

MINI REVIEW

HMGB1-mediated apoptosis and autophagy in ischemic heart diseases

Eleonora Foglio¹, Laura Pellegrini^{2,3}, Antonia Germani⁴, Matteo Antonio Russo^{5,6} and Federica Limana^{7,8}

¹Department of Experimental Medicine, Sapienza University of Rome, Rome, Italy

²Institute of Oncology Research (IOR), Bellinzona, Switzerland

³Università della Svizzera Italiana, Lugano, Switzerland

⁴Laboratory of Vascular Pathology, Istituto Dermopatico dell'Immacolata, IDI-IRCCS, Fondazione Luigi Maria Monti, Rome, Italy

⁵IRCCS San Raffaele Pisana, San Raffaele Open University, Rome, Italy

⁶MEBIC Consortium, San Raffaele Open University, Rome, Italy

⁷Laboratory of Cellular and Molecular Pathology, IRCCS San Raffaele Pisana, Rome, Italy

⁸San Raffaele Open University, Rome, Italy

Correspondence should be addressed to F Limana: fe_limana@hotmail.com

Abstract

Acute myocardial infarction (MI) and its consequences are the most common and lethal heart syndromes worldwide and represent a significant health problem. Following MI, apoptosis has been generally seen as the major contributor of the cardiomyocyte fate and of the resultant myocardial remodeling. However, in recent years, it has been discovered that, following MI, cardiomyocytes could activate autophagy in an attempt to protect themselves against ischemic stress and to preserve cardiac function. Although initially seen as two completely separate responses, recent works have highlighted the intertwined crosstalk between apoptosis and autophagy. Numerous researches have tried to unveil the mechanisms and the molecular players involved in this phenomenon and have identified in high-mobility group box 1 (HMGB1), a highly conserved non-histone nuclear protein with important roles in the heart, one of the major regulator. Thus, the aim of this mini review is to discuss how HMGB1 regulates these two responses in ischemic heart diseases. Indeed, a detailed understanding of the crosstalk between apoptosis and autophagy in these pathologies and how HMGB1 regulates them would be of tremendous help in developing novel therapeutic approaches aimed to promote cardiomyocyte survival and to diminish tissue injury following MI.

Key Words

- ▶ HMGB1
- ▶ apoptosis and autophagy
- ▶ HMGB1 and heart repair
- ▶ molecular and cellular rehabilitation

High mobility group box 1 (HMGB1) is a small protein, highly conserved in evolution across mammalian species and considered the prototype of damage-associated molecular patterns (DAMPs). DAMPs usually have defined roles inside the cell but once passively released or actively secreted by stressed cells, can activate inflammation and eventually tissue repair through the interaction with different cellular receptors, mainly pattern recognition receptors (PPRs) (1). Inside the cell, HMGB1 is involved

in a number of DNA-related activities such as DNA repair, transcription and recombination and in the regulation of apoptosis/autophagy balance. Once in the extracellular space, it is involved in a variety of different processes such as inflammation, proliferation, differentiation, migration, invasion and tissue regeneration (2). The ability to translocate from a cellular compartment to another is mainly due to its post-translational modifications, in particular to acetylation and phosphorylation (3).

Moreover, the extracellular activity of HMGB1 is regulated by its redox state. Indeed, HMGB1 can act as a chemokine in its reduced form while as a pro-inflammatory cytokine when it is partially oxidated (2).

Role of HMGB1 in the immuno-inflammatory-reparative response

A large number of papers describe HMGB1 as a leaderless cytokine (similar to other pleiotropic immuno-inflammatory modifiers, such as IL-1 and FGF family members), underscoring its multiple roles in the complex response to cell damage (4). However, unlike other inflammatory cytokines, HMGB1 is constitutively expressed in almost all cell types and its functional adaptation to different physiopathological inflammatory conditions is mainly achieved by changes in its cellular localization (2).

The response to cell damage includes at least six main interrelated sequential aspects in all of which HMGB1 may play a central role.

1. Sensing the cell damage or the damaging agents:

HMGB1 is mostly localized into the nucleus but, upon cellular stress and damage, it can translocate to the cytoplasm and be actively secreted from the cell. In sublethally stressed cells or during apoptosis, HMGB1 can be released in the extracellular space docked in vesicles of various size, macrovesicles and exosomes. In the presence of necrosis, HMGB1 is passively released in the extracellular space (2).

2. Signaling and alerting close/distant cells and tissue:

Once in the extracellular space, HMGB1 can interact with different receptors, such as RAGE, TLRs and CXCR4 among others, present on the surface of a variety of cells, in particular on that of the immune compartment (5).

3. Activating natural immunity and inflammatory response:

Activated HMGB1 receptors typically generate a signal cascade that ends up in the activation of proinflammatory master transcription factors (TFs), such as NF κ B, AP1, CREB, STAT3, NFAT and myogenin, all involved in the transcription of genes that participate in the immune-inflammatory-reparative response such as cytokines and chemokines and their receptors, metalloproteinases, adhesion molecules, phagocytic apparatus and cytoskeletal constituents (6).

4. Modulating all lymphoid cell of immune response:

Both the humoral and cellular forms of the adaptive immune response are affected by different subpopulations of lymphoid cells, whose function is in turn regulated by HMGB1 (7).

