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**High-mobility group box 1 protein (HMGB1) in ischemic heart disease:
Beneficial or deleterious?**

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Editorial

High-mobility group box 1 protein (HMGB1; also known as amphoterin) was initially identified more than 30 years ago as a non-chromosomal nuclear protein that maintains the nucleosome structure and regulates gene transcription.¹ HMGB1 is highly conserved among species, has over 98% identity among all mammals, and is ubiquitously expressed in almost all types of cells. HMGB1-deficient mice are viable but die shortly after birth due to hypoglycemia, suggesting that its nuclear functions are essential for survival. In 1999, Wang et al.² discovered that HMGB1 functions as a delayed mediator in inflammatory responses in sepsis and showed that the inhibition of HMGB1 confers significant protection against the lethal effects of endotoxin, indicating the importance of extracellular HMGB1 in inflammation.

HMGB1 is released into the extracellular milieu by 2 different routes: passive release from necrotic cells and active secretion from activated innate immune cells such as monocytes and macrophages. In necrotic cells, HMGB1 leaks into the extracellular environment via disrupted plasma membranes. In contrast, apoptotic cells retain chromatin-bound HMGB1. Therefore, HMGB1 is thought to contribute to the inflammation elicited by necrosis. Active secretion of HMGB1 from monocytes or macrophages occurs in response to proinflammatory stimuli, such as lipopolysaccharide (LPS), tumor necrosis factor- α (TNF- α), and interleukin-1 β (IL-1 β). HMGB1 has been reported to transduce its signals by interacting with at least 3 receptors: receptor for advanced glycation end products (RAGE), Toll-like receptor 2 (TLR2), and TLR4. RAGE-signaling induces the activation of the nuclear factor- κ B (NF- κ B) pathway as well as signal transduction mediated by extracellular-regulated protein kinase1/2 (ERK1/2) and p38, which promote cytokine production and cell survival. In contrast, TLR2- and TLR4-signaling activate the NF- κ B pathway via a myeloid differentiation primary response protein 88 (MyD88)-dependent mechanism. The recent discovery of

extracellular HMGB1 as a proinflammatory mediator has opened up avenues to help study the role of HMGB1 in inflammatory diseases.

A growing body of evidence suggests that inflammatory responses are involved in the pathophysiology of ischemic heart disease; therefore, it is postulated that HMGB1 acts as an inflammatory mediator in ischemic heart disease. Moreover increased levels of serum HMGB1 has been demonstrated in patients with acute coronary syndrome.³ In the current issue of *Cardiovascular Research*, Kitahara et al.⁴ have reported a study on the role of HMGB1 secreted from the infarcted myocardium in cardiac function and remodeling after myocardial infarction (MI) using transgenic mice with cardiac overexpression of HMGB1 under the control of the α -cardiac myosin heavy chain promoter (α MHC/HMGB1-Tg mice). Necrosis was elicited in cardiomyocytes by the ligation of the left anterior descending (LAD) coronary artery, and these cells released HMGB1 into the circulation. As was expected, the plasma HMGB1 levels in the α MHC/HMGB1-Tg mice after MI were significantly higher than those in the wild-type littermate mice. α MHC/HMGB1-Tg mice exhibited smaller infarct areas and improved cardiac function and survival rates after MI as compared to wild-type littermate mice. Moreover, these transgenic mice exhibited enhanced capillary and arteriole formation after MI. The findings obtained by Kitahara et al.⁴ indicate that the release of cardiac HMGB1 has beneficial effects on the heart after MI. Supporting this, Limana et al.⁵ showed that HMGB1 induced myocardial regeneration and improved cardiac dysfunction and remodeling after MI when administered locally into the infarcted murine heart tissue. Restoration of the infarcted heart tissue was thought to be at least in partly due to the proliferation of resident cardiac c-kit⁺ stem cells and their differentiation into myocytes. In contrast, Andrassy et al.⁶ recently demonstrated that the systemic administration of HMGB1 causes an increase in the inflammatory responses worsens the cardiac dysfunction and remodeling after myocardial ischemia-reperfusion injury. Conversely, treatment with a functional antagonist of HMGB1 (HMGB1 box-A)

conferred protection against ischemia-reperfusion injury. Furthermore, *in vitro* experiments have suggested that HMGB1-induced inflammatory responses are mediated by the infiltrated macrophages and not by cardiomyocytes. Interestingly, HMGB1 or HMGB1-boxA administration in RAGE-deficient mice produces no effects, indicating the critical role of HMGB1-RAGE pathway in ischemia-reperfusion injury of the heart.

From the above discussion, it is clear that there is currently a disagreement regarding the role of HMGB1 in ischemic heart disease; the former two studies indicate that it is beneficial, while the latter suggests the contrary. The reason for this discrepancy remains unclear at present; however, we should consider the differences in the experimental conditions in these studies. First, in the former 2 studies, permanent ligation of the LAD artery was performed, i.e., permanent MI was induced; however, the latter study, the heart was reperfused after a 30-min ligation of the LAD artery. There is a substantial difference between the pathophysiologies of permanent MI and ischemia-reperfusion injury. Reperfusion is accompanied by the release of an excessive amount of oxygen-derived free radicals that cause reperfusion injury. The inflammatory responses, including neutrophil and macrophage infiltration, are much more severe in reperfusion injury than in infarction, suggesting that ischemia-reperfusion injury could enhance inflammatory cell activity. Furthermore, HMGB1 stimulates the biological responses in endothelial cells and fibroblasts,⁷⁻⁹ which participate in the healing process after MI. Indeed, Kitahara et al.⁴ showed that angiogenesis after infarction was enhanced in α MHC/HMGB1-Tg mice. Several investigations have revealed that HMGB1 functions as an angiogenic cytokine in endothelial cells.^{7,8} In particular, Chavakis et al.⁹ have suggested that HMGB1 activates integrin-dependent recruitment of endothelial progenitor cells and contributes to angiogenesis in response to ischemia. In addition, Rossini et al.¹⁰ have reported that HMGB1 exerts paracrine effects on cardiac fibroblasts to stimulate the production of inflammatory cytokines and growth

factors, which in turn, modulated the proliferation and differentiation of cardiac c-kit⁺ stem cells. Thus, HMGB1 may influence myocardial injury or repair by exerting the above-mentioned effects on endothelial cells and fibroblasts. Second, the doses and routes of HMGB1 administration employed in these studies were different. Kitahara et al.⁴ used transgenic mice overexpressing HMGB1 in the myocardium. In their study, Limana et al.,⁵ injected low doses of HMGB1 (200 ng/mouse) into the ventricular wall bordering the viable myocardium 4 h after inducing MI. Andrassy et al.⁶ administered high doses of HMGB1 (10 µg/mouse) intraperitoneally 1 h before inducing ischemia-reperfusion injury. In terms of dosage, it has been reported that HMGB1 administration is beneficial in low doses and detrimental in high doses,¹ suggesting that the effects of HMGB1 are dose-dependent.

Although the 2 recent investigations by Kitahara et al.⁴ and Andrassy et al.⁶ significantly enhance our understanding regarding the role of HMGB1 in ischemic heart disease, there still remains a disagreement as to whether HMGB1 has beneficial or deleterious effects. Therefore, further investigations are necessary in order to understand the precise mechanism of HMGB1 action and its therapeutic potential in ischemic heart disease.

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