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Cardioprotective Mechanisms Activated in Response to Myocardial Ischemia

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Abstract: Myocardial ischemia, a disorder causing myocardial infarction and malfunction, can activate various adaptive mechanisms that protect cardiomyocytes from ischemic injury. During the early hours post myocardial ischemia, injured cardiac cells can release several molecules, including adenosine, opioids, and bradykinin, which promote myocardial survival by activating the G protein signaling pathways. During a later phase about several days, myocardial ischemia induces upregulation of growth factors and cytokines, including VEGF, ILGF, HGF, and SDF-1, in the injured myocardium, contributing to cardioprotection. In addition to the injured heart, the liver participates in cardioprotection. In response to myocardial ischemia, the liver upregulates and releases secretory proteins, including FGF21 and TFF3, both of which promote cardiomyocyte survival. The liver also provides a reservoir of hepatic cells that mobilize to the site of myocardial ischemia, potentially contributing to cardioprotection. Taken together, the early and late mechanisms act coordinately in a time-dependent manner, ensuring effective cardioprotection post myocardial infarction. Investigations on these innate cardioprotective mechanisms have provided insights into the development of cardioprotective strategies for treating myocardial infarction. In this article, the authors review the innate mechanisms of cardioprotection in myocardial ischemia.

1 Introduction

Myocardial ischemia is a prevalent disorder causing myocardial infarction and impairment of cardiac function. As cardiomyocyte death is a principal cause of cardiac deficits, two fundamental strategies have been established for treating myocardial ischemia: protecting myocardium from death and promoting myocardial regeneration. Nature has established various mechanisms for myocardial protection and regeneration. Examples include upregulation and release of secretory proteins

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for myocardial protection [1-5] and activation of stem cells for myocardial regeneration [6, 7]. These naturally occurring protective and regenerative mechanisms provide a foundation for developing cardiac therapeutic strategies. In recent years, myocardial regeneration has become a popular research topic. The mechanisms and therapeutic strategies for myocardial regeneration have been discussed extensively in literature [6-13]. Compared to myocardial regeneration, myocardial protection has not been given much attention, although cardioprotective therapies can rescue injured cardiomyocytes and alleviate myocardial infarction. In this article, the authors address a fundamental aspect of cardioprotection: the innate cardioprotective mechanisms.

2 The concept of myocardial protection

Myocardial protection is to reduce myocardial infarction by rescuing cardiomyocytes at risk. In myocardial ischemia caused by coronary artery insufficiency, cardiomyocytes in the core ischemic region rapidly die of extreme hypoxia, whereas cardiomyocytes in the peripheral ischemic region experience various levels of injury along the gradient of hypoxia. The goal of cardioprotective therapies is to rescue the injured cardiomyocytes from death, reducing the degree of myocardial infarction. There are a number of naturally occurring secretory proteins and substances that are upregulated and released in response to myocardial ischemia. These proteins and substances may act on injured cardiomyocytes to activate cell survival signaling pathways and establish cardiomyocyte tolerance to hypoxia, alleviating myocardial infarction. Pharmacological agents may be developed based on these ischemia-induced proteins and substances for cardioprotective treatment in human patients.

The innate cardioprotection concept stems from observations in the experimental model of ischemic preconditioning, a procedure inducing mild ischemic episodes in the heart or a remote organ, resulting in alleviation of subsequent myocardial infarction [14, 15]. The cardioprotective effect of this procedure has been repeatedly demonstrated in experimental and clinical investigations [5, 16-19]. These observations suggest that ischemic preconditioning activates innate cardioprotective factors and signaling mechanisms, which in turn support myocardial survival. Previous investigations have demonstrated that there are two types of innate cardioprotective factors, classified based on the activation time post myocardial ischemia: early and late cardioprotective factors. The early factors include, but are not limited to, adenosine, opioids, and bradykinin [20-33]. These factors are released from injured cells during the first several hours post myocardial ischemia without *de novo* protein synthesis. The late factors include growth factors [34-36] and hepatic secretory proteins [1-3, 37] that are upregulated via protein synthesis within serveral

days post myocardial ischemia. Taken together, the early and late factors cover the time window (several hours to several days) during which cardiomyocyte death occurs post myocardial ischemia. Such a protective time window may be sufficient for establishing myocardial tolerance to ischemic insults. In the following sections, the early and late factors are discussed with a focus on the cardioprotective role and mechanisms of action.