5. Regulating resolution and/or persistence of inflammation:

HMGB1 is a potent modulator of the inflammatory response. It can, indeed, stop the response leading to resolution or reinforce and prolong it, thus driving to chronic inflammation. The persistence of the inflammatory response is responsible for the inflammation-associated damage and chronic repair and leads to fibrosis and scar (2).

6. Inducing regeneration/repair of tissue damage:

HMGB1 activates several genes involved in cell/tissue repair. This process includes three different aspects in which HMGB1 is involved: (a) Disposal of intracellular damaged components and necrotic cell debris. (b) Proliferation and differentiation of stem cells to replace dead cells, as well as the transcription and synthesis of components (such as cytoskeletal, mitochondrial, enzymatic proteins) to recover the function of sublethally damaged cells. (c) Activation of TGF-gene family for extracellular matrix (ECM) molecule synthesis. Importantly, in chronic inflammation, this pathway is abnormally regulated or prolonged leading to scar, fibrosis and tissue/organ functional insufficiency (8).

Apoptosis/autophagy cross talk

In the past years, programmed cell death (PCD) was synonymous with apoptosis; however, recently, alternative cell death mechanisms have been described (9) as well as their interconnection.

Apoptosis has a fundamental role in physiological and pathological conditions (10) and may be triggered either by extrinsic or intrinsic stimuli, resulting in a cascade of molecular events (9) that leads to the proteolytic destruction of the cell (10).

However, apoptosis is not acting by itself in regulating the complex and well-controlled process of cell death. Accumulating evidence reveals that apoptosis can indeed cooperate, antagonize or assist autophagy, thus influencing differentially the destiny of the cell (11).

Autophagy is a self-degradative process that, under nutrient deprivation or stress, selectively delivers

cytoplasmic material and intracellular organelles to lysosomes for degradation or recycling of cellular components (12). A low level of constitutive autophagy has an important housekeeping role in the normal turnover of long-lived proteins and whole organelles and is therefore crucial for promoting cell survival during stress conditions (13) (Figs 1 and 2).

Autophagy and apoptosis often occur in the same cell: autophagy is induced in response to different stresses that could trigger apoptosis, in an attempt of the cells to survive (11). Thus, autophagy seems to be a cytoprotective mechanism against cell death (14). Nevertheless, it might also become harmful and promote cell death under specific conditions (12).

The crosstalk between apoptosis and autophagy may represent an evolutionary advantage to cells, since it allows a more controlled response to a given stress signal and homeostasis maintenance (11). However, the decision to switch from the autophagic process to the apoptotic state is complex and not completely understood. Multiple direct and indirect interactions between these processes have been described, and it is likely that each pathway diverts and readjusts proteins from the other to promote its own mechanism (11).

HMGB1's impact on apoptosis and autophagy

HMGB1 is a critical regulator of the crosstalk between apoptosis and autophagy and participates in their modulation in a location- and post-translational modification-dependent manner (Fig. 3). In the nucleus, in response to stress stimuli, HMGB1 regulates the expression of the heat shock protein β -1 (HSPB1), responsible for the intracellular trafficking during mitophagy and autophagy, thus attenuating the apoptotic response (15). In the cytosol of immortalized embryonic fibroblasts and cancer cells, disulfide HMGB1 forms complexes with Beclin-1, determining its dissociation from BCL-2 and thus sustaining autophagy (16). In intestinal epithelial cells, instead, HMGB1 regulates the crosstalk autophagy/apoptosis by directly interacting with Beclin1 and ATG5 and blocking their calpain-mediated cleavage during inflammation, therefore maintaining their pro-autophagic functions (17). Indeed, in the absence of HMGB1, Beclin-1 and ATG5 are cleaved and transformed in pro-apoptotic effectors.

Finally, in cancer cells, exogenous HMGB1 can induce autophagy when reduced and promote apoptosis in its oxidized form (18).

