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High mobility group box 1 protein as a potential drug target for infection- and injury-elicited inflammation

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Abstract

In response to infection or injury, a ubiquitous nucleosomal protein, HMGB1 is secreted actively by innate immune cells, and / or released passively by injured/damaged cells. Subsequently, extracellular HMGB1 alerts, recruits, and activates various innate immune cells to sustain a rigorous inflammatory response. A growing number of HMGB1 inhibitors ranging from neutralizing antibodies, endogenous hormones, to medicinal herb-derived small molecule HMGB1 inhibitors (such as nicotine, glycyrrhizin, tanshinones, and EGCG) are proven protective against lethal infection and ischemic injury. Here we review emerging evidence that support extracellular HMGB1 as a proinflammatory alarmin(g) danger signal, and discuss a wide array of HMGB1 inhibitors as potential therapeutic agents for sepsis and ischemic injury.

Keywords

innate immune cells; phagocytes; inflammation; cytokines; sepsis; antibodies; HMGB1; tanshinones

1. INTRODUCTION

The innate immune cells (such as monocytes and neutrophils) continuously patrol the body to search for invading pathogens or damaged tissues. Equipped with pattern recognition receptors (such as Toll-like receptors, TLR 2, 4, and 9) [1–3], they can recognize various pathogen-associated molecular patterns (PAMPs, e.g., bacterial peptidoglycan, endotoxin, and CpG-DNA) [3–6], or damage-associated molecular patterns (DAMPs, such as HMGB1) [7–9]. Subsequently, innate immune cells infiltrate into infected/injured tissues [10], and release various cytokines (such as TNF, IL-1, IL-6, and IL-12) and chemokines (such as IL-8, MIP-1s, MIP-2 and MCP-1) [11–13]. These biological responses to microbial infection or injury, collectively termed “inflammation” (“*set on fire*”, in Greek), serve to remove invading pathogens and to heal the wound [14]. In case of severe infection or injury, the inflammatory responses may become dys-regulated, resulting in excessive accumulation of potentially injurious proinflammatory mediators (such as HMGB1). Here we briefly review evidence to support extracellular HMGB1 as a potential therapeutic target for inflammatory diseases.

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2. NUCLEAR HMGB1 AS A TRANSCRIPTION FACTOR

HMG-1 was first purified from nuclei in the 1970's, and termed "high mobility group" (HMG) protein to reflect its rapid mobility on SDS-PAGE electrophoresis gels [15]. Recently, HMG-1 was renamed as high mobility group box 1 (HMGB1) by a nomenclature committee [16]. It is constitutively expressed in many types of cells, and a large "pool" of preformed HMGB1 is stored in the nucleus, possibly due to the presence of two lysine-rich nuclear localization sequences [17]. As an evolutionarily conserved protein, HMGB1 shares 100% homology (in amino acid sequence) between mouse and rat, and a 99% homology between rodent and human [18–20]. It contains two internal repeats of positively charged domains ("HMG boxes" known as "A box" and "B box") in the N-terminus (Figure 1), and a continuous stretch of negatively charged (aspartic and glutamic acid) residues in the C-terminus. These HMG boxes enable HMGB1 to bind chromosomal DNA, and fulfill its nuclear functions including determination of nucleosomal structure and stability, and regulation of gene expression [21]. The tertiary structure of full-length HMGB1 is still unknown, because the highly charged properties of the C-terminal tail make it difficult to crystallize the full-length protein.

3. ACTIVE RELEASE

In response to exogenous microbial products (such as endotoxin or CpG-DNA) [5,6], or endogenous host stimuli (e.g., TNF, IFN- γ , or hydrogen peroxide) [5,22,23], innate immune cells actively release HMGB1 into the extracellular space. Lacking a leader signal sequence, HMGB1 can not be actively secreted via the classical ER-Golgi secretory pathway [5]. Instead, activated macrophages/monocytes acetylate lysine residues within the nuclear localization sequences, leading to sequestration of HMGB1 within cytoplasmic vesicles and subsequent release [17,22,24]. In addition, phosphorylation of serine residues may also be important for endotoxin-induced nucleo-cytoplasmic translocation of HMGB1 [25]. This process is potentially mediated by protein kinases such as the Calcium/Calmodulin-Dependent Protein Kinase (CaMK) IV [26] and calcium-dependent protein kinase C (cPKC) [27].

