HMGB1-Mediated Neuroinflammatory Responses

Subjects: Clinical Neurology

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Brain injuries are devastating conditions, representing a global cause of mortality and morbidity, with no effective treatment to date. Increased evidence supports the role of neuroinflammation in driving several forms of brain injuries. High mobility group box 1 (HMGB1) protein is a pro-inflammatory-like cytokine with an initiator role in neuroinflammation that has been implicated in Traumatic brain injury (TBI) as well as in early brain injury (EBI) after subarachnoid hemorrhage (SAH). Herein, we discuss the implication of HMGB1-induced neuroinflammatory responses in these brain injuries, mediated through binding to the receptor for advanced glycation end products (RAGE), toll-like receptor4 (TLR4) and other inflammatory mediators. Moreover, we provide evidence on the biomarker potential of HMGB1 and the significance of its nucleocytoplasmic translocation during brain injuries along with the promising neuroprotective effects observed upon HMGB1 inhibition/neutralization in TBI and EBI induced by SAH. Overall, this review addresses the current advances on neuroinflammation driven by HMGB1 in brain injuries indicating a future treatment opportunity that may overcome current therapeutic gaps.

high mobility group box 1 (HMGB1) traumatic brain injury (TBI) subarachnoid hemorrhage (SAH) neuroinflammation biomarker

1. Introduction

Traumatic brain injury (TBI) is a devastating disorder associated with a major cause of morbidity and mortality leading to significant direct and indirect costs to society. TBI refers to a complex disorder associated with several degrees of contusion, diffuse axonal injury (DAI), hemorrhage, and hypoxia [1]. These effects cumulatively initiate biochemical and metabolic changes causing gradual tissue damage and cell death [2]. Although several agents have shown promising neuroprotective effects against TBI in pre-clinical settings, they failed to improve outcome in clinical trials [3][4]. These poor effects may be attributed to the disparate nature of TBI, with differences in clinical variables such as tissue biomechanics site and severity of injury [5][6].

Despite the extensive advances in clinical and pre-clinical research, the pathomechanism underlying TBI are still elusive. However, there is evidence on the contribution of neuroinflammation and blood–brain barrier (BBB) disruption in driving the secondary damage after brain injury [7]. Improved neurological effects have been reported in experimental TBI upon inhibition of post-traumatic neuroinflammatory response [8][9].

Targeting neuroinflammation to inhibit the secondary damage post-TBI represents an effective strategy in recent days. One of the inflammatory mediators that seem to be implicated in brain injury is the high mobility group box 1 (HMGB1) protein, a pro-inflammatory-like cytokine released actively after activation of other cytokines and passively during cell death [10]. HMGB1 released from stressed and dying brain cells acts as an effective neuroinflammatory mediator [11]. HMGB1 is mainly expressed in all cell types, including neurons and glial cells. Overexpression of extracellular HMGB1 was observed in several neuroinflammatory conditions, including TBI [12], Subarachnoid hemorrhage (SAH) [13], Epilepsy [14], Alzheimer's diseases (AD) [15], Amyotrophic lateral sclerosis (ALS) [16][17] Parkinson's diseases (PD) [18], etc.

Furthermore, HMGB1-mediated neuroinflammatory response has been implicated in several types of brain injury including TBI [19] and early brain injury (EBI) after SAH [13] as well as in cerebral ischemia-induced and hypoxic ischemic (HI)-induced brain injuries [20].

HMGB1 was demonstrated to drive the neuroinflammatory response after TBI leading to secondary damage as evident by its up-regulated expression and release after experimental TBI induction (Figure 1) [21][22]. Similarly, HMGB1-mediated neuroinflammatory response contributes to early brain injury (EBI) after SAH [13]. Active HMGB1 translocation from nuclei to the extracellular space after TBI and SAH was shown to activate neuroinflammatory cascades and rupture the BBB. Of interest, HMGB1-targeted therapies such as an anti-HMGB1 monoclonal antibody (mAb) and the pharmacological inhibitor Glycyrrhizin were effective experimentally in inhibiting the neuroinflammatory response post-TBI and in minimizing the EBI after SAH. This reflects that HMGB1 might be a promising extracellular target against these conditions leading to new treatment opportunities.