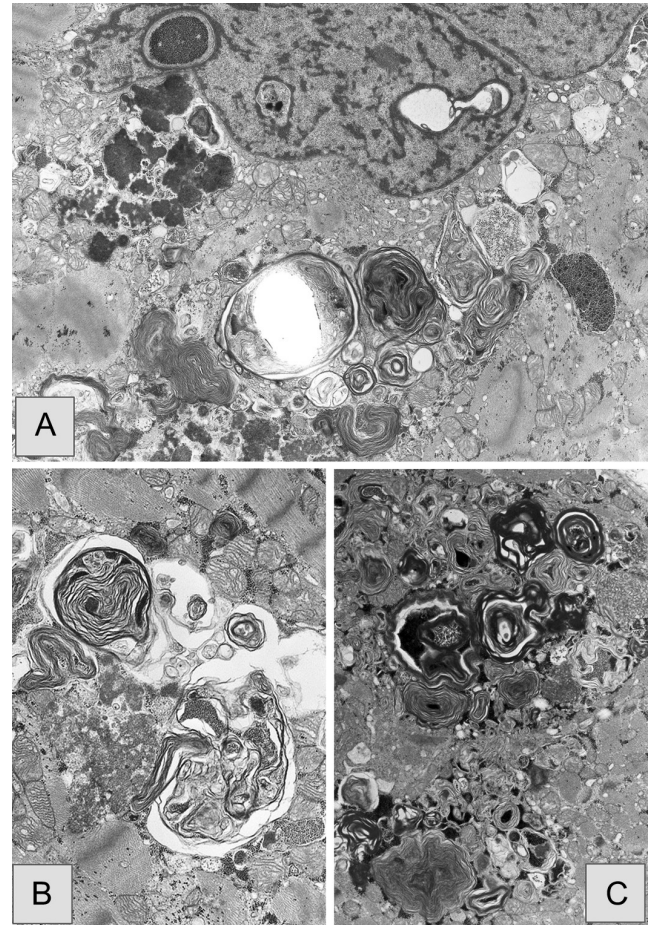


Figure 1

Autophagy in ischemic/hypoxic myocardial damage. (A) Massive autophagy in a myocardiocyte after 4–5 h of ischemia. Autophagosomes contain a large variety of cell components, including degraded membranes (lipoperoxidation?), glycogen β -particles, ribosomes and indigestible material (lipofuscins). Sarcomeres are disorganized. (B) Detail of fusing phagosomes: some components may derive from disorganized and digested mitochondria. (C) Myocardium from a patient treated with Adriamycin (doxorubicin). Autophagocytosis of ROS-damaged subcellular components is massive and comparable to that induced by ischemia, shown in (A).

The role of HMGB1 in the regulation of cell fate appears to be common across the majority of cell types. However, the study of Huebener *et al.* (19) showed that in cells characterized by an elevated number of mitochondria, such as hepatocytes or cardiomyocytes, HMGB1 deletion did not alter the structure of the mitochondria and the long-term survival of the cell. However, as suggested by Zhu *et al.* (17), this could be due to the fact that basal level of autophagy does not require HMGB1 or that these cells in normal condition could compensate for its loss. Indeed, in the model of infectious or inflammatory disease in different organs, HMGB1 deletion leads to an increase in cell death (20, 21, 22).

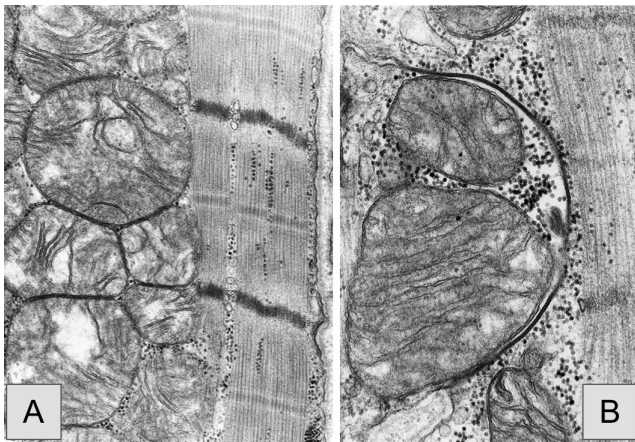


Figure 2 Mitophagy in ischemic/hypoxic myocardial damage. (A) After 3-h ischemia, mitochondria show the earliest signs of damage, including moderate-to-severe swelling with inhomogeneous, vacuolated and/or fragmented cristae. In contrast, the sarcomeres are still well organized (moderately relaxed as shown by visible I-band!) suggesting that sarcolemma and sarcoplasmic reticulum are intact with a still preserved cytosolic Ca⁺⁺ homeostasis. (B) Mitophagy: detail of an early formation of phagosome in which double membrane is segregating two moderately swollen mitochondria.

HMGB1-mediated modulation of apoptosis and autophagy in ischemic heart diseases

A crosstalk between autophagy and apoptosis has been documented in different heart diseases (14), where the multiple features of apoptosis, necrosis and autophagy have been simultaneously observed. However, while the detrimental effects of apoptosis have been widely demonstrated, the role of autophagy is still controversial. Indeed, it is still unclear whether autophagy represents a signal for cardiomyocyte repair or a suicide pathway for failing cardiomyocytes. This is especially true in the context of ischemia/reperfusion injury (23). Considering the debated role of HMGB1 in cardiovascular diseases (24) and in the regulation of cell fate, the main aim of this review is to provide an overview of the effects of HMGB1-mediated apoptosis and autophagy in the setting of different heart diseases.

In the context of acute myocardial infarction (MI), the effect of HMGB1 on cardiomyocyte survival has been investigated in two *in vivo* studies with different conclusions. Fei-Qi and colleagues demonstrated in transgenic mice overexpressing the angiotensin-converting enzyme 2 (ACE2), that, under acute MI, reduced levels of circulating HMGB1 and of its downstream pro-inflammatory cytokines were associated with decreased infarct size and apoptosis (25).