3 Early-phase cardioprotective factors

Cardioprotective factors activated during the early phase (several hours) are a group of small molecules from injured cells in the heart or other organs in response to acute ischemia. These factors include, but are not limited to, adenosine, opioids, and bradykinin [20-33]. These factors can act on G-protein-coupled receptors to activate intracellular signaling pathways involving phosphoinositide 3 kinase (PI3K), protein kinase C (PKC), mitogen-activated proteins kinases (MAPKs), and/or mitochondrial K_{ATP} channels. The activation of these molecules suppresses cell death and supports myocardial survival [5, 38, 39]. Thus, adenosine, opioids, and bradykinin have been considered pharmacological agents for myocardial protection under an ischemic condition.

Adenosine is a purine nucleoside, composed of adenine and a ribose sugar molecule, the core structure of the energy transfer molecules ADP and ATP. In addition to energy transfer, adenosine participates in other important biological processes, including cytoprotection, suppression of inflammatory responses, relaxation of vascular smooth muscle cells, and inhibition of the central nerve activities [40, 41]. Adenosine exerts these diverse activities by acting on different G protein-coupled receptors, including adenosine receptor A1, A2A, A2B, and A3 [40]. Among these four receptors, receptors A1 and A3 are associated with inhibitory G_i proteins that suppress the activity of adenylate cyclase, an enzyme catalyzing the formation of cAMP from ATP. The A1 receptor also regulates the activity of several kinase pathways involving PKC, PI3K, and MAPK [42]. The receptor A2A and A2B are associated with stimulatory G_s proteins that activate adenylate cyclase, resulting in formation of cAMP. In addition, the A2B and A3 receptors can interact with G_q proteins and activate the phospholipase Cβ signaling pathway, resulting in an increase in cytoplasmic calcium and activation of PKC and MAPK [43]. In myocardial ischemia, adenosine is rapidly accumulated in the lesion of myocardial ischemia [44, 45] and acts on the A1 receptor to exert a cardioprotective effect. This role is supported by the observations that pharmacological inhibition of the A1 receptor reduces the cardioprotective effect of adenosine [26] and administration of A1 receptor agonists contributes to cardioprotection [29, 30]. However, during coronary artery reperfusion following myocardial ischemia, adenosine acts

on a different receptor, the A2A receptor, to initiate cardioprotective activities [46]. Administration of A2A receptor agonists prior to reperfusion alleviates myocardial infarction [47, 48], whereas inhibition of the A2A receptor reduces the cardioprotective effect of adenosine during reperfusion [49]. The A2B receptor has also been reported to participate in the regulation of adenosine-mediated myocardial protection during reperfusion [50, 51]. Given the opposing roles of the A1 and A2 receptors in regulating the activity of adenylate cyclase, it seems difficult to interpret the aforementioned observations. It is possible that the adenosine receptors can interact with not only the inhibitory G_i and stimulatory G_s protein pathways, but also with other signaling pathways that promote cytoprotective responses [42].

Endogenous opioids are a group of peptides including dynorphins, enkephalins, endorphins, endomorphins, and nociceptin. These molecules are known to serve as analgesics, acting through interaction with G protein-coupled receptors [52, 53]. There are four major types of opioid receptors, including deferens (δ or DOP), ketocyclazocine (κ or KOP), morphine (μ or MOP), and nociception (NOP) receptors. These receptors are primarily found in the central and peripheral sensory neurons [53]. Activation of the opioid receptors enhances tolerance to pain. Opioids have also been implicated in the regulation of cardiomyocyte survivability. Myocardial ischemia induces rapid release of opioids, which in turn act on the δ and κ opioid receptors to promote cardiomyocyte survival [5]. The cardioprotective action of these receptors is supported by the observations that administration of δ or κ opioid receptor agonists results in alleviation of myocardial infarction [54]. Thus, opioid analogues have been considered pharmacological agents for cardioprotection.

