Liver-Mediated Myocardial Protection in Experimental Myocardial Infarction

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Abstract

Myocardial ischemia activates innate cardioprotective mechanisms, including expression of growth factors, activation of cardiac resident stem cells and mobilization of bone marrow cells to the ischemic myocardium, which alleviate ischemic injury and promote cardiomyocyte regeneration. This review addresses a newly recognized cardioprotective mechanism involving the liver. Myocardial ischemia induces liver responses, including mobilization of hepatic cells to the circulatory system and upregulation of hepatic secretory proteins, such as fibroblast growth factor 21 (FGF21) and trefoil factor 3 (TFF3). The mobilized hepatic cells either engraft to the ischemic myocardium or disintegrate in the circulatory system, facilitating delivery of the hepatic secretory proteins. These proteins contribute to myocardial protection, alleviating myocardial infarction. These investigations provide new information for understanding the innate cardioprotective mechanisms and developing therapeutic strategies for myocardial infarction.

Keywords: Myocardial infarction; Myocardial protection; Liver; Hepatic cell mobilization; Hepatic secretory proteins; FGF21; TFF3

Introduction

Myocardial ischemia is a prevalent disorder causing myocardial infarction and dysfunction. As cardiomyocyte death is the principal cause of myocardial deficits, a recognized treatment strategy is to protect the ischemic myocardium from death. Given the necessity of cardioprotection in myocardial ischemia, extensive investigations have been conducted to search for cardioprotective pharmacological agents. While numbers of such agents have been identified and tested in experimental investigations [1-6], few have been proven effective for myocardial protection in human patients [7,8]. There exist various innate mechanisms that are activated in response to myocardial ischemia to protect the heart from injury. Such mechanisms were first recognized in the experimental model of ischemic preconditioning, a procedure inducing mild ischemic episodes in the heart or a remote organ and resulting in alleviation of subsequent myocardial infarction [9,10]. The cardioprotective effect of this procedure has been repeatedly demonstrated in experimental and clinical investigations [11-15]. These observations suggest that ischemic preconditioning may activate innate signaling mechanisms, which in turn support myocardial protection. These innate mechanisms may include, but are not limited to; ischemia-induced upregulation of growth factors to support cardiomyocytes urvival and angiogenesis [16-19], activation of cardiac resident stem cells [20-26] and mobilization of bone marrow cells to the heart to stimulate cardiomyocyte regeneration [27-32]. Investigations on these natural protective mechanisms provide insights into the development of cardioprotective strategies for treatment of myocardial infarction.

A mammalian individual consists of multiple organs operating in a highly coordinated manner. An injury event in one organ often involves other organs through induced environmental changes and hormone regulation, resulting in systemic responses. The inter-organ responses may be considered adaptive processes for boosting the protection and repair 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, resulting in activation of two protective processes: (1) mobilization of hepatic cells to the circulatory system [33,34] and (2) upregulation and release of hepatic secretory proteins [35,36]. Both processes contribute to myocardial protection under ischemic conditions. In this article, the authors address the mechanisms of these liver responses and the role of hepatic cells and secretory proteins in myocardial protection. It should be noted that there are other innate cardioprotective mechanisms activated in response to myocardial ischemia as mentioned above. These mechanisms have been discussed extensively in the literature. The reader may refer to references 16 - 32 for these mechanisms.

Hepatic Cell Mobilization and Regulatory Mechanisms

Mobilization of hepatic cells to the circulatory system in myocardial ischemia

Hepatic cell mobilization 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 (Figure 1) [33]. 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 lox P-flanked stop sequence that blocks EYFP

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expression [34]. 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 between the loxP sites 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 with sham operation (Figure 1) [34]. The population of circulating EYFP+ hepatic cells increased to a peak at day 5 post myocardial ischemia and reduced afterwards, as demonstrated by fluorescence microscopy. These observations were confirmed by flow cytometry [34]. 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 [34]. These observations suggest that hepatic cells can be mobilized to the circulatory system in response to myocardial ischemia.

It is important to note that mobilized hepatic cells can only be found in blood samples collected from the thoracic portion of the inferior vena cava, right heart and left heart, but rarely found in the peripheral arteries or peripheral veins of Cre-EYFP mice with myocardial ischemia [33]. These observations suggest that the mobilized hepatic cells can survive the blood flow environment from the hepatic vein to the left ventricle, but not the environment of the arterial system. The disappearance of hepatic cells in the peripheral arteries indicates that the hepatic cells are disintegrated in the arterial system. A possible cause for hepatic cell disintegration is the shearing effect of rapid arterial blood flow.