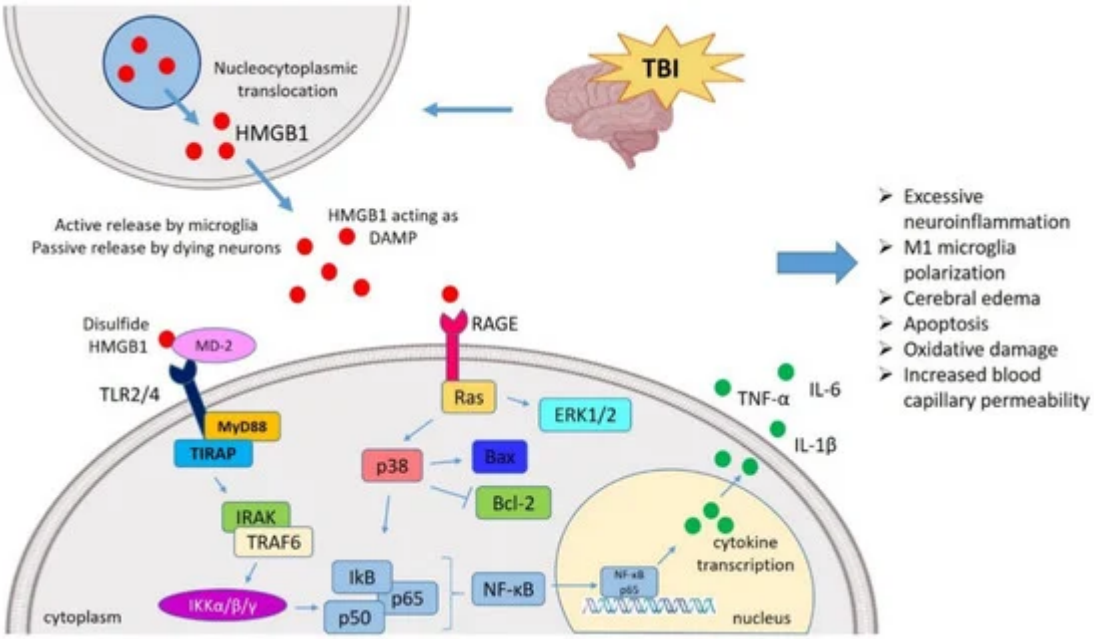

Figure 1. HMGB1 mediated neuroinflammatory response in TBI. TBI induces nucleocytoplasmic translocation of HMGB1 resulting into the release of HMGB1 in extracellular milieu. The extracellular HMGB1 may be partially oxidized at the two cysteine residues generating the disulfide form of HMGB1. The disulfide HMGB1 further binds to its prominent receptor system such as TLR4 and RAGE which in turn interacts with MD-2 and initiates the

MyD88 dependent pathway. It also binds to Ras to initiate the ERK pathway, respectively. HMGB1-TLR4 axis can activate NF-κB signaling both directly and through TRAF6. These pathways ultimately interact with the NF-κB lead to the generation of neuroinflammatory response by producing several pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6). In this way, HMGB1 might mediate the TBI-induced secondary injury where HMGB1 is understood to amplify vicious neuroinflammation, M1 polarization, apoptosis, oxidative damage, cerebral edema, increased BBB permeability. TBI, Traumatic brain injury; HMGB1, High mobility group box 1; TLR4, Toll-like receptor 4; MD-2, Myeloid differentiation factor-2; MyD88, Myeloid differentiation response protein 88; ERK, Extracellular signal-related kinase; NF-κB, Nuclear factor κ light chain enhancer of activated B cells; TNF-α, Tumor necrosis factor-α; IL, Interleukin; BBB, Blood–brain barrier; TRAF6, TNF receptor-associated factor 6.

Herein, we address the role of HMGB1-mediated neuroinflammatory response as a mechanism of secondary injury after TBI and HMGB1-mediated neuroinflammation in EBI after SAH. Moreover, we discuss the emerging preclinical findings of HMGB1-targeting studies demonstrating inhibition of both TBI-induced secondary damage and EBI after SAH.

2. Insights into the HMGB1 Biology

HMGB1 is a 25-kDa DNA-binding protein composed of 215 amino acids, consisting of two DNA bindings sites (Box A and Box B) and a negatively charged *C*-terminal [23][24]. HMGB1 can be secreted extracellularly through active and passive release that occurs in both somatic and immune cells [25]. After reaching to the extracellular milieu, HMGB1 serves as a damage-associated molecular pattern (DAMP) protein and exerts a compelling inflammatory response, engaging several inflammatory mediators. Upon release from neurons and astrocytes, HMGB1 begins the production of several inflammatory markers, including TNF-α, IL-6, and IL-1β [26]. Extracellular HMGB1 interacts with several pathogen recognition receptors (PRRs), namely the toll-like receptor 4 (TLR4) and the receptor for advanced glycation end products (RAGE), to initiate cell migration and production of cytokines [27]. The ability of HMGB1 to interact with several receptors depends on the post-translational redox modifications (PTMs) of the three cysteine residues (at positions 23, 45, and 106 in the box A and B domains of HMGB1) [28].