In vivo, bacterial infection induced HMGB1 nucleo-cytoplasmic shuttling in tissue (alveolar macrophages, which was associated with a decrease in TNF production [28], a phenomenon termed "macrophage suppression". This immunosuppression may be attributable to HMGB1 depletion from the nucleus, where HMGB1 regulates TNF gene transcription by binding to a cis-acting regulatory element (spanning from –157 to –137 bp of the 5'-flanking region) of the TNF gene [29].

The mechanisms underlying the regulation of endotoxin-induced HMGB1 release are poorly understood. The important roles for endotoxin receptors (such as CD14 and TLR4) are supported by the observations that endotoxin induced less HMGB1 release in CD14-deficient Balb/C or TLR4-defective C3H/HeJ murine macrophages (Figure 2) [5,27]. The JNK mitogen-activated protein kinase (MAPK) has been implicated in the regulation of HMGB1 release, because specific inhibitors for JNK (but not p38 and ERK1/2) MAPK partly attenuated endotoxin- or hydrogen peroxide-induced HMGB1 release [30,31]. Early proinflammatory cytokines (e.g., TNF, IFN- γ or IFN- β) may contribute to HMGB1 release, because inhibition of their expression (by gene knock-out) or activities (by neutralizing antibodies) partially attenuates endotoxin-induced HMGB1 release (Figure 2) [31–33]. Although some late proinflammatory mediators [e.g., the 14 kDa phospholipase A₂ (PLA₂) and inducible nitric oxide synthase (iNOS)] have also been implicated in the regulation of endotoxin-induced HMGB1 release [24,30,34–36], it is not yet known whether endotoxin induced less HMGB1 release in PLA₂- or iNOS-deficient macrophages. Conversely, HMGB1 could activate expression of PLA₂, and consequently enhance production of lipid mediators (such as prostaglandin E₂) in vascular smooth muscle cells [37]. The HMGB1-mediated activation of

lipid mediator pathways became more dramatic if these cells were pre-sensitized with proinflammatory cytokines (such as IL-1 β), which elevated expression of HMGB1 receptors [including the receptor for advanced glycation end products (RAGE), and toll-like receptors 2 (TLR2 and TLR4)] in these vascular smooth muscle cells [37].

4. PASSIVE LEAKAGE

In addition, HMGB1 can be passively released from necrotic cells [38], or cells infected by viruses (e.g., West Nile, Salmon anemia, Dengue, and influenza viruses) [39–42] or mycobacteria [43,44]. It is possible that HMGB1 passively released by necrotic cells functions as a damage-associated molecular pattern (DAMP) that allows innate immune cells to respond to injury. Notably, necrotic cells also release other HMG box-containing proteins such as the mitochondrial transcription factor A, TFAM, which has recently been shown to similarly amplify innate immune response to necrotic cells [45].

5. EXTRACELLULAR HMGB1 AS AN ALARMIN SIGNAL

Recently, a number of ubiquitous, structurally and functionally diverse host proteins [such as HMGB1 and heat shock protein 72 (Hsp72)] have been categorized as “alarmins” based on the following shared properties [46] (Figure 3).

5.1. Stimulating cell migration

Accumulating evidence indicate that HMGB1 can stimulate migration of various types of cells including neurite [47], smooth muscle cells [48], tumor cells [49], mesoangioblast stem cells [50,51], monocytes [52], dendritic cells [53,54], and neutrophils [55,56] (Figure 3). It raises a possibility that extracellular HMGB1 may facilitate recruitment of innate immune cells to sites of infection or injury [57], thereby functioning as a potential host cell-derived chemotactic factor [58].

5.2. Facilitating innate recognition of microbial products

Emerging evidence have suggested that HMGB1 can bind and facilitate innate recognition of various bacterial products (e.g., CpG-DNA or LPS) by innate immune cells (such as macrophages and dendritic cells) to ensure a more pronounced inflammatory response [6,59, 60]. For instance, HMGB1 co-localizes with TLR9 and CpG-DNA in macrophage cytoplasmic vesicles [6], and augments CpG-DNA-driven cytokine production in macrophage cultures [6, 59]. It thus appears that innate immune cells have evolved a mechanism to utilize HMGB1 to detect low levels of bacterial products to initiate a rigorous inflammatory response. In addition, HMGB1 may also bind many endogenous molecules such as thrombomodulin [61], immunoglobulin (e.g., IgG1) [62], IL-1 [63], or nucleosomes derived from apoptotic cells [64]. Different host factors, on the other hand, affect HMGB1-mediated inflammatory responses in a negative [61] or positive fashion [63,64].