Regulation of hepatic cell mobilization

A fundamental question for the aforementioned discovery is how hepatic cells are mobilized in myocardial ischemia. It is well recognized that the collagen matrix supports the liver integrity. Hepatic cell mobilization occurs only when the collagen matrix is degraded, a process requiring activation of collagenases. Recent investigations have demonstrated that myocardial ischemia induces upregulation of the collagenase MMP2 in the liver as tested by gelatin zymography [34], suggesting a role for MMP2 in mediating hepatic cell mobilization. The role of MMP-2 was confirmed by administration of an anti-MMP2 antibody to Cre-EYFP mice with myocardial ischemia. This treatment

Figure 1: Mobilization of hepatic cells to the circulatory system in experimental myocardial ischemia. (**A**) Fluorescence micrograph showing hepatocytes in the liver marked with an anti-albumin antibody (red). (**B**) Presence of albumin+ hepatic cells (red) in a blood sample from the vena cava (from the liver to the right atrium) in myocardial ischemia. (**C**) Fluorescence micrograph showing EYFP+ hepatocytes and biliary epithelial cells (green). Red: CK19. pv: portal vein. *: bile duct. (**D**) Presence of EYFP+ hepatic cells (green) in a blood sample from the vena cava (from the liver to the right atrium) in myocardial ischemia. Blue: cell nuclei for all panels. Scale: 10 μm for all panels.

significantly reduced hepatic cell mobilization post myocardial ischemia compared to administration of an isotype-matched control antibody [34]. These observations suggest that MMP2 contributes to hepatic cell mobilization.

There are two cell sources that possibly express MMP2 in the liver: hepatic cells and leukocytes [34]. RT-PCR tests demonstrated that hepatocytes, the largest hepatic cell population, from mice with myocardial ischemia did not express a significant level of MMP2 mRNA compared to hepatocytes from control mice. The remaining hepatic cell types did not exhibit increased MMP2 expression either. In search for cell sources that express MMP2 in the liver, it was found that the density of leukocytes retained in the liver was significantly increased in mice with myocardial ischemia. RT-PCR analyses demonstrated that the liver-retained leukocytes, isolated by anti-CD45 antibody-based magnetic separation, exhibited a significant upregulation of MMP2 mRNA in mice with myocardial ischemia [34]. These results were supported by fluorescence microscopy, demonstrating MMP2 expression in the liver-retained leukocytes. These observations suggest that liver-retained leukocytes contribute to MMP2 upregulation in the liver following myocardial ischemia.

This discovery leads to another question: what causes leukocyte retention in the liver. Given the anatomical relationship of the heart, leukocytes and liver, it is conceivable that inflammatory factors released from injured cardiac cells and activated leukocytes may mediate these processes. One of the common inflammatory factors is interleukin 6 (IL6), a cytokine upregulated in myocardial ischemia as observed in experimental [34] and clinical investigations [37,38]. This cytokine plays a role in leukocyte retention in the liver, presumably via enhanced leukocyte adhesion to the hepatic sinusoid endothelium. The role of IL6 is supported by the observations that while leukocyte retention in the liver increased significantly in Cre-EYFP mice with myocardial ischemia, the density of liver-retained leukocytes reduced significantly in Cre-EYFP-IL6-/- mice under identical myocardial ischemic conditions. Administration of mouse recombinant IL6 to Cre-EYFP-IL6-/- mice with myocardial ischemia restored leukocyte retention. These results suggest that IL6 stimulates leukocyte retention in the liver in myocardial ischemia.

While it remains to be investigated how IL6 regulates leukocyte retention in the liver in response to myocardial ischemia, it is possible that the mechanism involves IL6-mediated leukocyte adhesion to the endothelium, as demonstrated by previous studies [39,40]. IL6 can activate the leukocyte adhesion molecule L-selectin in response to fever-range thermal stress, promoting leukocyte-endothelial interaction [39,40]. IL6 can bind the soluble form of the IL6 receptor α subunit, which interacts with the transmembrane receptor gp130. This process activates JAK1, which induces tyrosine phosphorylation on gp130 in the cytoplasmic domain. Selected phosphotyrosines on gp130 can subsequently recruit SH2 domain-containing protein tyrosine phosphatase 2 (SHP2), which can also be phosphorylated by JAK1. Phosphorylated SHP2 can interact with SOS/Grb2, an adaptor protein complex that activates the MEK-ERK1/2 signaling pathway. ERK1/2 induces activation of the transcriptional factors AP1, CREB and/or Egr1, which trigger the expression of target genes encoding L-selectin and related regulatory proteins, resulting in enhanced leukocyte adhesion to the endothelium [39,40].