Moreover, three isoforms of HMGB1 have been identified: the fully reduced HMGB1, the disulfide HMGB1, and the sulphonyl HMGB1. The fully reduced isoform of HMGB1 binds to CXCL12, which further interacts with a great affinity to CXCR4. The extracellular TLR4 adaptor myeloid differentiation factor-2 (MD-2) forms a complex only with the disulfide isoform of HMGB1 (not to any other redox forms) and aggravates the expression of chemokines and cytokines [29] at a comparable level to lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines [30]. Disulfide HMGB1 signaling through TLR4 enhances the activation of nuclear factor-κ light chain enhancer of activated B cells (NF-κB) and consequently the transcription of pro-inflammatory cytokines [31]. Due to its cytokine-inducing potential, HMGB1 has gained increased attention in recent days and was extensively studied in several inflammatory diseases [23].

3. HMGB1 Targeted Therapies against Brain Injuries

In recent days, HMGB1 has emerged as an extracellular target against a diverse range of CNS disorders with HMGB1 involvement, including AD [15], PD [18], MS [32], Epilepsy [33], TBI [12], SAH [13], etc. Moreover, HMGB1 targeted therapy conferred neuroprotective effects against these diseases mainly by inhibiting its expression and release, blocking its translocation, and down-regulating the expression of inflammatory molecules. Common HMGB1 targeting strategies in experimental studies include the use of anti-HMGB1 mAb and the natural HMGB1 inhibitors Glycyrrhizin with its derivatives, and Ethyl pyruvate [34][35] which have recently gained increasing attention as potential therapeutic strategies against several diseases of CNS and of the peripheral nervous system (PNS) [36][37]. In the following sections, the promising outcomes of HMGB1 inhibition by the anti-HMGB1 mAb and Glycyrrhizin in TBI and SAH are discussed.

3.1. HMGB1 Neutralization against TBI

In FPI-induced TBI, treatment with anti-HMGB1 mAb was shown to significantly suppress the HMGB1 translocation in neurons and retain its immunoreactivity in the nuclei. The anti-HMGB1 mAb attenuated neuronal cell death as evidenced by the intact nissl-positive pyramidal neurons and protected BBB integrity based on the significant inhibition of Evans blue leakage (by 88%), impeding the leakage area to the primary lesion area. Moreover, treatment with anti-HMGB1 mAb improved motor functions as evident from the rotarod and cylinder test and suppressed the activation of inflammatory molecules (TNF-α, iNOS, HIF-1α, COX-2, VEGF-A_{189}, and VEGF-A_{165}) [12].

The anti-HMGB1 mAb treatment in the FPI-induced TBI model also suppressed microglia as evident by reduced CD68-positive cells, ameliorated neuronal cell death in the hippocampus based on low TUNEL positivity, inhibited HMGB1 translocation and reduced plasma HMGB1 levels. Moreover, anti-HMGB1 mAb exerted beneficial effects on the impairment of motor and cognitive functions that were present for 14 days post-TBI [38].

Similarly, in a model of intracerebral hemorrhage (ICH)-induced brain injury, the anti-HMGB1 mAb inhibited its translocation into the extracellular space, decreased its serum levels and reduced brain edema while it maintained the permeability of BBB. Moreover, the anti-HMGB1 mAb reduced microglial activation, reduced oxidative stress, ameliorated behavioral performance and down-regulated the expression of inflammation-related mediators (TNF-α, iNOS, IL-1β, IL-6, IL-8R, COX-2, MMP2, MMP9 and VEGF 121) [39]. All these promising findings indicate that anti-HMGB1 mAb therapy might be beneficial against TBI.