Bradykinin is a nine amino acid peptide generated from its precursor kininogen by kallikrein-mediated cleavage. Bradykinin is well known for its endothelium-dependent action inducing vascular smooth muscle cell relaxation and vasodilation, a process mediated by prostacyclin and nitric oxide. Bradykinin exerts these effects via interaction with G protein-coupled receptors [55]. There are two bradykinin receptors identified to date: bradykinin receptor B1 and B2. Both receptors can interact with G_q proteins, which activate the phospholipase Cβ signaling pathway, resulting in an increase in intracellular calcium and activation of the MAPK signaling pathway. These receptors may also interact with G_i proteins, which inhibit the activity of adenylate cyclase [55]. The B1 receptor is not significantly expressed under physiological conditions, but is upregulated in response to cell injury, participating in inflammatory responses [56]. The B2 receptor, in contrast, is constitutively expressed and is involved in the regulation of vasodilatory activities under physiological conditions [55]. In myocardial ischemia, bradykinin is released and plays a role in cardioprotection. Administration of bradykinin to ani-

mals with myocardial ischemia results in alleviation of myocardial infarction [20, 57], whereas administration of bradykinin antagonists reduces the cardioprotective effect of bradykinin [58]. Observations from bradykinin receptor B2 knockout mice further support the cardioprotective role of bradykinin [59]. Thus, bradykinin and agonists have been considered potential pharmacological agents for cardioprotection in myocardial ischemia.

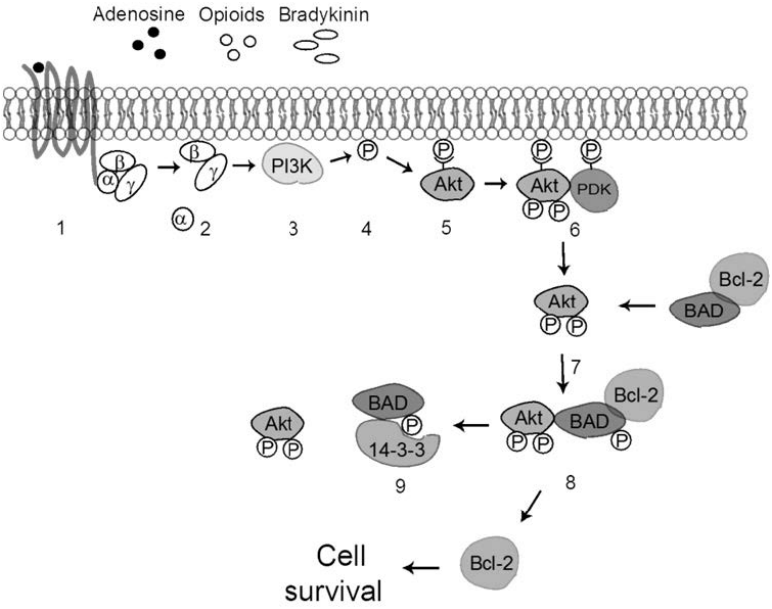

Figure 1: Signaling pathways possibly mediating the cardioprotective effect of adenosine, opioids, and bradykinin. PI3K: phosphoinositide-3-kinase. PDK (or PDPK): phosphoinositide dependent protein kinase. Bcl-2: B-cell lymphoma 2. BAD: Bcl-XL/Bcl-2-associated death promoter.

A fundamental question is how G protein activation leads to cardioprotective responses in myocardial ischemia. While the exact mechanisms remain an ongoing research topic, previous investigations have provided preliminary evidence to address this question (Fig. 1). A major signaling pathway that mediates G protein-dependent cell survival involves the protein kinases phosphoinositide 3-kinase (PI3K) and serine/threonine protein kinase B (PKB or Akt) [4]. In response to ligand binding, G proteins can release the $\beta\gamma$ subunits, which activate and recruit PI3K to the cell membrane [60-63]. Activated PI3K induces phosphorylation of phosphoinositides (PI), generating phospholipid molecules including phosphatidylinositol (3,4)-bisphosphate (PI(3,4)P_2) and phosphatidylinositol (3,4,5)-trisphosphate