5.3. Activating innate immune cells

A number of studies have suggested that extracellular HMGB1 binds to the receptor for advanced glycation end products (RAGE), and pattern-recognition receptors such as TLR2 and TLR4 [65,66]. Consequently, HMGB1 activates innate immune cells [29,65–69] or endothelial cells [70,71] to produce proinflammatory cytokines, chemokines [69], tissue factor [72] and adhesion molecules (Figure 3). *In vitro*, one of the DNA-binding domains of HMGB1, the “A box”, functions as an antagonist of HMGB1 [73–75]. In contrast, another DNA-binding domain, the “B box”, recapitulates the cytokine activity of full length HMGB1 [8,76]. Interestingly, oxidation of HMGB1 by reactive oxygen species (ROS) enables formation of disulfide bond involving thiol group of Cys¹⁰⁶, Cys²³ or Cys⁴⁵, and consequently abolishes

HMGB1-mediated immunostimulatory activities [77]. Because Cys¹⁰⁶ is located within the 18-amino acid cytokine domain of HMGB1 B box (Figure 1), it will be important to investigate whether oxidization similarly affects HMGB1-mediated cytokine production.

In addition to RAGE [78] and TLR2/4 [66,67], HMGB1 can also interact with other cell surface receptors such as CD24 [9], and this interaction enables innate immune cells to distinguish between DAMP- versus PAMP-elicited inflammatory response [9]. Furthermore, phage display assays revealed that HMGB1 can recognize peptide motifs in several oncogenes including p53 and the retinoblastoma susceptibility protein (pRb) [79,80]. The HMGB1-RB interaction is critical for HMGB1-mediated inhibition of tumor cell proliferation, because HMGB1 mutant lacking the Rb-binding consensus sequence (LXCXE) lost its anti-tumor properties [80]. Thus, HMGB1 may be involved in the stabilization and/or assembly of many multifunctional complexes through protein-protein interactions.

5.4. Inhibiting phagocytotic elimination of apoptotic neutrophils

During infection or injury, macrophages are also responsible for eliminating apoptotic cells, and rely on cell surface receptors for phosphatidylserine (PS) to recognize them. Interestingly, HMGB1 could interact with PS on cell surface of apoptotic neutrophils, and consequently inhibit phagocytotic elimination of apoptotic neutrophils by macrophages (Figure 3) [81]. Inefficient elimination of apoptotic cells may lead to excessive accumulation of late apoptotic and/or secondary necrotic cells, which may cause passive leakage of HMGB1 and other DAMPs [82]. Considered together, these studies indicate that extracellular HMGB1 can function as an alarmin signal to recruit, alert and activate innate immune cells, thereby sustaining a potentially injurious inflammatory response during infection or injury.

6. HMGB1 AS A LATE MEDIATOR OF EXPERIMENTAL SEPSIS

Sepsis refers to a systemic inflammatory response syndrome resulting from a microbial infection. A wide array of pro-inflammatory cytokines including TNF [83], IL-1 [84], IFN- γ [85], and macrophage migration inhibitory factor (MIF) [86,87] individually or in combination, contribute to the pathogenesis of lethal systemic inflammation. For instance, neutralizing antibodies against TNF [83], reduces lethality in an animal model of endotoxemic/bacteremic shock. However, the early kinetics of systemic TNF accumulation makes it difficult to target in clinical setting [83], prompting the investigation of other late proinflammatory mediators (such as HMGB1) as potential therapeutic target for inflammatory diseases.