The natural small molecule Glycyrrhizin binds directly to both HMG boxes, inhibiting its chemoattractant and mitogenic properties [34] and exerting protective effects against experimental TBI. Glycyrrhizin treatment ameliorated TBI-induced brain edema and beam walking distance, inhibited the translocation of HMGB1 and down-regulated TBI-induced up-regulation of HMGB1, RAGE, TLR4, and NF-κB. It further inhibited cell apoptosis and reduced TBI-induced up-regulation of inflammatory cytokines (TNF-α, IL-1β, IL-6) [40]. Glycyrrhizin conferred neuroprotection in a preclinical model of pediatric TBI by decreasing brain HMGB1 levels, edema, and preventing impairment of spatial and motor learning [21]. Thus, targeting HMGB1 by Glycyrrhizin might reduce the inflammatory cascades and ameliorate the motor function after TBI.

Please note that binding of Glycyrrhizin to HMGB1 is concentration-dependent with an equilibrium dissociation constant (Kd) value of 4.03 μM. Glycyrrhizin blocks HMGB1 binding to its receptor RAGE and inhibits TBI. In turn it inhibits the TBI-induced HMGB1 translocation and suppresses the reduction of HMGB1 from the site of injury. It further inhibits the BBB permeability and down-regulates the expression of inflammatory molecules (TNF-α, IL-1β, IL-6) [41]. It is worth noting that Glycyrrhizin has a wide therapeutic window, as shown by the fact that it is effective even at 6 h post-TBI [41].

It further affects microglial polarization, exerting a neuroprotective effect. In a preclinical model, Glycyrrhizin treatment inhibited HMGB1 expression and release after TBI as evident by the presence of scarce HMGB1 in several nuclei which was completely lost in the injured area of TBI rats. Moreover, it inhibited M1 phenotype and induce M2 phenotype activation of microglia/macrophages (5 days post-TBI) as evident by the reduction in CD86, iNOS, TNF-α, and IL-1β (M1 phenotype markers and functional cytokine mRNA levels) and up-regulation in the markers and the functional cytokine mRNA levels of the M2 phenotype (CD206, Arg1, Ym1, and IL-10) [42].

Ethyl pyruvate is an aliphatic ester developed from an endogenous metabolite [43] that can counteract HMGB1 and neutralize it, further exhibiting biological effects in several diseases [44][45][46]. Its therapeutic potential against experimental TBI relies mainly on HMGB1 inhibition. In a modified Feeney's weight drop model of TBI, treatment with ethyl pyruvate was shown to inhibit TBI-induced up-regulation of HMGB1 and TLR4 expression. Moreover, it inhibited NF-κB DNA-binding activity, reduced expression of inflammatory molecules (TNF-α, IL-1β, IL-6) and ameliorated locomotor function as evident by beam walking performance, along with cerebral edema and cortical apoptotic cell death [47].

Immunohistochemical findings and western blot analysis from the TBI model in rodents revealed that Ethyl pyruvate treatment reduced HMGB1, TLR4 and RAGE expression after TBI in the pericontusional cerebral tissue. Moreover, the treatment protected BBB permeability as evidenced by increased Occludin, Claudin-5, and ZO-1 levels of BBB tight junction binding proteins, increased total antioxidant status, decreased total oxidant status and oxidative stress index [48].

Omega-3 polyunsaturated fatty acid (ω-3 PUFA) possess a neuroprotective effect which is attributed to the modulation of inflammatory pathways [49]. Supplements of ω-3 PUFA have exerted beneficial effects against clinical [50] and experimental model of TBI [51]. Recently, ω-3 PUFA were shown to exert neuroprotective effects against experimental TBI mainly through the modulation of HMGB1 pathway. In an experimental Feeney DM TBI model, ω-3 PUFA supplementation inhibited TBI-induced activation of microglia by fostering a change from the M1 to the M2 phenotype and decreased the inflammatory response (as evident by down-regulation of TNF-α, IL-1β, IL-6, and IFN-γ). It also reduced neuronal apoptosis post-TBI and enabled neuronal recovery mainly by inhibiting HMGB1 release and translocation as well as HMGB1-mediated stimulation of the TLR4/NF-κB signaling axis [52][51].

Altogether these studies indicate that HMGB1 inhibition by the anti-HMGB1 mAb, Glycyrrhizin, Ethyl pyruvate and ω-3 PUFA exhibit promising effects in experimental TBI mainly by inhibiting the secondary damage. However, the safety profile of these HMGB1-targeted therapies needs to be further explored since mAb treatment has been

associated with immunotoxicity and difficult penetration of BBB [53], thus limiting its translational implication. The studies of HMGB1 targeting therapies in TBI are summarized in Table 1.

Table 1. Studies targeting HMGB1 in TBI.