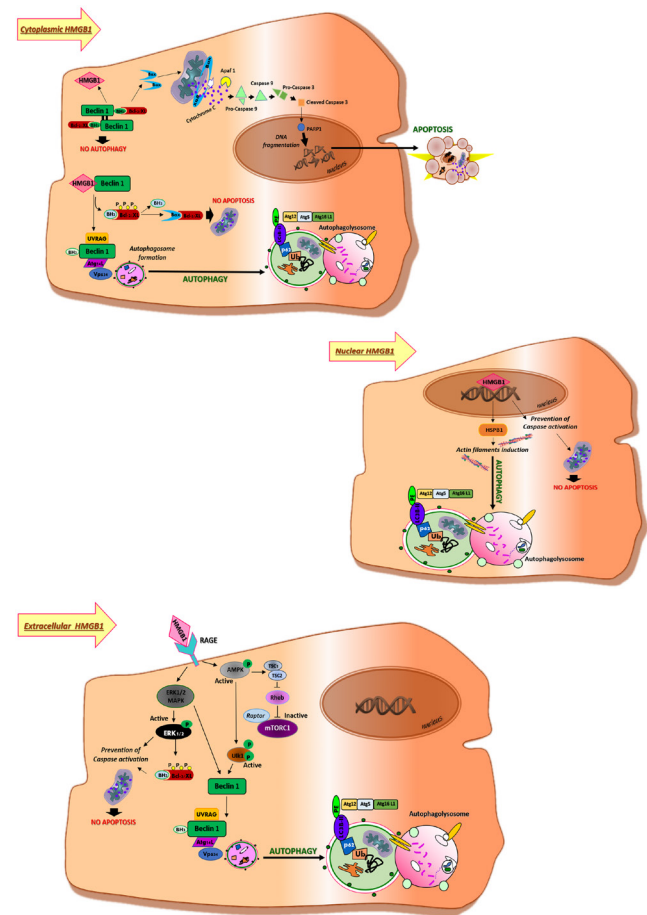


Figure 3 Critical role of HMGB1 in the complex interplay between apoptosis and autophagy. HMGB1 participates in the modulation of apoptosis and autophagy through different pathways depending on its cellular localization. In the cytosol, HMGB1 forms complexes with Beclin-1, determining its dissociation from the anti-apoptotic protein Bcl-2 and thus inducing autophagy and inhibiting apoptosis. In the nucleus, HMGB1 regulates the expression of the heat shock protein β -1 (HSPB1), responsible for the intracellular trafficking during autophagy and the attenuation of the apoptotic response through prevention of caspases activation. Finally, extracellular HMGB1 regulates the crosstalk autophagy/apoptosis by binding to the Receptor for Advanced Glycation End products (RAGE), which inhibits the mammalian target of rapamycin (mTORC) and activates the ERK1/2/MAPK pathway.

These cardioprotective effects of ACE2 were attributed to a decreased inflammation. However, all the analyses were performed 4 weeks following MI, a time point when the inflammatory response is resolved and the reparative process prevails. On the other end, the study of Foglio *et al.* showed that HMGB1 treatment, immediately after coronary artery ligation, induced cardiac repair not only by stimulating cardiac regeneration but also by inducing cardiomyocyte survival (26). Specifically, 3 days following MI, HMGB1 determined the attenuation of cardiomyocyte apoptosis and the induction of autophagy,

protective during acute MI, leading to an improvement in cardiac function 1 week after MI.

The reason of this discrepancy could be most likely attributed to the different experimental conditions: endogenous vs exogenous HMGB1, late vs early HMGB1-mediated effects, detection of apoptosis 4 weeks vs 3 days post MI. In the first study, HMGB1 exerted its effects during the inflammatory response, possibly exacerbating the detrimental effects triggered by inflammation, while in the second study it played its role before the onset of inflammation.

HMGB1 is likely to play a role in attenuating apoptosis of myocardial cells also in the failing heart. Indeed, after doxorubicin-induced cardiomyopathy, cardiac apoptosis was significantly reduced in mice with cardiac-specific overexpression of HMGB1 (HMGB1-Tg) compared with WT mice. This attenuation was related to the ability of HMGB1 to increase the expression of the HSPB1, thus preventing caspase activation (27).

Apoptosis has a major role also in the context of myocardial I/R injury. It occurs, indeed, during ischemia and is boosted by the reperfusion event (28). Different studies have proposed to target endogenous HMGB1 to reduce cardiac apoptosis following I/R injury. Accordingly, the cardioprotective role exerted by several drugs in the model of I/R injury has been attributed to their ability to inhibit endogenous HMGB1 expression. For instance, pretreatment with minocycline, an antibiotic with anti-apoptotic properties, determined a reduction in the levels of HMGB1 in a rat model of I/R. Since, *in vitro*, exogenous HMGB1 significantly decreased cell viability and promoted the apoptosis of neonatal rat ventricular myocytes in a dose-dependent manner, the authors suggested that minocycline could protect against cardiomyocytes apoptosis and myocardial I/R injury by inhibiting HMGB1 expression (29). Similar results were obtained with picoside II pretreatment, an active compound that has already been reported to significantly improve the neurobehavioral function and inhibit neurocytes apoptosis through the inhibition of the TLR-4/NfκB pathway in a rat model of middle cerebral artery occlusion and reperfusion (30). Interesting, treatment with picoside II before ischemia was able to protect from I/R injury also the myocardium as showed by the decrease in infarct size, cardiomyocyte apoptosis and activity of CK and LDH. According to the authors, these effects were partly due to the inhibition of the HMGB1-RAGE/TLR2/TLR4-NfκB signaling pathway (31). The same pathway has been found to be affected by ethyl pyruvate (EP). Indeed, EP treatment, by inhibiting the HMGB1-RAGE/TLR-NfκB

axis, decreased infarct size and apoptosis following I/R. In rat myocardium, upon reperfusion following a period of ischemia, EP treatment reduced the Bax/Bcl-2 ratio both under normoglycemic and hyperglycemic conditions and determined a decrease in the expression levels of TNF-α, IL-1β and IL-6 only under hyperglycemia (32, 33).