IL6 may also be one of the cytokines that stimulate MMP2 upregulation in liver-retained leukocytes. In vivo investigations demonstrated that, while Cre-EYFP liver-retained leukocytes exhibited upregulation of MMP2 mRNA in myocardial ischemia, Cre-EYFP-
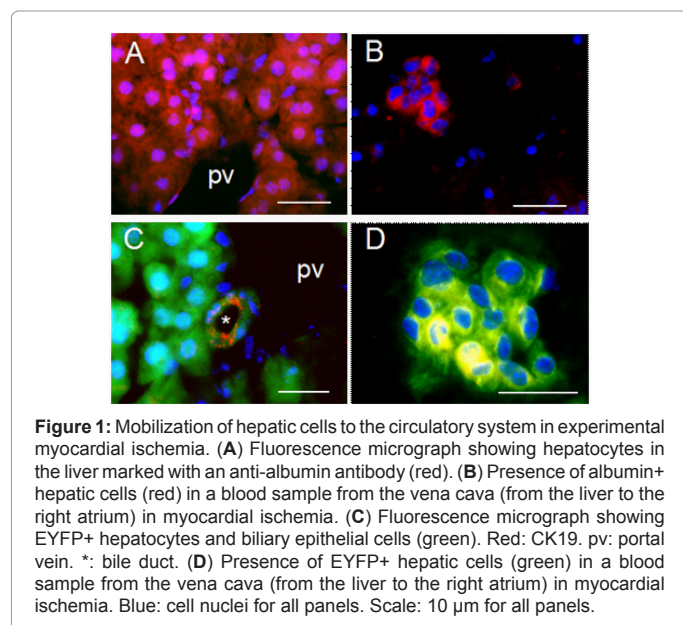
IL6-/- liver-retained leukocytes did not show a significant change in the relative expression of MMP2 mRNA [34]. Administration of mouse recombinant IL6 to Cre-EYFP-IL6-/- mice with myocardial ischemia restored MMP2 expression in liver-retained leukocytes [34]. These observations suggest that IL6 stimulates MMP2 expression in liver-retained leukocytes in myocardial ischemia. This mechanism was further supported by results from in vitro experimental tests. In cultured liver specimens, the presence of IL6 alone did not induce MMP2 upregulation, whereas the presence of leukocytes induced MMP2 upregulation. Application of IL6 to liver specimens in the presence of leukocytes significantly enhanced MMP2 expression in the liver specimens, confirming the role of IL6 in mediating MMP2 expression in leukocytes.

The IL6Rα–gp130–SHP-2–ERK1/2 signaling pathway may play a role in mediating IL6-induced MMP2 expression. IL6 induces ERK1/2 activation and ERK1/2 in turn activates the transcription factor AP1 [41,42]. AP1 acts on the AP1 responsive site of the MMP2 gene, inducing MMP2 expression [43,44]. IL6 itself is expressed in activated leukocytes, inducing inflammatory processes such as leukocyte transmigration and fever [45,46]. The physiological significance of MMP2 upregulation in response to IL6 is to facilitate leukocyte transmigration to the site of injury and inflammation by degrading extracellular matrix. In myocardial ischemia, such inflammatory processes may be activated in the liver, resulting in hepatic cell mobilization.

The aforementioned experimental observations suggest that hepatic cell mobilization in myocardial ischemia is a regulated process involving IL6, leukocytes and MMP2. Potential regulatory mechanisms of hepatic cell mobilization in myocardial ischemia can be summarized as follows: myocardial ischemia induces IL6 upregulation in the ischemic lesion and IL6 release into the circulatory system, IL6 stimulates leukocyte retention in the liver as well as expression of MMP2, and MMP2 in turn degrades extracellular matrix and induces hepatic cell mobilization. These results and analyses provide a basis for understanding the significance of hepatic cell mobilization, a process contributing to cardioprotection in myocardial ischemia.

Role of mobilized hepatic cells in myocardial protection

Mobilized hepatic cells may contribute to myocardial protection in two ways: (1) disintegration in the circulatory system to release cell contents [33]; 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 hypoxia and protect myocardium from injury. Hepatic cell disintegration in the circulatory system was first recognized from the observations that circulating hepatic cells only appeared 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 [33]. These observations suggest that hepatic cells are mobilized into the vena cava and survive the environment of the venous and pulmonary vascular systems. The disappearance 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 vaso-activity 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 [33]. Similar results were observed when hepatocytes isolated from mice with acute myocardial ischemia were used for transplantation instead of liver extract 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 vasoactive factors post myocardial infarction, hepatic cell mobilization and disintegration may lead to a more rapid accumulation of these factors within 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, 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 mobilized hepatic cells engrafted to the ischemic myocardium, whereas the majority of 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.

