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Apelin-13 infusion salvages the peri-infarct region to preserve cardiac function after severe myocardial injury

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ABSTRACT

Background: Apelin-13 (A13) regulates cardiac homeostasis. However, the effects and mechanism of A13 infusion after an acute myocardial injury (AMI) have not been elucidated. This study assesses the restorative effects and mechanism of A13 on the peri-infarct region in murine AMI model.
Methods: 51 FVB/N mice (12 weeks, 30 g) underwent AMI. A week following injury, continuous micro-pump infusion of A13 (0.5 μg/g/day) and saline was initiated for 4-week duration. Dual contrast MRI was conducted on weeks 1, 2, 3, and 5, consisting of delayed-enhanced and manganese-enhanced MRI. Four mice in each group were followed for an extended period of 4 weeks without further infusion and underwent MRI scans on weeks 7 and 9.
Results: A13 infusion demonstrated preserved LVEF compared to saline from weeks 1 to 4 (21.9 ± 3.2% to 23.1 ± 1.7%* vs. 23.5 ± 1.7% to 16.9 ± 2.8%, *$p = 0.02$), which persisted up to 9 weeks post-MI (+1.4%* vs. −9.4%, *$p = 0.03$). Mechanistically, dual contrast MRI demonstrated significant decrease in the peri-infarct and scar % volume in A13 group from weeks 1 to 4 (15.1 to 7.4% and 34.3 to 25.1%, $p = 0.02$, respectively). This was corroborated by significant increase in 5-ethynyl-2′-deoxyuridine (EdU^+) cells by A13 vs. saline groups in the peri-infarct region (16.5 ± 3.1% vs. 8.1 ± 1.6%; $p = 0.04$), suggesting active cell mitosis. Finally, significantly enhanced mobilization of $CD34^+$ cells in the peripheral blood and up-regulation of APJ, fibrotic, and apoptotic genes in the peri-infarct region were found.
Conclusions: A13 preserves cardiac performance by salvaging the peri-infarct region and may contribute to permanent restoration of the severely injured myocardium.

1. Introduction

Coronary artery disease continues to carry high mortality and morbidity despite introduction of novel therapeutics, including stem cell therapy [1–3]. Although recent clinical stem cell trials showed promise in ischemic cardiomyopathy, there are still many unresolved debates regarding the ideal cell-type, mode of delivery, restorative effects, and mechanism of repair [2–4].

Apelin-13 (A13) is a recently discovered endogenous peptide associated with G-protein coupled receptor APJ in both human and murine species [5]. A13 is a potent inotropic and vasodilatory agent, demonstrating the beneficial preclinical effects in myocardial injury models [6–8]. In addition, the Apelin-APJ system influences the heart field development and cardiac differentiation of pluripotent stem cells as we have reported recently [9,10]. Other groups have reported that A13 may modulate endogenous stem cell function after acute myocardial injury (AMI) [11,12]. However, there is no definitive tissue characterization of the restorative effects on the injured myocardium. Moreover, the potential mechanisms underlying A13, including mobilization of bone marrow derived stem cells (BMSCs) and cardiac stem cells (CSCs), have not been delineated.

Precise characterization of the myocardium is critical to understand the impact of A13 on cardiac function and remodeling. The gold standard for infarct evaluation, delayed-enhancement MRI (DEMRI), using gadolinium (Gd^{2+})-based contrast agent does not provide direct cell viability information because of its non-specific distribution in the extracellular space and does not delineate the peri-infarct region [13–16]. On

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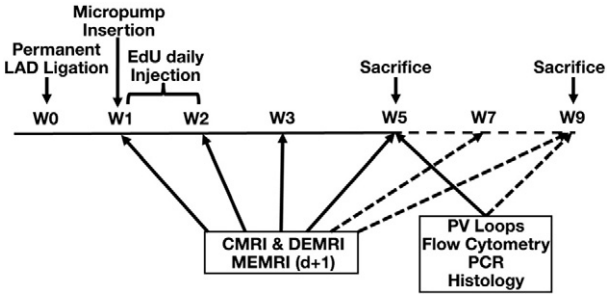

Fig. 1. Study design. LAD indicates left anterior descending coronary artery; EdU, 5-ethynyl-2′-deoxyuridine; CMRI, cardiac magnetic resonance imaging; DEMRI, delayed enhanced magnetic resonance imaging; MEMRI, manganese enhanced magnetic resonance imaging; PCR, polymerase chain reaction. Broken arrow indicates the infusion-free extension period from weeks 5–9.

the other hand, manganese-enhanced MRI (MEMRI), a viability-specific contrast agent, employing Mn^{2+}-based contrast agent enters only the viable cells via voltage-gated calcium channels [13]. Used in combination, MEMRI-positive viable myocardium overlaps the DEMRI-positive injured myocardium to generate a clear delineation of at-risk but viable peri-infarct myocardium as we have described previously [14].