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
1	FPI-induced TBI in adult male Wistar rats	Anti-HMGB1 mAb (1 mg/kg, I.V.) was administered at 5 min and 6 h after TBI	• Anti-HMGB1 mAb prevented neuronal death, attenuated accretion of activated microglia in the rat cortex, inhibited translocation of HMGB1 and ameliorated motor function. • Treatment with anti-HMGB1 mAb exerted beneficial effects on motor and cognitive function (only for 2 weeks after TBI).	[38]
2	ICH-induced brain injury in male Wistar rats	Anti-HMGB1 mAb (1 mg/kg, I.V.) was administered immediately and 6 h after ICH.	• Anti-HMGB1 mAb inhibited the HMGB1 release into the extracellular space, decreased serum HMGB1 levels and reduced brain edema by protecting BBB integrity, reduced activated microglia and decreased the expression of inflammation-related factors (TNF-α, iNOS, IL-1β, IL-6, IL-8R, COX-2, MMP2, MMP9 and VEGF 121). • Anti-HMGB1 mAb administration suppressed oxidative stress and ameliorated behavioral performance.	[39]
3	FPI-induced TBI in adult male Wistar rats	Anti-HMGB1 mAb (1 mg/kg, I.V.) was administered at 5 min and 6 h after TBI	• Anti-HMGB1 mAb reduced FPI-induced brain edema, inhibited translocation of HMGB1, protected BBB integrity, suppressed expression of inflammatory molecules (TNF-α, iNOS, HIF-1α, COX-2, VEGF-A189, and VEGF-A165) and improved motor function.	[12]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
4	CCI-induced TBI in male C57Bl/6 mice	GL (for acute recovery study) (50 mg/kg, I.P.) was administered 1 h, 6 h, 1 d and 2 d post-injury, plus 1 h pre-injury. GL (for chronic recovery study) was administered 1 h pre-injury, at 1 h, 6 h post-injury, plus once daily for 7 added days for 1 week.	• GL reduced brain HMGB1 levels and brain edema at an acute time point of 3 days post-injury (acute outcomes upon HMGB1 neutralization). • Treatment with GL ameliorated short-term spatial memory and motor learning impairments as well as reduced an elevation in hippocampal microglial reactivity (chronic outcomes on HMGB1 inhibition).	[21]
5	TBI induced by modified Feeney's free weight drop method in male SD rats	GL (10 mg/kg, I.V.) administered 30 min after TBI	• GL administration reduced overexpression of HMGB1, TLR4, and RAGE, NF-κB DNA-binding activity and inhibited expression of inflammatory cytokines (TNF-α, IL-1β, and IL-6). • GL treatment decreased brain edema and ameliorated the motor function as evident by beam walking test.	[40]
6	FPI-induced TBI in adult male Wistar rats	GL (0.25, 1.0 or 4.0 mg/kg, I.V.) was administered 5 min after injury	• GL inhibited the translocation of HMGB1 in neurons at the injured area, protected the BBB permeability and ameliorated motor functions. • GL administration inhibited TBI-induced up-regulation of inflammatory molecules (TNF-α, IL-1β, and IL-6) post-TBI.	[41]
7	ICH-induced injury in male SD rats	GL (50 mg/kg) was administered 20 min post-ICH, and then once daily for 3 days.	• GL reduced ICH-induced increase of the brain water content, ameliorated neurological deficit induced by ICH. • Treatment with GL ameliorated ICH-induced neuron loss inside hematoma as evident by	[54]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
			an increased number of NeuN-positive cells.	
8	DAI-induced brain injury in adult SD rats	Glycyrrhizic acid (GA) (10 mg/kg, I.V.) administered 30 min before DAI	• Pre-treatment with GA ameliorated motor and cognitive deficits, inhibited DAI-induced extracellular expression of HMGB1, reduced neuronal apoptosis, protected BBB integrity and inhibited expression of pro-inflammatory cytokines (TNF-α, MMP-9, and IL-6).	[55]
9	Weight-dropping TBI model in male adult SD rats	Ethyl pyruvate (EP) (75 mg/kg, I.P.) prepared at 30 min, 1.5 h, and 6 h	• EP treatment decreased the post-traumatic up-regulation in HMGB-1, TLR4 and RAGE expressions, reduced brain edema, increased BBB permeability as evident by increased expression of occludin, claudin-5 and ZO-1 expression (tight junction proteins of BBB). • EP suppressed proapoptotic bax and active caspase 3 expression, increased anti-apoptotic bcl-2 levels, decreased total oxidant status and oxidative stress and increased total antioxidant status post-TBI.	[48]
10	Feeney's weight drop model in male SD rats	EP (75 mg/kg, I.P.) administered 5 min, 1 h, and 6 h post-TBI	• EP treatment ameliorated performance in beam walking, brain edema, and cortical apoptotic cell death. • EP treatment inhibited expression of HMGB1 and TLR4, NF-κB DNA-binding activity and down-regulated expression of inflammatory mediators (IL-1β, TNF-α, and IL-6).	[47]
11	CCI-induced TBI in male SD rats	Minocycline (90 mg/kg, I.P.) was administered 10 min and 20 h after injury	• Minocycline treatment attenuated nuclear to cytosolic translocation of HMGB1, reduced activation of microglia (in the ipsilateral	[56]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
			cortex, hippocampus, and thalamus), inhibited neurodegeneration (FJB-positive neurons) and delayed motor recovery and improved spatial memory acquisition as evident by MWM test.	
12	Feeney DM TBI model in adult male SD rats	ω-3 PUFA (2 mL/kg, I.P.) was administered 30 min after TBI, each day for 1 week.	• Treatment with ω-3 PUFA demonstrated neuroprotection against TBI by manipulating microglial polarization via SIRT1-mediated deacetylation of the HMGB1-NF-κB signaling axis. • ω-3 PUFA suppressed nucleocytoplasmic translocation of HMGB1, down-regulated acetylation of HMGB1, reduced TBI-induced expression of inflammatory mediators (HMGB1, TNF-α, IL-1β, IL-6), and protected TBI-induced neuronal apoptosis.	[52]
13	Feeney DM TBI model in adult male SD rats	ω-3 PUFA (2 mL/kg, I.P.) was administered 30 min post-TBI, each day for 1 week. [57]	• ω-3 PUFA supplementation inhibited HMGB1 nuclear translocation, reduced the secretion and expression of HMGB1 in neurons and microglia in the lesioned areas. • ω-3 PUFA supplementation reduced TBI-mediated activation of microglia and expression of inflammatory mediators (TNF-α, IL-1β, IL-6, and IFN-γ), lowered brain edema, reduced neuronal apoptosis, and ameliorated neurological functions post-TBI.	[51]

