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Original article

The novel role of mast cells in the microenvironment of acute myocardial infarction

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ABSTRACT

Mast cells are multifunctional cells containing various mediators, such as cytokines, tryptase, and histamine, and they have been identified in infarct myocardium. Here, we elucidated the roles of mast cells in a myocardial infarction (MI) rat model. We studied the physiological and functional roles of mast cell granules (MCGs), isolated from rat peritoneal fluid, on endothelial cells, neonatal cardiomyocytes, and infarct heart (1-hour occlusion of left coronary artery followed by reperfusion). The number of mast cells had two peak time points of appearance in the infarct region at 1 day and 21 days after MI induction in rats ($p<0.05$ in each compared with sham-operated heart). Simultaneous injection of an optimal dose of MCGs modulated the microenvironment and resulted in the increased infiltration of macrophages and decreased apoptosis of cardiomyocytes without change in the mast cell number in infarct myocardium. Moreover, MCG injection attenuated the progression of MI through angiogenesis and preserved left ventricular function after MI. MCG-treated cardiomyocytes were more resistant to hypoxic injury through phosphorylation of Akt, and MCG-treated endothelial cells showed enhanced migration and tube formation. We have shown that MCGs have novel cardioprotective roles in MI via the prolonged survival of cardiomyocytes and the induction of angiogenesis.

1. Introduction

Myocardial infarction (MI) is associated with an intense inflammatory response and activation of the host innate immune program for inflammatory cell mobilization, cardiac repair, and remodeling [1]. The inflammatory reaction in MI is regulated by neutrophils, monocytes, and macrophages. After MI, infiltration of leukocytes and macrophages into the injured myocardium takes place within several hours. Marginated leukocytes contribute to the removal of dead tissue through the release of proteolytic enzymes and produce fibrinogenic and angiogenic mediators, leading to the proliferative phase of infarct healing.

Mast cells are another inflammatory cell type recently found in patients with congestive heart failure [2] and in the MI rat model [3]. Classically, mast cells have been described as essential effector cells of immediate hypersensitivity and chronic allergic reactions that contribute to asthma, atopic dermatitis, and other allergic disease.

In several reports, mast cells have been regarded as a harmful regulator in the heart because mast cells participate in inducing the apoptosis of cardiomyocytes in vitro [4], in the myocardial fibrosis in patients with congestive heart failure [2], in the evolution to congestive heart failure in mast cell-deficient mice [5], and in the pathogenesis of viral myocarditis in mice [6,7]. The density of cardiac mast cells increases dramatically following MI (1.8 ± 0.3 to 26.3 ± 7.4 cells/mm^2) [3].

Recent studies have begun to use mast cell (c-kit)-deficient mice to determine the role of mast cells in ischemia–reperfusion injury. In the ischemia–reperfusion injury model, the amount of viable myocardium is significantly greater in mast cell (c-kit)-deficient mice [8]. However, male W/Wv mice show no difference in survival rate compared with wild-type mice at 35 days after MI. Also, Levick et al. investigated the contribution of mast cells to the post-MI remodeling in W/Wv mice at 7 days after MI. Their preliminary results indicated that chamber dilation was significantly greater in wild-type hearts than in W/Wv hearts with increased wall thinning and collagen deposition in the viable myocardium [9].

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Even though mast cells are important in ischemia–reperfusion injury, it is difficult to determine the importance of mast cells in ventricular remodeling after MI.

Mast cells also release proinflammatory cytokines [interferon-gamma (IFN-γ); tumor necrosis factor-alpha (TNF-α); and interleukin (IL)-1, IL-3, IL-4, I-5, IL-6, and IL-13] and chemokines [monocyte chemoattractant protein-1 (MCP-1), IL-8, and stem cell factor (SCF)], which may participate in MI [10–16].

In patients with MI [17,18] and animal MI model [19–23], the changes of cytokine and chemokine expression in the blood and myocardium can affect in the myocardial survival and remodeling, trigger of angiogenesis, and regulation of inflammation [19,24,25]. Cytokine and chemokine have diverse effects. SCF induces the proliferation and differentiation of mast cells, which are regarded as a harmful agent. However, SCF also has therapeutic effects on MI by recruiting stem cells or triggering angiogenesis [22,23,26]. MCP-1 contributes to the initiation and progression of cardiac dysfunction and remodeling of the extracellular matrix in the heart [27,28]. In vitro evidence has indicated that MCP-1 can protect cultured mouse neonatal cardiomyocytes (CMs) from hypoxia-induced cell death [29].

Mast cells are incompletely understood and have multi-dimensional effects. They regulate inflammation and house chemokines and cytokines. Therefore, in the present study, we aimed to elucidate the function of mast cells in angiogenesis, inflammatory process, and the adaptation of cardiac regeneration pathways in acute MI by use of an MI rat model.

2. Materials and methods

2.1. Isolation of mast cell granules (MCGs)

All experiments were conducted in accordance with the institutional guidelines for the use and care of laboratory animals, which conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Also, animal protocols were approved by the Chonnam National University Animal Care and Use Committee (Number = CNU IACUC-H-2009-18).