Ding and colleagues firstly explored the role of the HMGB1-TLR4 axis in triggering cardiomyocytes apoptosis by using a TLR4-mutant mouse model (34, 35). When the hearts of these transgenic mice were subjected to 30 min of ischemia followed by 6 h of reperfusion, the number of neutrophils and the levels of HMGB1 and inflammatory cytokines were lower compared to that of WT mice, leading to a marked decrease in the ischemic injury. Further, results showed decreased apoptotic indices, increased expression of Bcl-2 and decreased expression of Bax prompting the authors to suggest that HMGB1 could mediate neutrophil recruitment and worsen myocardial I/R injury by activating the TLR4-dependent pathway and triggering cardiomyocyte apoptosis.

Very recently, the cardioprotective effects of another compound with potent anti-inflammatory and anti-tumor activities, that is celastrol, has been attributed to the activation of PI3/Akt pathway and to the consequent inhibition of HMGB1. In a rat model of myocardial I/R injury, celastrol treatment determined the attenuation not only of myocardial apoptosis but also of autophagy, whose activation has been considered deleterious in the context of I/R injury (36).

Different *in vitro* studies have also analyzed HMGB1-mediated autophagy in the model of hypoxia and reoxygenation.

Rat cardiomyocytes, overexpressing HMGB1 and subjected to hypoxia and reoxygenation injury, were characterized by enhanced apoptosis and autophagy (37, 38). Interestingly, another study showed that endogenous HMGB1, generated by cardiomyocytes subjected to anoxia and reoxygenation (A/R), a model that correlates *in vitro* to I/R, promoted I/R-induced myocardial apoptosis by potentiating the effect of TNFα/JNK (39).

Another anti-inflammatory cytokine, interleukin-33, showed protective effects against myocardial I/R injury by inhibiting inflammatory responses and attenuating cardiomyocyte apoptosis as indicated by an increase in the ratio of Bcl-2 to Bax and a reduction in the cleaved-caspase-3 expression and in the apoptotic index. The authors proposed that IL33 attenuated cardiac apoptosis by inhibiting the release of HMGB1 and suggested a possible involvement of the P38 MAPK signaling pathway in this effect (40).

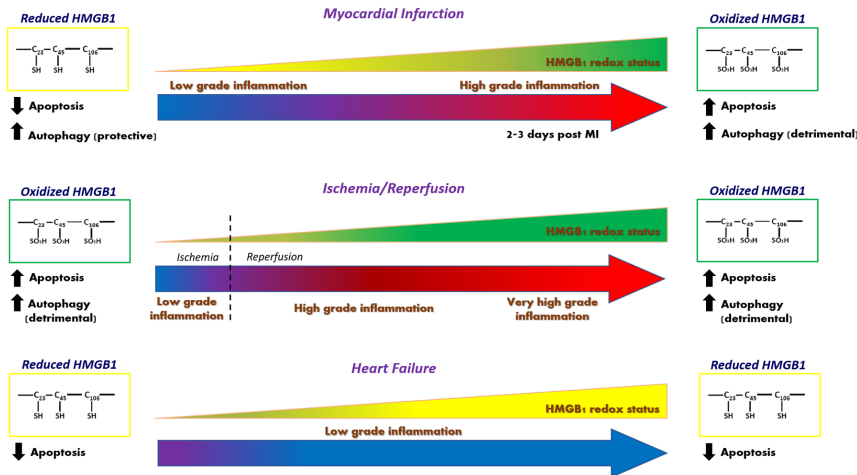


Figure 4

A schematic representation of the role of post-ischemic inflammatory response in determining HMGB1 action in heart diseases. After ischemic insults, inflammation grade is crucial to determine HMGB1 redox status, and, as a result, whether HMGB1 exerts protective or detrimental effects in heart diseases. In particular, in an environment characterized by the presence of high-grade inflammation (i.e. in the first 2–3 days following MI or during I/R), HMGB1 is present in its oxidized form and induces both apoptosis and autophagy, worsening cardiac function. On the contrary, when inflammation is low grade (i.e. immediately after MI or during HF), HMGB1 is present in its reduced form and it exerts anti-apoptotic and (even just following acute MI) pro-autophagic effects, promoting cardiac repair.

The inhibition of endogenous HMGB1, by means of two specific HMGB1 inhibitors, that is glycyrrhizin and BoxA, has demonstrated to attenuate apoptosis. Specifically, glycyrrhizin is a natural compound that inhibits the chemotactic and mitogenic functions of HMGB1 and therefore could have great therapeutic potential to prevent I/R injury in various organs. Zhai and colleagues showed that in the hearts of rats undergoing 30 min of ischemia and 24 h of reperfusion, glycyrrhizin treatment, just before reperfusion, induced a smaller infarct size and a reduction in the serum levels of HMGB1 and of the related inflammatory markers as TNF α and IL6. Importantly, extracellular HMGB1 inhibition resulted in the reduction of myocardial apoptosis and JNK/Bax pathway activation. All these results were reversed by administration of recombinant HMGB1 (41).

BoxA is a domain that has been shown to antagonize the extracellular activity of the full-length HMGB1 (41) and has been recently used to demonstrate a possible involvement of miR21 in mediating the effects of BoxA following I/R injury. Indeed, treatment with recombinant BoxA showed a remarkable protective effect against I/R injury and suppressed the activation of the proinflammatory cascade (42). Since preinjection of miR-21 *in vivo* attenuated ischemia-induced cardiomyocyte damage by decreasing apoptosis, Han and colleagues hypothesized that recombinant BoxA pretreatment in rats undergoing I/R injury could attenuate cardiomyocyte apoptosis and, therefore, myocardial I/R damage, through miR21. This hypothesis was confirmed by the dramatic increase of myocyte apoptosis following treatment with BoxA and antagonism of miR21, demonstrating a synergistic effect between HMGB1, BoxA and miR21 (43).