($PI(3,4,5)P_3$) [61]. These phospholipids can interact with the serine/threonine protein kinase Akt, resulting in Akt recruitment to the plasma membrane and Akt conformational changes. The phospholipids $PI(3,4)P_2$ and $PI(3,4,5)P_3$ also stimulate interaction of a protein kinase known as 3-phosphoinositide-dependent protein kinase-1 (PDPK1 or PDK1) with Akt, resulting in Akt phosphorylation on Thr-308 and Ser-473 [64]. Activated Akt in turn phosphorylates the protein Bcl-XL/Bcl-2-associated death promoter (BAD) on Ser-136. BAD is known a pro-apoptotic factor that belongs to the Bcl-2 family [65]. When dephosphorylated, BAD binds to and sequesters the anti-apoptotic proteins Bcl-2 and Bcl-XL, rendering the pro-apoptotic factors BAX and BAK relatively more active [66, 67]. BAX and BAK induce pore formation in the mitochondrial outer membrane, allowing cytochrome C to escape from the mitochondria into the cytoplasm. Cytochrome C in turn binds to Apaf1 (apoptotic protease activating factor 1), activating downstream caspases and resulting in apoptosis. When phosphorylated, BAD dissociates from Bcl-2 and Bcl-XL. Free Bcl-2 and Bcl-XL become active and exert an inhibitory effect on cell apoptosis, promoting cell survival. Phosphorylated BAD in turn forms complexes with an adaptor protein known as 14-3-3, which prevents BAD from dephosphorylation and reduces BAD-mediated apoptotic activities [64]. Thus, activation of G protein-coupled receptors results in cell survival via a mechanism involving the phosphorylation of BAD and downregulation of its pro-apoptotic activities (Fig. 1).

4 Late-phase cardioprotective factors

4.1 Growth factors and cytokines

The aforementioned cardioprotective responses involve adenosine, opioids, and bradykinin that are released from injured cardiac cells during the early hours post myocardial ischemia without de novo gene expression. Following the early period, there is a second cardioprotective response, which occurs within several days post myocardial ischemia. This late response requires de novo protein synthesis [68, 69]. A number of growth factors and cytokines, including vascular endothelial growth factor (VEGF) [34-36], insulin-like growth factor (ILGF), hepatocyte growth factor (HGF), and stromal cell derived factor 1 (SDF-1), are upregulated in injured cardiac cells [7, 70]. These factors may activate cell survival and angiogenic signaling mechanisms, protecting cardiomyocytes from ischemic injury and promoting myocardial regeneration. VEGF, ILGF, and HGF are ligands that interact with and activate the protein tyrosine kinase receptor signaling pathways [43]. These signaling pathways in turn interact with the PI3K-Akt signaling pathway, leading to myocardial protection as discussed in the previous section. SDF-1 is a cytokine also known as chemokine (C-X-C motif) ligand 12 (CXCL12). This

cytokine binds to the G protein-coupled receptor CXCR4, which is expressed in hematopoietic cells (neutrophils, monocytes, T cells, B cells, and macrophages) and vascular endothelial cells. SDF-1 and CXCR4 play a role in activating and recruiting endothelial progenitor cells from the bone marrow to ischemic lesions, inducing and enhancing angiogenesis [71], a critical process for myocardial protection and regeneration. VEGF has also been shown to promote angiogenesis in ischemic myocardium by promoting the formation of endothelial cells from endothelial progenitor cells and inducing capillary sprouting [34-36]. In addition to the cardioprotective role, these growth factors and cytokines may activate cardiac resident stem cells, which in turn transform to functional cardiomyocytes in myocardial ischemia, an important mechanism for myocardial regeneration [6, 7, 9-13].

4.2 Ischemia-induced hepatic secretory proteins

In an integrated physiological system, an injury event in one organ may induce systemic responses in other organs through environmental changes and hormone regulation. Such responses may boost the protection of the injured organ, a possible mechanism critical to the survival of vital organs, especially, those with a limited capacity of protection and regeneration such as the heart. Recent investigations have demonstrated that experimental myocardial ischemia induces liver responses, leading to activation of two protective processes: (1) upregulation of hepatic secretory proteins [1, 37] (Fig. 2); and (2) mobilization of hepatic cells into the circulatory system [1]. Both processes contribute to myocardial protection under ischemic conditions.