Rat peritoneal mast cells were isolated by a method described previously [4] with minor modifications. Briefly, 8- to 9-week-old male Sprague–Dawley (SD) rats (Samtaco) were used as the source of peritoneal mast cells. Mast cell viability was determined by trypan blue exclusion. Mast cells isolated by this procedure were more than 90% pure on the basis of staining with 0.05% Toluidine blue O. Under sterile conditions, mast cell granules (MCGs) were prepared from purified mast cells by stimulation with compound 48/80 as described previously [4] with minor modifications. Optimal stimulation and release were achieved by incubating rat peritoneal mast cells (1×10^6 cells/ml) for 10 minutes with 10 μg/ml compound 48/80 at 37 °C. Thereafter, cold DMEM was added to stop the reaction and dilute the solution 4 times to a final concentration of compound 48/80 of 2.5 μg/ml. Aliquots of the cell suspension were used as 100 % MCGs. All experiments were performed with compound 48/80 and culture medium as a control. In addition, modified MCGs [MCGs ± protease inhibitor (PI)] were prepared by incubation at 4 °C with PI cocktail (Pierce Biotechnology) and soy bean trypsin inhibitor 10 μg/ml (SBTI, Sigma-Aldrich) for 12 hours and with modification as described previously [4]. Aliquots of the inhibitor treated MCGs were used to identify the role of the protease.

2.2. Cell culture

The investigation conformed to the principles outlined in the Declaration of Helsinki for the use of human tissues. Regarding the use of human umbilical vein endothelial cells (hUVECs) in our study, approval was granted by a Chonnam National University Hospital Ethics Review Board (Number = I-2008-06-067). hUVECs were isolated from human umbilical veins as described previously [30]. The culture medium was Endothelial Cell Basal Medium-2 (EBM-2, Lonza). Rat CMs (rCMs) were isolated as described previously [31]. The methods for the isolation and characterization of rat cardiac microvessel endothelial cells (rCMECs) from adult rat ventricular tissue have been described in detail elsewhere [32], and the detailed method is described in the Supplemental methods.

2.3. Induction of hypoxic condition

The hypoxic condition was induced by a hypoxic chamber (95% N_2 and 5% CO_2) with serum starvation for 6 hours. Re-oxygenation was performed for 18 hours by replacement with complete media with serum and further incubation in an incubator under 95% air and 5% CO_2.

2.4. Apoptosis cell detection by annexin V and PI fluorescence-activated cell sorting analysis

Cell apoptosis was assayed by staining with the FITC annexin V apoptosis detection kit (Becton Dickinson (BD) Pharmingen) and was analyzed by fluorescence-activated cell sorting (FACS, BD). In the in vivo study, apoptosis was assayed by TUNEL staining (ApopTag peroxidase in situ apoptosis detection kit, Millipore) and was analyzed by microscopy analysis. Apoptosis of cardiomyocytes in infarct heart was analyzed by modified fluorescent TUNEL stain (Dead End TM Fluorometric TUNEL system, Promega) and fluorescence staining against anti-troponin antibody (Ab) and was observed by confocal microscopy.

2.5. Western blot analysis

Western blots were performed as previously described [33] by using an Akt sampler kit (Cell Signaling Technology) and anti troponin-I Ab (Santa Cruz Biotechnology). Equal protein loading was determined by the Bradford assay; blots were stripped and reprobed with anti β-actin Ab (Sigma-Aldrich) as an internal loading control. A single strong band at the appropriate size was assumed to indicate specificity.

2.6. Reverse transcription–polymerase chain reaction (RT–PCR)

We prepared $1–5\times10^5$ purified cells and total RNA was isolated from rCMs by using Trizol (Life Technologies). Reverse transcription was performed with 10 μg total RNA with oligo (dT) and 500 U MMLV reverse transcriptase (Superscript II, Invitrogen Corporation) according to the manufacturer's protocol. Fifty microliters of PCR reactions contained 0.2 mM of each dNTP, 20 nM of each primer, 1.25 U HotStar Taq polymerase, 5 μl 10× concentrated PCR buffer, 10 μl solution Q, and 2.5 mM $MgCl_2$. Amplification was performed for 35 cycles according to the manufacturer's protocol. Oligonucleotide primers were used as follows: c-kit, 5′-GGC CCA CCC TGG TCA TTA CAG AAT-3′ (forward) and 5′-TTC CTT GAT CAT CTT GTA GAA CTT-3′ (reverse); GAPDH, 5′-GGC CAA GGT CAT CCA TGA-3′ (forward) and 5′-TCA GTG AGC CCA GGA TG-3′ (reverse).

2.7. Angiogenesis assay

Culture wells were coated by fibrin gel (In Vitro Angiogenesis Assay, Millipore) for tube formation assay on ice. After 2 hours incubation at 37 °C, hUVECs or rCMECs were seeded on top and the dishes were further incubated for 24 hours in the presence of EBM-2 with 2 μl/ml MCGs or 2 μg/ml bovine serum albumin (BSA, Invitrogen