All the studies investigating the role of HMGB1 in myocardial I/R injury showed that HMGB1 always induces apoptosis and autophagy, therefore, exerting detrimental effects on the heart. Most likely, these findings could be explained by the fact that the inflammatory response, present during the reperfusion, is much more severe than after permanent ligation, both in the acute and in the late phase. In this context, HMGB1, both endogenous and exogenous, is oxidized and promotes apoptosis and autophagy contributing to worsen ventricular function.

In conclusion, we hypothesize (Fig. 4) that when inflammation is sustained, that is in the first 2–3 days following MI or during I/R, HMGB1 is present in its oxidized form and induces both apoptosis and autophagy worsening cardiac function. On the contrary, in an environment where inflammation is at low grade, i.e. immediately after MI and during heart failure, HMGB1 is also present in other redox forms as the reduced one and it exerts an anti-apoptotic and (following acute MI) a pro-autophagic effect promoting cardiac repair.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

Funding

The authors are supported by Ministero della Salute (Italian Ministry of Public Health) and by Fondazione ROMA.

Acknowledgments

The authors apologize to the many researchers whose work was not cited in this review.

References

- 1 Venereau E, Ceriotti C & Bianchi ME. DAMPs from cell death to new life. *Frontiers in Immunology* 2015 **6** 422. (<https://doi.org/10.3389/fimmu.2015.00422>)
- 2 Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, Huang J, Yu Y, Fan XG, Yan Z, *et al.* HMGB1 in health and disease. *Molecular Aspects of Medicine* 2014 **40** 1–116. (<https://doi.org/10.1016/j.mam.2014.05.001>)
- 3 Andersson U, Antoine DJ & Tracey KJ. The functions of HMGB1 depend on molecular localization and post-translational modifications. *Journal of Internal Medicine* 2014 **276** 420–424. (<https://doi.org/10.1111/joim.12309>)
- 4 Bianchi ME, Crippa MP, Manfredi AA, Mezzapelle R, Rovere Querini P & Venereau E. High-mobility group box 1 protein orchestrates responses to tissue damage via inflammation, innate and adaptive immunity, and tissue repair. *Immunological Reviews* 2017 **280** 74–82. (<https://doi.org/10.1111/imr.12601>)
- 5 Yang H, Wang H, Chavan SS & Andersson U. High Mobility Group Box Protein 1 (HMGB1): the prototypical endogenous danger molecule. *Molecular Medicine* 2015 **21** (Supplement 1) S6–S12. (<https://doi.org/10.2119/molmed.2015.00087>)
- 6 Naglova H & Bucova M. HMGB1 and its physiological and pathological roles. *Bratisl Lek Listy* 2012 **113** 163–171. (https://doi.org/10.4149/BLL_2012_039)
- 7 Li G, Liang X & Lotze MT. HMGB1: the central cytokine for all lymphoid cells. *Frontiers in Immunology* 2013 **4** 68. (<https://doi.org/10.3389/fimmu.2013.00068>)
- 8 Gurtner GC, Werner S, Barrandon Y & Longaker MT. Wound repair and regeneration. *Nature* 2008 **453** 314–321. (<https://doi.org/10.1038/nature07039>)
- 9 Galluzzi L, Vitale I, Aaronson SA, Abrams JM, Adam D, Agostinis P, Alnemri ES, Altucci L, Amelio I, Andrews DW, *et al.* Molecular mechanisms of cell death: recommendations of the Nomenclature Committee on Cell Death 2018. *Cell Death and Differentiation* 2018 **25** 486–541. (<https://doi.org/10.1038/s41418-017-0012-4>)
- 10 Barman J, Kumar R, Saha G, Tiwari K & Dubey VK. Apoptosis: mediator molecules, interplay with other cell death processes and therapeutic potentials. *Current Pharmaceutical Biotechnology* 2018 **19** 644–663. (<https://doi.org/10.2174/1389201019666180821093239>)
- 11 Booth LA, Tavallai S, Hamed HA, Cruickshanks N & Dent P. The role of cell signalling in the crosstalk between autophagy and apoptosis. *Cellular Signalling* 2014 **26** 549–555. (<https://doi.org/10.1016/j.cellsig.2013.11.028>)
- 12 Yonekawa T & Thorburn A. Autophagy and cell death. *Essays in Biochemistry* 2013 **55** 105–117. (<https://doi.org/10.1042/bse0550105>)
- 13 Nikolettou V, Markaki M, Palikaras K & Tavernarakis N. Crosstalk between apoptosis, necrosis and autophagy. *Biochimica et Biophysica Acta* 2013 **1833** 3448–3459. (<https://doi.org/10.1016/j.bbamcr.2013.06.001>)
- 14 Nishida K, Yamaguchi O & Otsu K. Crosstalk between autophagy and apoptosis in heart disease. *Circulation Research* 2008 **103** 343–351. (<https://doi.org/10.1161/CIRCRESAHA.108.175448>)
- 15 Tang D, Kang R, Livesey KM, Kroemer G, Billiar TR, Van Houten B, Zeh 3rd HJ & Lotze MT. High-mobility group box 1 is essential for mitochondrial quality control. *Cell Metabolism* 2011 **13** 701–711. (<https://doi.org/10.1016/j.cmet.2011.04.008>)