In response to experimental myocardial ischemia induced by coronary artery ligation, hepatocytes upregulate nine genes encoding secretory proteins, as demonstrated by cDNA microarray analyses, including α-1-acid glycoprotein 2 (AGP2), bone morphogenetic protein binding endothelial cell regulator (BMPER), chemokine (C-X-C motif) 13 (CXCL13), fibroblast growth factor 21 (FGF21), neuregulin 4 (NRG4), proteoglycan 4 (PRG4), trefoil factor 3 (TFF3), serum amyloid A1 (SAA1), and SAA2 [1]. Given the association of gene upregulation with myocardial ischemia, the proteins encoded by these genes might be involved in cardioprotective responses. Recently, we have identified two hepatic secretory proteins, including FGF21 and TFF3, as cardioprotective proteins. We are currently conducting a screening process in a mouse model of myocardial ischemia for identifying additional cardioprotective proteins based on the upregulated hepatic secretory protein genes. The cardioprotective hepatic secretory proteins may serve as a basis for developing pharmacological agents for alleviating myocardial infarction.

Fibroblast growth factor 21 (FGF21) is a 209 amino acid protein with a molec-

ular weight about 22.3 kDa [72]. This protein belongs to the fibroblast growth factor family that is composed of 22 members [73, 74]. FGF21 is primarily expressed in the liver and, to a lesser degree, in the thymus, adipose tissue [72, 75, 76], and islet β cells of the pancreas [77]. Among the FGF family proteins, while the majority play a role in regulating cell proliferation and differentiation, FGF21 has been reported to regulate glucose and lipid metabolisms [74, 75, 78] with the following specific functions: (1) stimulating insulin-independent glucose uptake in adipocytes and potentiating insulin-induced metabolic activities [78, 79]; (2) enhancing insulin expression and secretion from the β cells of the pancreas and reducing the plasma level of fasting glucose; (3) promoting lipolysis in adipocytes and conversion of fatty acids to ketones in the liver [80-82]; and (4) reducing plasma LDL and increasing plasma HDL levels [79, 83]. FGF21 regulates the aforementioned cell activities possibly via interaction with the FGF Receptor 2α, resulting in upregulation of the glucose transporter GLUT1 [75] and activation of the ERK1/2 and Akt signaling pathways, which in turn mediate glucose and lipid metabolism [79, 84-86]. These previous investigations suggest that FGF21 regulates carbohydrate and lipid metabolism and may be used as a therapeutic agent for the treatment of diabetes, lipid disorders, and obesity.

In a recent investigation, we found that, in association with hepatic upregulation of the FGF21 gene, the FGF21 protein level was elevated in the liver (hepatocytes) as well as the serum in myocardial ischemia [2]. Administration of recombinant FGF21 to mice immediately post myocardial infarction resulted in a significant reduction in myocardial infarction at day 1 and 10 in association with improved

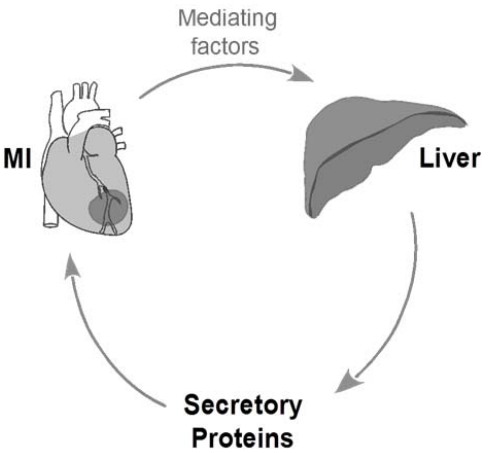

Figure 2: Schematic demonstration of liver response to myocardial ischemia.

left ventricular dp/dt. In FGF21 overexpression mice, the degree of myocardial infarction was significantly lower than that in wildtype mice. Further investigations demonstrated that FGF21 administration to healthy mice induced phosphorylation of FGF Receptor 1 (FGFR1), phosphoinositide 3 kinase (PI3K), Akt, and BAD in cardiomyocytes within 10 - 30 min. These molecules were also phosphorylated in cardiomyocytes within 1 day post myocardial infarction, suggesting a potential role for these molecules in mediating the cardioprotective effect of FGF21 [2]. These observations support the cardioprotective role of FGF21 in myocardial ischemia.