6.1. Delayed systemic HMGB1 accumulation

The prevailing theories of sepsis as a dys-regulated systemic inflammatory response are supported by extensive studies employing various animal models of sepsis, including endotoxemia and peritonitis induced by cecal ligation and puncture (CLP) [14,88]. In murine models of endotoxemia and sepsis, HMGB1 is first detectable in the circulation eight hours after the onset of diseases, subsequently increasing to plateau levels from 16 to 32 hours [5, 73]. Meanwhile, tissue HMGB1 mRNA levels are increased in various tissues such as muscle, liver, and lung during endotoxemia [89] or burn-induced sepsis [90]. This late appearance of circulating HMGB1 precedes and parallels with the onset of animal lethality from endotoxemia or sepsis, and distinguishes itself from TNF and other early proinflammatory cytokines [91] (Figure 4). Although circulating HMGB1 levels in un-fractionated crude serum of septic patients did not correlate with disease severity [92,93], its levels in the < 100 kDa sub-fraction (following ultrafiltration through filters with M.W. cut-off) correlated well with the lethal outcome of human sepsis [5,14]. It supported the notion that HMGB1 can interact with other serum components such as thrombomodulin [61], immunoglobulin (e.g., IgG1) [62] to form large (> 100 kDa) complexes [14].

6.2. HMGB1-mediated injurious inflammatory responses

Administration of exogenous HMGB1 to mice recapitulates many clinical signs of sepsis including fever [94], derangement of intestinal barrier function [95], and tissue injury [96–99]. In the brain, exogenous HMGB1 induces the release of proinflammatory cytokines [100] and excitatory amino acids (such as glutamate) [101] and fever [94]. In the lung, HMGB1 induces neutrophil infiltration and acute injury [96–99]. Focal administration of HMGB1 near the sciatic nerve induces unilateral and bilateral low threshold mechanical allodynia [102]. Similarly, intraperitoneal injection of HMGB1 induces peritoneal infiltration of neutrophils [56], and accumulation of cytokines (e.g., TNF and IL-6) and chemokines (e.g., MCP-1).

6.3. Protective effects of anti-HMGB1 antibodies

The pathogenic role of HMGB1 as a late mediator of lethal endotoxemia was previously examined using HMGB1-specific neutralizing antibodies, which conferred a dose-dependent protection against lethal endotoxemia [5], and reversed endotoxin-induced gut barrier dysfunction [103]. In a more clinically relevant animal model of sepsis (induced by CLP), delayed administration of HMGB1-specific neutralizing antibodies beginning 24 h *after* the onset of sepsis, dose-dependently rescued mice from lethal sepsis [73,104]. Taken together, these experimental data establish extracellular HMGB1 as a critical late mediator of experimental sepsis, with a wider therapeutic window than early proinflammatory cytokines (Figure 4).

7. HMGB1 AS AN EARLY MEDIATOR OF ISCHEMIC INJURY

Emerging evidence have suggested that HMGB1 can be released from ischemic, damaged, or dying cells and tissues during ischemia/reperfusion, and trigger a potentially injurious innate immune response [105] (Figure 4). In contrast to the delayed systemic HMGB1 accumulation in experimental sepsis, HMGB1 release occurs quickly in patients with hemorrhagic shock [106] or traumatic injury [107]. Consequently, circulating HMGB1 levels are elevated within 2–6 hours after onset of hemorrhagic shock and traumatic injury [102,103]. In animal models of hepatic ischemic/reperfusion injury, prophylactic administration of HMGB1-neutralizing antibody conferred protection against hepatic I/R injury in mice [108–112]. Similarly, treatment with HMGB1 antagonist (such as HMGB1 box A) significantly reduced myocardial [113] and cerebral [114,115] ischemic injury. Notably, anti-HMGB1 agents are not protective in TLR4-defective [111] or RAGE-deficient mutants [113,116], indicating a potential role for TLR4 or RAGE in HMGB1-mediated ischemic injury. The potential involvement of RAGE in HMGB1-mediated ischemic injury was further supported by the observation that genetic RAGE deficiency and the decoy soluble RAGE receptor similarly reduced cerebral ischemic injury [115].

In addition, HMGB1-specific neutralizing antibodies have been proven protective against ventilator-induced acute lung injury [117], severe acute pancreatitis [118], and hemorrhagic shock [106], supporting a pathogenic role for extracellular HMGB1 in various inflammatory diseases. Although elevated serum HMGB1 levels were associated with adverse clinical outcomes in patients with myocardial infarction [119], prolonged blockade of HMGB1 with neutralizing antibodies (for 7 days) impaired healing process in animal models of myocardial ischemia/reperfusion. Therefore, like other cytokines, there may be protective advantages of extracellular HMGB1 when released at low amounts [120,121]. Indeed, HMGB1 is capable of attracting stem cells [50], and may be important for tissue repair and regeneration [14,120]. It is thus important to pharmacologically modulate, rather than abrogate, systemic HMGB1 accumulation to facilitate resolution of potentially injurious inflammatory response.