- 16 Tang D, Kang R, Livesey KM, Cheh CW, Farkas A, Loughran P, Hoppe G, Bianchi ME, Tracey KJ, Zeh 3rd HJ, *et al.* Endogenous HMGB1 regulates autophagy. *Journal of Cell Biology* 2010 **190** 881–892. (<https://doi.org/10.1083/jcb.200911078>)
- 17 Zhu X, Messer JS, Wang Y, Lin F, Cham CM, Chang J, Billiar TR, Lotze MT, Boone DL & Chang EB. Cytosolic HMGB1 controls the cellular autophagy/apoptosis checkpoint during inflammation. *Journal of Clinical Investigation* 2015 **125** 1098–1110. (<https://doi.org/10.1172/JCI76344>)
- 18 Kang R, Tang D, Schapiro NE, Livesey KM, Farkas A, Loughran P, Bierhaus A, Lotze MT & Zeh HJ. The receptor for advanced glycation end products (RAGE) sustains autophagy and limits apoptosis, promoting pancreatic tumor cell survival. *Cell Death and Differentiation* 2010 **17** 666–676. (<https://doi.org/10.1038/cdd.2009.149>)
- 19 Huebener P, Gwak GY, Pradere JP, Quinzii CM, Friedman R, Lin CS, Trent CM, Mederacke I, Zhao E, Dapito DH, *et al.* High-mobility group box 1 is dispensable for autophagy, mitochondrial quality control, and organ function in vivo. *Cell Metabolism* 2014 **19** 539–547. (<https://doi.org/10.1016/j.cmet.2014.01.014>)
- 20 Huang H, Nace GW, McDonald KA, Tai S, Klune JR, Rosborough BR, Ding Q, Loughran P, Zhu X, Beer-Stolz D, *et al.* Hepatocyte-specific high-mobility group box 1 deletion worsens the injury in liver ischemia/reperfusion: a role for intracellular high-mobility group box 1 in cellular protection. *Hepatology* 2014 **59** 1984–1997. (<https://doi.org/10.1002/hep.26976>)
- 21 Kang R, Zhang Q, Hou W, Yan Z, Chen R, Bonaroti J, Bansal P, Billiar TR, Tsung A, Wang Q, *et al.* Intracellular Hmgb1 inhibits inflammatory nucleosome release and limits acute pancreatitis in mice. *Gastroenterology* 2014 **146** 1097–1107. (<https://doi.org/10.1053/j.gastro.2013.12.015>)
- 22 Yanai H, Matsuda A, An J, Koshiba R, Nishio J, Negishi H, Ikushima H, Onoe T, Ohdan H, Yoshida N, *et al.* Conditional ablation of HMGB1 in mice reveals its protective function against endotoxemia and bacterial infection. *PNAS* 2013 **110** 20699–20704. (<https://doi.org/10.1073/pnas.1320808110>)
- 23 Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, Levine B & Sadoshima J. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. *Circulation Research* 2007 **100** 914–922. (<https://doi.org/10.1161/01.RES.0000261924.76669.36>)
- 24 Pellegrini L, Foglio E, Pontemezzo E, Germani A, Russo MA & Limana F. HMGB1 and repair: focus on the heart. *Pharmacology and Therapeutics* 2019 **196** 160–182. (<https://doi.org/10.1016/j.pharmthera.2018.12.005>)
- 25 Qi YF, Zhang J, Wang L, Shenoy V, Krause E, Oh SP, Pepine CJ, Katovich MJ & Raizada MK. Angiotensin-converting enzyme 2 inhibits high-mobility group box 1 and attenuates cardiac dysfunction post-myocardial ischemia. *Journal of Molecular Medicine* 2016 **94** 37–49. (<https://doi.org/10.1007/s00109-015-1356-1>)
- 26 Foglio E, Puddighinu G, Germani A, Russo MA & Limana F. HMGB1 inhibits apoptosis following MI and induces autophagy via mTORC1 inhibition. *Journal of Cellular Physiology* 2017 **232** 1135–1143. (<https://doi.org/10.1002/jcp.25576>)
- 27 Narumi T, Shishido T, Otaki Y, Kadowaki S, Honda Y, Funayama A, Honda S, Hasegawa H, Kinoshita D, Yokoyama M, *et al.* High-mobility group box 1-mediated heat shock protein beta 1 expression attenuates mitochondrial dysfunction and apoptosis. *Journal of Molecular and Cellular Cardiology* 2015 **82** 1–12. (<https://doi.org/10.1016/j.yjmcc.2015.02.018>)
- 28 Eefting F, Rensing B, Wigman J, Pannekoek WJ, Liu WM, Cramer MJ, Lips DJ & Doevendans PA. Role of apoptosis in reperfusion injury. *Cardiovascular Research* 2004 **61** 414–426. (<https://doi.org/10.1016/j.cardiores.2003.12.023>)
- 29 Hu X, Zhou X, He B, Xu C, Wu L, Cui B, Wen H, Lu Z & Jiang H. Minocycline protects against myocardial ischemia and reperfusion injury by inhibiting high mobility group box 1 protein in rats. *European Journal of Pharmacology* 2010 **638** 84–89. (<https://doi.org/10.1016/j.ejphar.2010.03.059>)
- 30 Liu Z, Li P, Zhao D, Tang H & Guo J. Protective effect of extract of *Cordyceps sinensis* in middle cerebral artery occlusion-induced focal cerebral ischemia in rats. *Behavioral and Brain Functions* 2010 **6** 61. (<https://doi.org/10.1186/1744-9081-6-61>)
- 31 Li JZ, Xie MQ, Mo D, Zhao XF, Yu SY, Liu LJ, Wu C & Yang Y. Picroside II protects myocardium from ischemia/reperfusion-