This study hypothesizes that A13 salvages the injured myocardium through direct effects on the peri-infarct region by mobilization of BMSCs and up-regulation of LV remodeling genes. Specifically, dual contrast cardiac MRI studies assess the restorative effects of A13 on the peri-infarct region in murine AMI model.

2. Methods

2.1. Murine myocardial injury model

All animal studies were approved by the Stanford University Administrative Panel on Laboratory Animal Care. Animals were housed in a temperature-controlled (20 ± 2 °C) and humidity-controlled (60%) room under a 12 h light/cycle 6:00 am/6:00 pm. Male FVB/N mice at 12–14 weeks (Charles River Inc., Hollister, CA) were used.

Myocardial injury and MRI were performed in a total of 51 adult FVB/N mice (Fig. 1) [5]. Briefly, the mice were anesthetized with 2–3% inhalational isoflurane and intraperitoneal sodium pentobarbital. They were intubated to achieve positive pressure ventilation with oxygen/isoflurane mixture and thoracotomy was performed. Peak inspiratory pressure was maintained between 10 and 14 cm H_2O. The lung was retracted and the pericardium was incised. The left anterior descending (LAD) coronary artery was ligated until blanching of the distal left ventricle. Following complete hemostasis, the chest wall was closed in four layers. The animal was weaned from the ventilator, extubated, and monitored in the recovery area. Thirty-two of 51 (35% mortality, n = 19) mice were randomly assigned to either apelin-13-treated group or NS (normal saline) control group at the time of micro-pump insertion at 1 week after AMI.

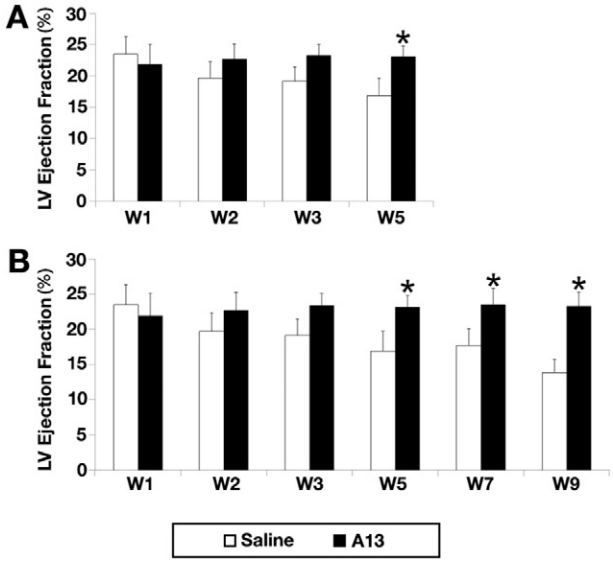

Fig. 2. Changes in left ventricular ejection fraction (LVEF) between apelin-13 (A13) infusion (n = 16, black) and 0.9% saline infusion (n = 16, white). A. Although LVEF of 0.9% saline infusion group was significantly decreased, A13 infusion group showed preserved LVEF through 4 weeks (weeks 1–5) of infusion (21.9 ± 3.2% to 23.1 ± 1.7%* vs. 23.5 ± 1.7% to 16.9 ± 2.8%, *$p = 0.02$) and B. In the extended group, infusion was stopped at the end of week 5 for 4 weeks from weeks 6–9. A13 group consistently demonstrated preservation of LVEF while the saline group showed reduction of LVEF during both the 4 weeks of infusion (weeks 1–5) and the 4 weeks of extended period without infusion. LVEF change at week 9 between A13 vs. control demonstrated significant increase in A13 group (+1.4%* vs. −9.4%, *$p = 0.03$).