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Exploring and developing therapeutic agents that reduce EBI and delay cerebral vasospasm (CVS) post-SAH have emerged as effective therapeutic strategies. The anti-HMGB1 mAb attenuated the progression of delayed cerebral vasospasm (CVS) mainly by inhibiting HMGB1 translocation in the VSMCs and reducing SAH up-regulated plasma HMGB1 levels. Also, it suppressed the up-regulation of vasoconstriction-mediating molecules (PAR1, TXA2, AT1, ET_A). Moreover, the anti-HMGB1 mAb treatment reduced SAH-induced up-regulation of inflammatory molecules (TLR4, IL-6, TNF-α, and iNOS) and ameliorated the neurological symptoms [13], indicating that anti-HMGB1 mAb interrupts the CVS and brain injury after SAH.

In a preclinical model, Glycyrrhizin was shown to suppress the inflammatory response post-SAH mainly by inhibiting the expression of HMGB1. Other mechanisms underlying Glycyrrhizin treatment include reduction of SAH-induced neuronal apoptosis, reduction of inflammatory cytokines (TNF-α and IL-1β), inhibition of BBB permeability post-SAH and reduction of SAH-induced neuronal degeneration [60]. These findings indicate that Glycyrrhizin protects the brain injury of SAH and may present a potential therapy against HMGB1-induced brain inflammation and injury.

In a double-hemorrhage model of SAH, Glycyrrhizic acid ameliorated neurological outcome, inhibited CVS as evident by the increased diameter of BA, and reduced the thickness of the vascular wall. It reduced HMGB1 expression in the BA, and down-regulated the expression of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) [61], further suggesting that Glycyrrhizic acid exerted neuroprotection on CVS post-SAH, mainly through inhibition of HMGB1 expression and release, and reduction of pro-inflammatory cytokines.