- induced injury through inhibition of the inflammatory response. *Experimental and Therapeutic Medicine* 2016 **12** 3507–3514. (<https://doi.org/10.3892/etm.2016.3841>)
- 32 Lin Y, Chen L, Li W & Fang J. Role of high-mobility group box-1 in myocardial ischemia/reperfusion injury and the effect of ethyl pyruvate. *Experimental and Therapeutic Medicine* 2015 **9** 1537–1541. (<https://doi.org/10.3892/etm.2015.2290>)
- 33 Soh S, Jun JH, Song JW, Shin EJ, Kwak YL & Shim JK. Ethyl pyruvate attenuates myocardial ischemia-reperfusion injury exacerbated by hyperglycemia via retained inhibitory effect on HMGB1. *International Journal of Cardiology* 2018 **252** 156–162. (<https://doi.org/10.1016/j.ijcard.2017.11.038>)
- 34 Ding HS, Yang J, Gong FL, Yang J, Ding JW, Li S & Jiang YR. High mobility group (corrected) box 1 mediates neutrophil recruitment in myocardial ischemia-reperfusion injury through toll like receptor 4-related pathway. *Gene* 2012 **509** 149–153. (<https://doi.org/10.1016/j.gene.2012.07.072>)
- 35 Ding HS, Yang J, Chen P, Yang J, Bo SQ, Ding JW & Yu QQ. The HMGB1-TLR4 axis contributes to myocardial ischemia/reperfusion injury via regulation of cardiomyocyte apoptosis. *Gene* 2013 **527** 389–393. (<https://doi.org/10.1016/j.gene.2013.05.041>)
- 36 Tong S, Zhang L, Joseph J & Jiang X. Celastrol pretreatment attenuates rat myocardial ischemia/reperfusion injury by inhibiting high mobility group box 1 protein expression via the PI3K/Akt pathway. *Biochemical and Biophysical Research Communications* 2018 **497** 843–849. (<https://doi.org/10.1016/j.bbrc.2018.02.121>)
- 37 Xu W, Jiang H, Hu X & Fu W. Effects of high-mobility group box 1 on the expression of Beclin-1 and LC3 proteins following hypoxia and reoxygenation injury in rat cardiomyocytes. *International Journal of Clinical and Experimental Medicine* 2014 **7** 5353–5357.
- 38 Ouyang F, Huang H, Zhang M, Chen M, Huang H, Huang F & Zhou S. HMGB1 induces apoptosis and EMT in association with increased autophagy following H/R injury in cardiomyocytes. *International Journal of Molecular Medicine* 2016 **37** 679–689. (<https://doi.org/10.3892/ijmm.2016.2474>)
- 39 Xu H, Yao Y, Su Z, Yang Y, Kao R, Martin CM & Rui T. Endogenous HMGB1 contributes to ischemia-reperfusion-induced myocardial apoptosis by potentiating the effect of TNF- α /JNK. *American Journal of Physiology: Heart and Circulatory Physiology* 2011 **300** H913–H921. (<https://doi.org/10.1152/ajpheart.00703.2010>)
- 40 Ruisong M, Xiaorong H, Gangying H, Chunfeng Y, Changjiang Z, Xuefei L, Yuanhong L & Hong J. The protective role of interleukin-33 in myocardial ischemia and reperfusion is associated with decreased HMGB1 expression and up-regulation of the P38 MAPK signaling pathway. *PLoS ONE* 2015 **10** e0143064. (<https://doi.org/10.1371/journal.pone.0143064>)
- 41 Zhai CL, Zhang MQ, Zhang Y, Xu HX, Wang JM, An GP, Wang YY & Li L. Glycyrrhizin protects rat heart against ischemia-reperfusion injury through blockade of HMGB1-dependent phospho-JNK/Bax pathway. *Acta Pharmacologica Sinica* 2012 **33** 1477–1487. (<https://doi.org/10.1038/aps.2012.112>)
- 42 Andrassy M, Volz HC, Igwe JC, Funke B, Eichberger SN, Kaya Z, Buss S, Autschbach F, Pleger ST, Lukic IK, *et al.* High-mobility group box-1 in ischemia-reperfusion injury of the heart. *Circulation* 2008 **117** 3216–3226. (<https://doi.org/10.1161/CIRCULATIONAHA.108.769331>)
- 43 Han Q, Zhang HY, Zhong BL, Zhang B & Chen H. Antiapoptotic effect of recombinant HMGB1 A-box protein via regulation of microRNA-21 in myocardial ischemia-reperfusion injury model in rats. *DNA and Cell Biology* 2016 **35** 192–202. (<https://doi.org/10.1089/dna.2015.3003>)

Received in final form 18 July 2019

Accepted 12 August 2019

Accepted Preprint published online 12 August 2019