2.2. Continuous systemic administration of A13 and NS using micro-osmotic pump

Half-life of A13 is approximately 30 min, requiring continuous micro-osmotic infusion pump (0.25 μL/h, 14 days; Alzet Direct, Cupertino, CA) of A13 (0.5 μg/g/day; American Peptide, Sunnyvale, CA) and NS for 4-week duration, following 1 week of convalescence post-LAD ligation in A13 treatment (n = 16) and in NS control (n = 16) groups [8]. The animals underwent DEMRI and MEMRI on consecutive days at 1, 2, 3 and 5 weeks after AMI. A subset of mice was followed for an extended period from weeks 6 to 9 without any treatment (n = 4 per group), additional scans were performed on weeks 7 and 9. All mice (n = 3 per group at week 2; n = 8 per group at week 5; and n = 4 per group at week 9) were sacrificed to perform ex vivo assays on explanted heart tissue.

2.3. In vivo MEMRI and DEMRI

To prepare for scanning, anesthesia was induced with 2% and maintained with 1.25–1.5% isoflurane. ECG leads were inserted subcutaneously to assess the heart rate while the body temperature was maintained at 37 °C and respiratory rate was monitored. Using a 3T GE Signa Excite whole-body scanner with a dedicated mouse coil (Rapid MR International, Germany), multiple cardiac function parameters were obtained on weeks 1, 2, 3 and 5 after AMI and additionally on weeks 7 and 9 for the extended survival group without the infusion treatment. The following sequences were performed for MRI acquisitions: (1) DEMRI — IP injection of 0.2 mmol/kg gadopentetate dimeglumine (Magnevist, Berlex Laboratories) and EKG gated fast gradient echo inversion recovery (fGRE-IR) sequences with FOV 4 cm, slice thickness 1 mm, matrix 256 × 256, TE 5 ms, TI 200–300 ms, NEX 2, and FA 30° and (2) MEMRI — IP injection of 0.7 cc/kg of manganese contrast agent (EVP1001-1, Eagle Vision Pharmaceutical) and EKG gated fGRE-IR sequence with FOV 4 cm, slice thickness 1 mm, matrix 256 × 256, TE 3.4 ms, FA 30°, 2R-R acquisition, TI 300–500 ms, and NEX2 at 24 h following DEMRI acquisition and (3) cardiac MRI — LV volumes and function were performed on the day of DEMRI using fGRE with FOV 5 cm, slice thickness 1 mm, matrix 256 × 256, TE 5 ms, and FA 30°. Coronal and axial scout images were used to position a 2-dimensional imaging plane along the short axis of the LV cavity.

2.4. MRI image analysis

MRI image analysis was performed as described previously [14]. Briefly, for each short-axis slice, planimetry measurements of the LV myocardium were conducted off-line by tracing the epicardial and endocardial borders at end-systole and -diastole with OsiriX software (OsiriX, open-source). The papillary muscles were considered part of the LV cavity. LV mass, LV end-diastolic volume (LVEDV), and LV end-systolic volume (LVESV) were measured to calculate the LV ejection fraction (LVEF). For infarct analysis, the MEMRI defect area and the DEMRI enhanced area were designated as scar tissue. These areas were traced in short-axis slices and integrated to determine scar volumes by MEMRI and DEMRI in matched mice hearts (n = 23). The % MEMRI scar volume = (MEMRI defect volume/total LV mass volume) × 100 and % DEMRI scar volume = (DEMRI scar volume/total LV mass volume) × 100. The difference between MEMRI and DEMRI defect volumes was defined as the peri-infarct volume [14].

2.5. Hemodynamic monitoring

For invasive evaluation of myocardial function, the mice were investigated using an impedance-micromanometer catheter [5]. Briefly, after catheterization via the right carotid artery, a 1.4 French catheter (SPR-839, Millar Instruments, Houston, TX) was introduced into the LV and pressure–volume (PV) loops were recorded. The method was based on measuring the time-varying electrical conductance signal of two segments of blood in the left ventricle from which the total volume is calculated. Raw conductance volumes were corrected for parallel conductance by the hypertonic saline dilution method.

2.6. Flow cytometry

Flow cytometry of the serum collected from the mice detected the presence of $CD34^+$ cells as described previously [14]. The serum was stained with FITC conjugated secondary antibody (1:500, Abcam, Cambridge, MA) for 30 min at room temperature. Expression of markers was determined by FACS Calibur (BD Bioscience, San Jose, CA) and FlowJo software (Tree Star, Ashland, OR) to quantify the percentage of $CD34^+$ cells. The cells were stained with mouse IgM isotype antibodies (Biolegend, San Diego, CA) to be used as the control group.