Several other molecules also demonstrated beneficial effects in experimental SAH, including Resveratrol, Purpurogallin, Melatonin, Rhinacanthin-C, and AG490 (inhibitor of JAK/STAT3). These agents mainly inhibit HMGB1 expression and release, as well as its nuclear to cytosolic translocation, further ameliorating neural apoptosis, brain edema and down-regulating SAH-induced inflammatory markers (TLR4, MyD88, and NF-κB, TNF-α, IL-6, and IL-1β) [62][63][64][65][66] (Table 2). It is, therefore, suggested that HMGB1-targeted therapy might regulate a complex series of inflammatory responses contributing to EBI post-SAH, mainly through suppression of the TLR4/NF-κB signaling cascade.

Table 2. Summaries of studies targeting HMGB1 against EBI induced by SAH.

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
1	Endovascular puncture model of SAH adult male Wistar rats	Anti-HMGB1 mAb (1 mg/kg, I.V.) was administered post-SAH, twice at an interval of 24 h.	• Anti-HMGB1 mAb suppressed nuclear translocation of HMGB1, suppressed up-regulation of inflammation-related factors (TLR4, IL-6, TNF-α, and iNOS) and inhibited vasoconstriction-mediating receptors (PAR1, TXA2, AT1, ET_A). • Anti-HMGB1 mAb administration ameliorated neurological symptoms and body weight post-SAH.	[13]
2	Endovascular perforation model of SAH adult male SD rats	Anti-HMGB1 mAb (1 mg/kg, I.V.) was administered post-SAH,	• Anti-HMGB1 mAb attenuated microglial activation, brain edema, and ameliorated neurological dysfunction.	[67]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
		twice at an interval of 24 h.	• Anti-HMGB1 mAb reversed the elevated expression of HMGB1 in the cortex after SAH and reversed VSMC phenotypic switching and vascular remodeling.	
3	Prechiasmatic cistern SAH model in male SD rats	Glycyrrhizin (GL) (15 mg/kg, I.P.) was administered immediately and then 6, 12 and 18 h post-SAH.	• Treatment with GL decreased HMGB1-positive cells, down-regulated mRNA and protein levels of HMGB1, preserved BBB permeability and attenuated neuronal cell death and apoptosis after SAH. • GL treatment suppressed the SAH-induced up-regulation of inflammatory molecules (TNF-α and IL-1β) and significantly improved neurological scores.	[60]
4	SAH in male SD rats	GL (5 mg/kg/day) was administered 24 h prior (precondition) and 1 h post-SAH (treatment).	• GL administration demonstrated anti-inflammatory effects in SAH-induced vasospasms. • Treatment with GL elevated the expression of PPAR-γ protein and mRNA (pre-conditioning) and PPAR-δ mRNA (treatment and preconditioning).	[68]
5	Modified double-hemorrhage SAH model in male SD rats	Glycyrrhizic acid (GA) (10 mg/kg, I.P.) was administered immediately after SAH and was continued for three consecutive days.	• Administration of GA improved neurological function post-SAH reduced SAH-induced increased expression of HMGB1 protein (in a basilar artery) and inflammatory mediators (TNF-α, IL-6, and IL-1β).	[61]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
6	Endovascular perforation induced SAH in male SD rats	AG490 (inhibitor of JAK2/STAT3) (2 mL, I.V.) was administered 30 min before SAH	• Treatment with AG490 after SAH significantly down-regulated JAK2/STAT3 phosphorylation, inhibited HMGB1 expression and its translocation, decreased cortical apoptosis, brain edema and ameliorated neurological deficits.	[62]
7	Prechiasmatic cistern SAH model in male SD rats	Resveratrol (60 mg/kg, I.P.) was administered at 2 and 12 h post-SAH.	• Treatment with Resveratrol exerted neuroprotection by inhibiting an up-regulated expression of HMGB1, TLR4, MyD88, and NF-κB post-SAH, ameliorating neural apoptosis, brain edema, and impairments in neurological behavior impairment.	[59]
8	Double-hemorrhage SAH model in male SD rats	Purpurogallin (100, 200 and 600 mg/kg/day) was administered 1 h after SAH.	• Purpurogallin demonstrated its neuroprotective effects by inhibiting IL-6 and TNF-α mRNA expression and decreasing HMGB1 expression (protein and mRNA). • Purpurogallin also exerted SAH-induced vasoconstriction (dose-dependent), ameliorated neurological deficit as evident by motor deficit index.	[64]
9	Prechiasmatic cistern SAH model in SD rats	Melatonin (150 mg/kg, I.P.) was administered 2 and 24 h after SAH.	• Melatonin exerted neuroprotective effects against SAH and attenuated neurofunctional dysfunction post-SAH mainly by inhibiting expression of HMGB1, TLR4, NF-κB, MyD88, IL-1β, TNF-α, IL-6, and iNOS.	[66]