8. POTENTIAL HMGB1-INHIBITING THERAPEUTIC AGENTS

With a limited number of effective therapies available for inflammatory diseases, it is important to search for other agents capable of inhibiting clinically accessible mediators. Below is a list of agents that have been proven protective against experimental sepsis and ischemic injury partly through attenuating systemic or local HMGB1 accumulation (Table 1).

8.1. Potential therapeutic agents for sepsis

8.1.1. Antibodies—In animal model of sepsis, intravenous administration of IFN- γ antibodies (1.2 mg/kg), immediately or 24 h after CLP decreased peritoneal and serum HMGB1 levels, and consequently attenuated CLP-induced animal mortality [32]. It suggests that specific inhibition of HMGB1-stimulating proinflammatory cytokines may attenuate sepsis-induced HMGB1 accumulation, thereby protecting animals against lethal sepsis.

In addition to cytokine-specific neutralizing antibodies, immunoglobulins (IgG, antibodies) pooled from the plasma of many healthy blood donors, the intravenous immunoglobulin (IVIG), have also been shown to be protective against sepsis-induced lung injury and lethality by attenuating systemic HMGB1 release [122]. Because human IgGs may potentially interact with HMGB1 in vitro [62], it is not known whether IVIG attenuates systemic HMGB1 accumulation, or merely interfere with ELISA detection of HMGB1 [14].

8.1.2. Anti-coagulant agents—Antithrombin inhibits the pro-coagulant activities of thrombin upon interaction with heparin or related glycosaminoglycans. Although antithrombin III (AT-III) failed to reduce mortality rate in large sepsis clinical trial [123], a recent study suggested that AT-III attenuated endotoxin-induced systemic HMGB1 accumulation, and reduced endotoxemic lethality [124]. Another anti-coagulant protein, thrombomodulin can bind thrombin to inhibit its pro-coagulant activities, and enhance its capacities to activate a plasma anticoagulant, activated protein C. Interestingly, soluble human thrombomodulin (ART-123) is capable of binding to HMGB1 protein [61], thereby inhibiting HMGB1-mediated inflammatory response. Furthermore, ART-123 conferred significant protection against lethal endotoxemia [61,125], and endotoxin-induced acute liver injury [125]. However, it is not yet known whether ART-123 is protective in clinically relevant animal models of sepsis.

8.1.3. Endogenous hormones—A number of endogenous hormones such as insulin [126], neuropeptides [e.g., vasoactive intestinal peptide (VIP), the pituitary adenylate cyclase-activating polypeptide (PACAP), and urocortin] [127], and ghrelin have been shown to be protective against lethal endotoxemia or sepsis partly by attenuating systemic HMGB1 accumulation. For instance, acute infusion of insulin conferred protection against lethal endotoxemia [126], although it is not yet known whether the protective effects are dependent on insulin's anti-inflammatory activities or glucose-modulating properties [128]. In animal models of sepsis induced by CLP, administration of VIP or urocortin attenuated systemic HMGB1 accumulation, and consequently reduced animal lethality [127]. Another member of the VIP family, the PACAP, was recently shown to be protective against lethal endotoxemia via a HMGB1-inhibiting mechanisms [129].

Ghrelin is a stomach-derived hormone responsible for regulating the appetite – increasing it before food intake and decreasing it afterward. Plasma ghrelin levels are decreased in septic animals [130], whereas administration of exogenous ghrelin promoted a dose-dependent protection against sepsis-induced acute lung injury and lethality [130–132]. Notably, ghrelin exerts its protective effects through multiple mechanisms, such as by attenuating systemic HMGB1 release, and by facilitating bacterial elimination [132,133]. Furthermore, ghrelin attenuates systemic accumulation of proinflammatory cytokines partly via the vagus nerve

[131,133], supporting the notion that pharmacologic stimulation of the vagus nerve may be an effective therapy for experimental sepsis.