Cardioprotective Role of Hepatic Secretory Proteins

Upregulation and release of hepatic secretory proteins

In response to experimental myocardial ischemia induced by coronary artery ligation, hepatocytes upregulate the expression of a number of genes encoding secretory proteins, as demonstrated by cDNA microarray analyses. These include 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 [33]. Given the association of gene upregulation with myocardial ischemia, the secretory proteins encoded by these genes might be involved in cardioprotective responses. Recently, we have identified two of these hepatic secretory proteins as cardioprotective proteins: FGF21 and TFF3. We are currently conducting a screening process in a mouse

model of myocardial ischemia to identify additional cardioprotective proteins based on the upregulated hepatic secretory protein genes. The hepatic cardioprotective secretory proteins may serve as a basis for developing pharmacological agents for alleviating myocardial infarction.

FGF21 is a 209 amino acid protein with a molecular weight of 22.3 kDa [47]. This protein belongs to the fibroblast growth factor family that is comprised of 22 members [48,49]. FGF21 is primarily expressed in the liver and, to a lesser degree, in the thymus, adipose tissue [47,50,51] and islet β cells of the pancreas [52]. 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 [49,50,53] with the following specific functions: (1) stimulating insulin-independent glucose uptake in adipocytes and potentiating insulin-induced metabolic activities [53,54]; (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 [55-57]; and (4) reducing plasma LDL and increasing plasma HDL levels [54,58]. 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 [54,59-61]. 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 myocardial ischemia, the FGF21 level was elevated in the liver (hepatocytes) as well as in the serum [35]. 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 left ventricular dp/dt. In mice over expressing FGF21, the degree of myocardial infarction was significantly lower than that in wild type 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 [35]. These observations support the cardioprotective role of FGF21 in myocardial ischemia.

TFF3 is a secreted protein with 80 amino acids and a molecular weight of 8.6 kDa [62]. This factor is characterized by the presence of a three-looped structure of the trefoil motif and is expressed primarily in mucus-secreting goblet cells of the gastrointestinal tract [63]. 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 [63,64]. 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 LAD coronary artery ligation in the mouse, the TFF3 protein level was elevated in both the liver and serum, in association with hepatic upregulation of the TFF3 gene [36]. In TFF3-/- mice, the degree of myocardial infarction was significantly larger than that in wild type mice at day 1 post myocardial infarction.

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 wild type mice. Administration of PI3K p110 siRNA to the LV anterior wall of wild type 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 infarction. 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 [36]. 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.

Potential role of hepatic secretory proteins in mediating the cardioprotective effect of ischemic preconditioning

Previous investigations have demonstrated that exposing the heart to episodes of mild myocardial ischemia results in alleviation of myocardial infarction in a subsequent heart attack, a phenomenon referred to as ischemic preconditioning-mediated cardioprotection [9-15]. In addition, transient ischemia in a remote organ, such as the skeletal muscle, has been shown to protect ischemic myocardium [10,12,65]. Further investigations have shown that ischemic preconditioning often induces early (1 – 3 hours) and late (days to weeks) protective responses [12,66]. The early-phase protection is thought to be the result of immediate release of cardioprotective factors, such as adenosine, bradykinin and opioids, which interact with G protein-coupled receptors, resulting in activation of protective signaling pathways involving PKC, PI3K and Akt [14,67-69]. The late phase protection has been thought a process involving gene expression and protein synthesis [66,68]. However, the protein types involved in the late process have not been completely identified and the regulatory mechanisms remain poorly understood [12]. The ischemia-upregulated hepatic secretory proteins are possible candidates for mediating the late phase cardioprotective effect of ischemic preconditioning. Experimental tests for the expression time course and the cardioprotective effect of the hepatic secretory proteins supported this possibility. Thus, ischemia-upregulated secretory proteins may be used as cardioprotective agents to boost innate myocardial protection.

Concluding Remarks

The liver has long been considered an organ responsible for metabolic processes, protein synthesis, hormone generation and detoxification. Our investigations have demonstrated that the liver also contributes to protection of ischemic myocardium by

mobilizing hepatic cells to the circulatory system and up regulating cardioprotective secretory proteins, including FGF21 and TFF3. The mobilized hepatic cells either engraft to the ischemic myocardium or disintegrate in the circulatory system, facilitating delivery of the cardioprotective proteins. While the upregulated hepatic secretory proteins contribute to myocardial protection, these proteins may not be sufficiently expressed during the early hours post myocardial ischemia. Administration of recombinant hepatic secretory proteins immediately post myocardial ischemia enhances myocardial protection. These investigations provide a foundation for understanding the innate cardioprotective mechanisms and developing cardioprotective agents based on the naturally expressed hepatic secretory proteins.

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