S.N.	Study Model	Intervention and Dosing Schedule	Observations	References
			• Melatonin treatment also ameliorated spatial learning and memory deficit as evident by the MWM test.	
10	Rodents SAH model in male SD rats	Rhinacanthin-C (RCT-C) (100, 200, and 400 µmol/kg/day) was administered orally 1 h after SAH and every 12 h.	• RCT-C treatment exerted neuroprotection by inhibiting the expression of HMGB1 (protein and mRNA), down-regulating the expression of inflammatory mediators (IL-1β, TNF-α, IL-6,) attenuating brain apoptosis post-SAH (reduced caspase-3- and caspase-9a). • RCT-C attenuated SAH-induced vasoconstriction, reduced GFAP+ microglia and increased NeuN+ neurons compared to SAH animals.	[65]
11	Double-hemorrhage SAH model in male SD rats	A 4OGOMV (100, 200 and 400 µg/kg/day) was administered 1 h post-SAH.	• 4. OGOMV exerted neuroprotective against SAH by attenuating SAH-induced vasoconstriction, ameliorating neurological deficit as evident by improved MDI, inhibiting expression of HMGB1 protein and pro-inflammatory mediators (IL-1β, IL-6, IL-8, and MCP-1).	[69]

GB1 mAb, eritoneal; itric oxide hancer of -activated eceptor-γ; cosyl-5-O- kinase 2;

Current research directions in TBI are mainly focused on preventing the secondary damage and on inhibiting the neuroinflammation that contributes to both acute and chronic stages. HMGB1 has been suggested to participate in the progression of TBI and might represent a promising target. During TBI, cellular damage/death might cause HMGB1 to translocate from the nucleus to extracellular space resulting in microglial activation and leading to further release of HMGB1 [39]. Elucidation of HMGB1 translocation and release process along with HMGB1-driven activation of the TLR4/NF-κB signaling pathway post-TBI may prove beneficial in understanding the mechanism of TBI-induced secondary brain damage [51].

HMGB1-based agents (anti-HMGB1 mAb and Glycyrrhizin) have been shown to exert neuroprotective effects in experimental TBI models including amelioration of neurological outcome, inhibition of translocation of HMGB1, down-regulation of TBI-induced neuroinflammatory responses, prevention of BBB breakdown and reduction of microglial activation [12][41]. Similarly, HMGB1 neutralization inhibited SAH-induced EBI mainly by protecting BBB permeability, blocking HMGB1 expression, release, and translocation, and down-regulating SAH-induced

neuroinflammatory response. These findings suggest that HMGB1 might represent an extracellular target against several forms of brain injury such as TBI and EBI induced by SAH. Nevertheless, when evaluating the beneficial therapeutic effect of HMGB1, it will be important to assess its effect on long-term cognitive function. The prolonged absence of HMGB1 might impair the cognitive function despite its beneficial effects on preventing secondary injury [70]. Moreover, assessment of the duration of beneficial effects upon HMGB1 neutralization/inhibition must be determined [12][38].

Despite the compelling findings from experimental studies, no clinical data reporting beneficial effects of HMGB1-targeted therapies against TBI and SAH-induced EBI are available. Moreover, extensive investigation of HMGB1 targeting agents against brain injuries is warranted since the degree, the location, and the duration of HMGB1 neutralization might be complex and variable due to different insult etiologies [70]. HMGB1 has therefore emerged as an attractive therapeutic target against several HMGB1-mediated inflammatory diseases [23][71]. A future challenge for the clinical translation of HMGB1-based therapies is the development of isoform-specific HMGB1 inhibitors that could suppress the damage without inhibiting tissue regeneration because the thiol-HMGB1 isoform may possess a tissue protecting role in inflammation, injury, and regeneration. All currently available HMGB1 antagonists bind to all the three redox forms of HMGB1 [23] and therefore future studies should be focused on blocking the harmful disulfide-HMGB1 isoform that exerts inflammatory role [23][25].

5. Conclusions

Altogether HMGB1 protein is a key mediator of neuroinflammatory response and contributes to the pathogenesis of TBI as well as to the secondary damage post-TBI and in the EBI following SAH. Moreover, HMGB1 can be used as a biomarker to predict functional outcome after TBI and EBI after SAH while its therapeutic neutralization may prove beneficial in inhibiting the secondary damage following these injuries.

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