps a new approach to interrupt this cascade of events and to induce a relaxed phenotype of smooth muscle cells, and consequently reduce the CVS and improve the clinical outcomes.

Interestingly, different isoforms of HMGB1 exist after the extracellular release with distinct functionalities and differences in their interactions with various receptors [11][12]. These differences owe to the redox states of three cysteine residues (C23, C45 and C106) in the Box B of the HMGB1 molecule. For instance, when all of these cysteine residues are in a reduced thiolated state, HMGB1 activates RAGE and promotes CXCL12/CXCR4 signaling [12]; whereas the oxidized form of HMGB1 with disulfide linkages between C23–C45 has a greater

propensity to activate TLR-2 and TLR-4 to strongly upregulate inflammation [12]. Furthermore, a fully oxidized and sulphonated form is seen during inflammation resolution and is inert [11]. Intriguingly, an oxidized form of HMGB1 has been shown to play a neuroprotective role during the recovery phase of the SAH (day 14 after the SAH), depicted by an inability to stimulate serum and CSF TNF-α upsurge and enhancing neurotrophin expression, as opposed to a reduced form of HMGB1 [13]. Furthermore, the inhibition of HMGB1 and RAGE signaling during this delayed recovery phase after the SAH (day 14 after the SAH) was associated with a decline in the neurotrophic growth factors (Nerve growth factor (NGF), Brain derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF)) and a reduction in neurogenesis as assessed by BrdU and DCX positive neurons [13]. The inhibition of RAGE by FPS-ZM1 and HMGB1 by ethyl pyruvate and glycyrrhizin also enhanced brain water content and the functional neurological impairment during this delayed recovery phase after SAH [13]. It would be quite interesting to study the dynamics of these distinct isoforms over the course of early brain injury and CVS after the SAH, and the impact of modulating these HMGB1 isoforms on inflammatory changes and neurological function after an experimental SAH. As mentioned earlier, HMGB1–RAGE signaling in macrophages has been shown to enhance ischemic brain damage as well as the secretion of anti-inflammatory cytokine (IL-10 secreted by M2-type macrophages). It would be also quite interesting to elucidate the dynamics of the structurally and functionally different isoforms of HMGB1 over the entire course of early and delayed brain injury in SAH patients, and how they modulate the activity of macrophages involving RAGE and other cognate receptors.

3. Findings

HMGB1 also contributes to coagulation, as depicted by the platelets' aggregation upon the ligation of RAGE, platelet activation and thrombus formation due to HMGB1/TLR-4 signaling and the enhanced expression of tissue factor in monocytes and endothelial cells [14]. Intriguingly, during an early brain injury after SAH, there is also evidence of microvasospasms and microthrombosis and the degree of arteriolar constriction correlates with microthrombotic frequency [15]. These mechanisms could compromise blood flow independently of cerebral perfusion pressure [15]. Furthermore, Clazosentan was also found to be ineffective towards relieving these microvasospasms and improving deficits in the experimental SAH rat model [16]. Previously, a failure of Clazosentan to improve the clinical outcomes of SAH patients led to a renewed interest in exploring the additional mechanisms of brain injury, other than angiographic vasospasm [17]. It might be a new beginning to explore the underpinnings of microvasospasms and exploring the role of inflammation, as inflammation and thrombosis cannot be segregated into distinct events independent of each other. It would be interesting to study the implications of HMGB1 and its cognate receptors in the microvasospasms and microthrombosis and the impact of modulating HMGB1 on the inflammation underlying these events. Thrombomodulin has also been shown to scavenge HMGB1 [18] and it may be employed to study its impact on CVS, microthrobosis and microvasospasms during EBI after aSAH. Altogether, the aforementioned pieces of evidence suggest an indispensable role of HMGB1 after an aSAH and its contribution to aSAH-led complications, especially CVS and poor neurological outcomes. Despite the shift in the traditional paradigm, i.e., from CVS leads to DCI and poor outcomes, towards a complex multifactorial pathophysiology involving varied contributions from EBI, cortical spreading depression and inflammation, there is still a population of CVS patients who develop DCI and poor outcomes [19]. Furthermore, inflammation is

associated with EBI, CVS, DCI and poor outcomes and the inhibition of HMGB1-mediated inflammation could be promising to benefit aSAH patients at increased risk of these complications and poor outcomes.

Due to the presence of an intact blood–brain barrier, pharmacological agents have limited excess to the brain. However, during pathological conditions including cerebral ischemia or subarachnoid hemorrhage, disrupted blood–brain barrier facilitates the access of pharmacological agents to the injured brain. Although there are differences in different rodent SAH models and also differences of the BBB among the various animals used for the SAH, the ischemic insult induces a massive disruption of the BBB [20][21][22]. Furthermore, the observance of the pharmacological effects of anti-HMGB1 antibodies at the CNS level also suggest their access through the permeable BBB. Reduction in the permeability of the BBB after the administration of anti-HMGB1 antibodies may also argue against any further access of these antibodies through the BBB, but may also reflect the culmination of the effects which may no longer be required, suggesting healing based self-termination of the CNS effects. However, pharmacokinetics of anti-HMGB1 antibodies and other anti-HMGB1 molecules remain to be elucidated in these SAH animal models. Recently, radiolabeled antibodies have been imaged to quantify the penetration through the blood–brain barrier. It is also known that antibody penetration across the BBB is more into the brain areas which are severely affected by tumors [23]. However, as mentioned above, strokes may lead to the massive disruption of the BBB and interestingly, ischemic brain regions have also shown greater penetration through the BBB [24]. Glycyrrhizic acid has been evaluated as an adjunctive anti-inflammatory agent to treat depression in a randomized-placebo-controlled clinical trial [25], but similar studies in aSAH could be possible. There is a need to characterize the pharmacokinetics and bioavailability of the drugs at the CNS level (e.g., in the CSF), particularly in patients who underwent extraventricular drain placement. Altogether, the experimental strategies antagonizing HMGB1 await clinical translation to benefit aSAH-afflicted patients by improving their outcomes, as pharmacological interventions in the context of aSAH are still scarce.

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