8.1.5. Vagus nerve stimulation—It has recently been suggested that the brain can attenuate peripheral innate immune response through efferent vagus nerve signals to tissue-resident macrophages [134]. This effect is mediated by the principle neurotransmitter of the vagus nerve, acetylcholine, which de-activates macrophages via nicotinic cholinergic receptors [134]. Indeed, stimulation of the vagus nerve by physical methods (e.g., electrical or mechanical) [135,136] or chemical agents (such as cholinergic agonists, nicotine, choline and GTS-21) [137–139] conferred protection against lethal endotoxemia and sepsis partly by attenuating systemic HMGB1 accumulation.

A chemical derivative of choline, stearyl lysophosphatidylcholine, was proven protective against experimental sepsis by stimulating neutrophils to destroy ingested bacteria in an H₂O₂-dependent mechanism [140]. However, stearyl LPC also conferred protection against lethal endotoxemia [140], implying that it might exert protective effects through an additional, bactericidal-independent mechanism [141]. We found that administration of stearyl LPC significantly attenuated circulating HMGB1 levels [34], indicating that stearyl LPC protects against experimental sepsis partly by facilitating elimination of invading pathogens, and partly by attenuating systemic HMGB1 accumulation [141].

8.1.6. Chinese medicinal herbs—Traditional herbal medicine has formed the basis of folk remedies for various inflammatory ailments. After screening several dozens of commonly used Chinese herbs [142], we found that aqueous extracts of Danggui (*Angelica sinensis*), Green tea (*Camellia sinensis*), and Danshen (*Salvia miltorrhiza*) efficiently inhibited endotoxin-induced HMGB1 release, and protected animals against lethal endotoxemia and sepsis [35, 36,143].

8.1.6.1. Danggui: Danggui has been regarded as the “ginseng” for women, and traditionally used to treat many gynecological disorders. We found that its aqueous extract dose-dependently inhibited LPS-induced HMGB1 release in macrophage and monocyte cultures, partly by interfering with HMGB1 cytoplasmic translocation [35]. Furthermore, Danggui extract rescued mice from lethal sepsis even when the first dose was given at 24 h post onset of disease [35]. The active components responsible for these beneficial effects remain to be characterized.

8.1.6.2. Green tea: Brewed from the leaves of the plant, *Camellia sinensis*, Green tea contains a class of biologically active polyphenols called catechins. As a major catechin, the epigallocatechin (EGCG), effectively inhibited endotoxin-induced HMGB1 release, and attenuated HMGB1-mediated production of nitric oxide by preventing accumulation/clustering of exogenous HMGB1 on macrophage cell surface [143]. Repeated administration of EGCG conferred a dose-dependent protection against lethal endotoxemia, and rescued animals from lethal sepsis even when the first dose of EGCG was given at +24 h after onset of sepsis [143, 144]. The protective effects were associated with a decrease in circulating levels of HMGB1, as well as two surrogate markers of experimental sepsis (such as IL-6 and KC) [143,145].

8.1.6.3. Danshen: Danshen, a medicinal herb widely used in China for patients with cardiovascular disorders [146,147], contains abundant red pigments (termed tanshinone I, tanshinone IIA, and cryptotanshinone). These tanshinones selectively attenuated LPS-induced HMGB1 release in macrophage cultures in a glucocorticoid receptor-independent mechanism [36], despite a structural resemblance (i.e., the presence of a four-fused-ring structure) between tanshinones and steroidal anti-inflammatory drugs (such as dexamethasone and cortisone). Administration of a water-soluble derivative (sodium sulfonate) of tanshinone IIA, TSN IIA-SS, beginning at +24 h, followed by additional doses at +48, +72 and +96 h after the onset of

sepsis, dose-dependently rescued mice from lethal sepsis [36]. Administration of TNS IIA-SS dose-dependently attenuated circulating HMGB1 levels in septic mice [36], suggesting that TSN IIA-SS confers protection partly by inhibiting systemic HMGB1 accumulation.