Trefoil factor 3 (TFF3) is a secreted protein with 80 amino acids and a molecular weight of 8.6 kDa [87]. This factor is characterized by the presence of a three-looped structure of the trefoil motif and expressed primarily in mucus-secreting goblet cells of the gastrointestinal tract [88]. Trefoil factor 3 has been shown to contribute to the maintenance of mucosal integrity under physiological conditions and facilitate mucosal healing after mechanical and chemical injury [88, 89]. In vitro tests have demonstrated that TFF3 may enhance aggregation of intestinal mucin glycoproteins by forming bridges between the mucin glycoproteins. This reaction results in the formation of a mechanically stable mucoviscous layer on the gastrointestinal epithelium. This layer plays a role in protecting the intestinal epithelium from chemical and mechanical injury.

In myocardial ischemia induced by coronary artery ligation in the mouse, the TFF3 protein level was elevated in the liver and serum, in association with hepatic upregulation of the TFF3 gene [3]. In TFF3-/- mice, the degree of myocardial infarction was significantly larger than that in wildtype mice at day 1 post myocardial ischemia. Administration of recombinant TFF3 to TFF3-/- mice immediately post myocardial infarction resulted in a significant reduction in myocardial infarction in association with a significant improvement of the left ventricular dp/dt. It was further demonstrated that TFF3 administration to healthy mice induced phosphorylation of PI3K p85 in cardiomyocytes within 30 min. PI3K was also phosphorylated in the ischemic cardiomyocytes of wildtype mice. The relative phosphorylation level of PI3K was reduced in the cardiomyocytes of TFF3-/- mice compared to that in the wildtype mouse. Administration of PI3K p110 siRNA to the left ventricular anterior wall of wildtype mice (at 6 locations about 2 mm apart) 3 days prior to myocardial infarction resulted in a reduction in the protein level of PI3K p110 within the region of siRNA administration at 1 day post myocardial ischemia. This modulation induced a decrease in the relative phosphorylation level of Akt and BAD, in association with an increase in the degree of myocardial infarction [3]. These observations suggest that upregulated TFF3 contributes to myocardial protection possibly via the PI3K-Akt-BAD signaling mechanisms.

The aforementioned investigations on FGF21 and TFF3 suggest that these proteins

possibly contribute to innate cardioprotective responses in myocardial ischemia. Recombinant FGF21 and TFF3 may be potentially used as cardioprotective agents. Although these proteins are upregulated post myocardial infarction, the timing of FGF21 and TFF3 expression (usually at or after 12 hrs post myocardial infarction) and the serum protein level may not be appropriate for effective cardioprotection. Thus, administration of exogenous FGF21 and/or TFF3 immediately post myocardial infarction may boost the innate cardioprotective mechanisms.

5 Ischemia-induced mobilization of hepatic cells

Another liver-mediated cardioprotective mechanism is hepatic cell mobilization to the circulatory system post myocardial ischemia. This phenomenon was first found in mice with coronary artery ligation-induced myocardial ischemia based on the presence of albumin-positive hepatocyte-like cells in blood samples collected from the thoracic portion of the inferior vena cava or the right heart [1]. This observation was confirmed in a transgenic mouse model expressing liver-specific EYFP, established by crossing a mouse strain expressing the albumin promoter-driven Cre recombinase (Alb-Cre) gene with a mouse strain conditionally expressing the EYFP gene controlled by a loxP-flanked stop sequence that blocks EYFP expression [90]. When the Alb-Cre gene is expressed in the liver of the mouse carrying the conditional EYFP gene (referred to as the Cre-EYFP strain), the stop sequence of the EYFP gene is deleted by the Cre recombinase, resulting in liver-specific EYFP expression.

In the Cre-EYFP model, EYFP+ hepatic cells were found in the circulatory system of mice with myocardial ischemia, but not in mice with sham operation. The population of circulating EYFP+ hepatic cells increased to a peak at day 5 post myocardial ischemia and reduced thereafter, as demonstrated by fluorescence microscopy. These observations were confirmed by flow cytometry [90]. The population of the circulating EYFP+ hepatic cells in Cre-EYFP mice with myocardial ischemia was significantly larger than that in sham control Cre-EYFP mice. These observations suggest that hepatic cells can be mobilized to the circulatory system in response to myocardial ischemia [90].