8.1.7. Others—Ethyl pyruvate (EP) is an aliphatic ester derived from pyruvic acid, a final product of glycolysis [148]. Like other HMGB1 inhibitors, EP dose-dependently inhibits LPS-induced HMGB1 release, and rescued mice from lethal sepsis even when the first dose was given at 24 hours after the onset of disease [149]. A platinum-based chemotherapy drug, cisplatin, can be converted into $[\text{PtCl}(\text{H}_2\text{O})(\text{NH}_3)_2]^+$, which can covalently binds to DNA and triggers apoptotic cell death. In a murine model of CLP-induced sepsis, early administration of cisplatin attenuated systemic HMGB1 accumulation, and reduced animal lethality [150]. A ubiquitous biogenic molecule, spermine, is passively released by damaged cells, and functions as a local feedback anti-inflammatory mechanism at sites of infection or injury [151–154]. Intriguingly, it confers significant protection against lethal sepsis only when the first dose was given immediately (0.5 h) post CLP [155]. The protective effects were associated with a significant reduction in peritoneal levels of HMGB1 and several surrogate markers of sepsis, including IL-6, KC, MCP-1, MIP-2, TIMP-1, and sTNFRs [155].

8.2. Potential therapeutic agents for ischemic injury

Many agents capable of inhibiting infection-elicited HMGB1 release are also protective against ischemia-elicited inflammatory responses. For instance, cisplatin was shown to be protective in a murine model of hepatic ischemia/reperfusion injury (Table 2) [156]. Similarly, ethyl pyruvate was also found to be protective against spinal cord ischemic injury [157] and kidney ischemia-reperfusion injury [158] partly through a HMGB1-inhibiting mechanism. In addition, a growing number of herbal components are shown to be effective in inhibiting HMGB1 release, and protecting animals against ischemic injury. For example, a major sweet component of a Chinese medicinal herb, liquorice, glycyrrhizin, protected rats against hepatic ischemia/reperfusion-injury [159] partly by reducing ischemia-elicited leukocyte adherence and neutrophil infiltration into ischemic liver tissue [159]. Similarly, cannabidiol, CBD, a non-psychoactive cannabinoid of Marijuana (Cannabis), was shown to be protective against cerebral ischemic injury partly by reducing circulating HMGB1 levels [160].

9. CONCLUSIONS AND PERSPECTIVES

Seemingly unrelated conditions, such as infection and injury, can converge on common processes such as inflammation that is orchestrated by various inflammatory mediators. Extensive pre-clinical animal studies have established HMGB1 as an early mediator of ischemic injury [107–110], and a late mediator of experimental sepsis [5,161,162]. Although the therapeutic window for HMGB1-inhibiting agents is rather narrow for ischemia-elicited inflammatory responses, many HMGB1-inhibiting agents could rescue mice from lethal experimental sepsis even when given in a delayed fashion (e.g., 24 h after onset of sepsis) (Figure 4). It is thus important to determine whether HMGB1 will ever become a clinically feasible therapeutic target for human sepsis in future clinical studies.

For complex systemic inflammatory diseases such as sepsis, it appears difficult to translate successful animal studies into clinical applications [14]. For instance, although neutralizing antibodies against cytokines (e.g., TNF) [83,163] are protective in animal models of endotoxemia, these agents failed in sepsis clinical trials [164–166]. This failure partly reflects the complexity of the underlying pathogenic mechanisms of sepsis, and the consequent heterogeneity of the patient population [167,168]. It may also be attributable to pitfalls in the selection of: 1) feasible therapeutic targets or drugs; 2) optimal doses and timing of drugs; and 3) non-realistic clinical outcome measures (such as mortality rates) [14,167–169].

One of the HMGB1 inhibitor, TSN IIA-SS, has been shown protective in animal models of myocardial ischemia/reperfusion injury [170], as well as transient or permanent focal cerebral ischemia [171,172]. Consequently, it has been widely used in China as a medicine for patients with cardiovascular disorders [146]. In animal models of sepsis, TSN IIA-SS reduced total peripheral vascular resistance, and yet increased cardiac stroke volume and cardiac output [36]. The dual effects of TSN IIA-SS in attenuating late inflammatory response and improving cardiovascular function make it a promising therapeutic agent for sepsis. It is thus important to further investigate the intricate mechanisms by which various agents attenuate systemic HMGB1 release, and explore their therapeutic potential in future clinical studies.

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1 MGKGDPPK PRGKMSSSYAFFVQTCREEHKKKHPDASVNFSEFSKKCSERWK 50
"A Box"

51 TMSAKEKGKFEDMAKADKARYEREMKTYI PPKGETKKKFKDPNA PKRPPS 100
"B Box"

106

101 AFFLF **CSEYRPKIKGEHPGLSIG** DVAKKLGEMWNNTAADDKQPYEKKA**A** 150
"Cytokine Domain"

151 **LKEYEKDIAAYR** **AKGKPDAAKKGVVKAESKK**KKEEEDEDEDEDEDEEE 200
"RAGE-binding domain"

201 EDEEDEDEEEDDDDE 215

Figure 1. Amino acid sequence of human HMGB1

The N-terminus of HMGB1 comprises two repeats of a positively charged domain of about 80 amino acids (termed HMG box A and B) (shown in box). The cytokine-stimulating motif (“Cytokine Domain”, shown in bold text) does not overlap with its RAGE-binding site (bold text), supporting the potential involvement of other cell surface receptors (such as TLR4) in HMGB1-mediated inflammatory responses.

Figure 2. Mechanisms underlying the regulation of endotoxin-induced HMGB1 release
The potential involvement of several signaling molecules (receptors and MAP kinases) and proinflammatory mediators in endotoxin-induced HMGB1 release is summarized.

Figure 3. Extracellular HMGB1 functions as an alarmin signal

HMGB1 is actively secreted by innate immune cells and passively released by damaged cells, and orchestrates an inflammatory response by: 1) stimulating cell migration; 2) facilitating innate recognition of bacterial products; 3) activating various innate immune cells; and 4) suppressing phagocytosis of apoptotic cells. Consequently, HMGB1 functions as an alarmin signal to recruit, alert and activate innate immune cells, thereby sustaining potentially injurious inflammatory response during infection or injury.

Figure 4. HMGB1 functions as late of sepsis, but an early mediator of ischemic injury
A). HMGB1 as a late mediator of experimental sepsis. Mice subjected to experimental sepsis (such as cecal ligation and puncture, CLP) succumb at latencies of up to 1–3 days, long after early proinflammatory cytokines (such as TNF) reach plateau levels in the circulation. In contrast, HMGB1 reach peak levels in the circulation in a delayed fashion, which parallels with septic lethality, thereby providing HMGB1 with a wider therapeutic window. **B). HMGB1 as an early mediator of ischemic injury.** Immediately following ischemic insult, HMGB1 is rapidly released by injured cells, and accumulates in the circulation within a few hours. Consequently, the therapeutic window for HMGB1-targeting strategies is relatively narrower as opposed to experimental sepsis.

Table 1

Protective effects of HMGB1-inhibiting agents in animal models of endotoxemia and sepsis.

Agents	Animal model of systemic inflammation	Via inhibiting HMGB1	References
Antibodies			
Anti-IFN- γ	Sepsis	release	32
Anti-HMGB1	Endotoxemia and Sepsis	activity	5, 73, 104
Intravenous immunoglobulin (IVIG)	Sepsis	release	122
Anti-coagulant agents			
Anti-thrombin III	Endotoxemia	release	124
Thrombomodulin	Endotoxemia	activity	61, 125
Endogenous hormones			
Insulin	Endotoxemia	release	126, 128
Vasoactive intestinal peptide	Endotoxemia and Sepsis	release	127, 129
Ghrelin	Sepsis	release	130, 133
Vagus nerve stimulation			
Electrical	Endotoxemia and Sepsis	release	134, 135
Chemical (nicotine, GTS-21, choline)	Endotoxemia and Sepsis	release	137, 139
Mechanical (transcutaneous)	Endotoxemia and Sepsis	release	136
Chinese herbal components			
Danshen (TSN IIA-SS)	Endotoxemia and Sepsis	release	36
Green tea (EGCG)	Endotoxemia and Sepsis	release	143, 144
Others			
Ethyl pyruvate	Endotoxemia and Sepsis	release	148, 149
Cisplatin	Sepsis	release	150
Spermine	Sepsis	release and activity	155

Table 2

Protective effects of HMGB1-inhibiting agents in animal models of ischemia.

Agents	Animal model of Ischemia	References
Antibodies		
Anti-HMGB1	Hepatic Ischemia/Reperfusion	108,112
	Cerebral Ischemia	114,115
Antagonist (A box)		
	Myocardial Ischemia	113
	Cerebral Ischemia	114,115
Chinese herbal components		
Glycyrrhizin	Hepatic Ischemia	159
Cannabidiol	Cerebral Ischemia	160
Others		
Ethyl pyruvate	Spinal Cord Ischemia	157
	Renal Ischemia	158
Cisplatin	Hepatic Ischemia/Reperfusion	156