Mobilized hepatic cells may contribute to myocardial protection in two ways: (1) disintegration in the circulatory system to release cell contents [1]; and (2) engraftment to the lesion of myocardial ischemia [Liu unpublished data]. Both hepatic cell disintegration and engraftment have been observed in experimental myocardial ischemia in the mouse. Although the exact mechanisms of myocardial protection remain to be investigated, these processes may be involved in delivery of protective factors to the ischemic myocardium. The delivered factors may help establish myocardial tolerance to ischemia and protect myocardium at risk from injury.

Hepatic cell disintegration in the circulatory system was first recognized from the observations that circulating hepatic cells were only presented in the blood samples collected from the thoracic portion of the inferior vena cava, right heart, and left heart, but not from the peripheral arteries and peripheral veins [1]. These observations suggest that hepatic cells are mobilized into the vena cava and can survive the environment of the venous and pulmonary vascular systems. The absense of hepatic cells in the peripheral arteries suggests that the cells are disintegrated in the arterial system. A possible cause of hepatic cell disintegration is the shearing effect of arterial blood flow, which is considerably higher than that in the venous and pulmonary vascular systems and may cause rapid rupture of circulating hepatic cells.

The significance of hepatic cell disintegration is to discharge the hepatic cell contents into the circulatory system, rapidly establishing a critical level of hepatic cell factors possibly required for effective myocardial protection and maintenance of cardiovascular functions in acute myocardial ischemia and heart failure. The hepatic cell factors may include, but are not limited to, secretory proteins and vasoactivity regulators (to be determined). Administration of liver extracts from donor mice with acute myocardial ischemia, but not with sham operation, to mice immediately post myocardial ischemia resulted in significant alleviation of myocardial infarction [1]. Similar results were observed when hepatocytes isolated from mice with acute myocardial ischemia were used for administration. These observations support the notion that mobilized hepatic cells in myocardial ischemia contain cardioprotective factors. Furthermore, administration of liver extracts from donor mice with acute myocardial ischemia to healthy mice resulted in progressive elevation of arterial blood pressure, suggesting the presence of vasoactive factors in the liver extract, although the factors remain to be identified [Liu unpublished data]. These factors may help maintain the arterial blood pressure in heart failure due to acute myocardial infarction. It is conceivable that, while hepatic cells may release cardioprotective and vaso-active factors post myocardial infarction, hepatic cell mobilization and disintegration may lead to a more rapid accumulation of these factors in the circulatory system. As cardiomyocyte injury and death occur rapidly following an ischemic insult, it is critical to achieve an early sufficient blood level of cardioprotective factors for effective myocardial protection.

Another liver-mediated cardioprotective response is engraftment of mobilized hepatic cells to the lesion of myocardial ischemia. This phenomenon was discovered by fluorescence microscopy in the experimental model of myocardial ischemia and reperfusion induced in the Cre-EYFP mouse [Liu unpublished data]. EYFP+ hepatic cells were found in the lesion of myocardial ischemia in Cre-EYFP mice, but not in the myocardium of Cre-EYFP mice with sham operation. These cells were

found at day 3 following the induction of myocardial ischemia and reperfusion, reached a peak population at day 5, and reduced in population size thereafter. These observations suggest that mobilized hepatic cells are able to engraft to the ischemic myocardium. It should be noted that only a small fraction of the mobilized hepatic cells engrafted to the ischemic myocardium, whereas the majority of the mobilized hepatic cells were disintegrated in the circulatory system. The significance of hepatic cell engraftment is possibly to deliver hepatic secretory proteins to the ischemic myocardium, but this action remains to be confirmed.

6 Concluding remarks

Myocardial ischemia activates innate mechanisms that protect cardiomyocytes from injury. These mechanisms include, but are not limited to: (1) release of protective factors within several hrs; (2) expression and release of growth factors and hepatic secretory factors within several days; and (3) mobilization of hepatic cells within several days post myocardial infarction. Understanding these innate mechanisms is essential for the development of cardioprotective strategies for alleviating myocardial infarction.

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