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Original article

Antagomir-92a impregnated gelatin hydrogel microsphere sheet enhances cardiac regeneration after myocardial infarction in rats☆

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ABSTRACT

Introduction: We investigated whether attachment of gelatin hydrogel microsphere (GHM) sheet impregnated with antagomir-92a on the infarcted heart promotes angiogenesis and cardiomyogenesis, and improves cardiac function after myocardial infarction (MI) in rats.
Methods: GHM sheet impregnated with antagomir-92a, its scramble sequence antagomir-control sheet or the sheet alone was attached on the area at risk of MI after the left anterior descending coronary artery ligation. Bromodeoxyuridine (BrdU) was included in the sheet to trace proliferating cells.
Results: The antagomir-92a sheet significantly increased capillary density in the infarct border zone 14 days after MI compared to the antagomir-control sheet or the sheet alone, associated with an increase in endothelial cells incorporated with BrdU. The antagomir-92a sheet significantly increased cardiac stem cells incorporated with BrdU 3 days after MI in the infarct border zone. This was associated with an increase in cardiomyocytes incorporated with BrdU 14 days after MI. Scar area was significantly reduced by the antagomir-92a sheet compared to the antagomir-control sheet or the sheet alone (12.8 ± 1.3 vs 25.2 ± 2.2, 24.0 ± 1.7% LV area, respectively) 14 days after MI. LV dilatation was inhibited, and LV wall motion was improved 14 days after MI in rats with the antagomir-92a sheet compared to the antagomir-control sheet or the sheet alone.
Conclusions: These results suggest that attachment of the GHM sheet impregnated with antagomir-92a on the area at risk of MI enhances angiogenesis, promotes cardiomyogenesis, and ameliorates LV function.

1. Introduction

Since current pharmacological and interventional approaches are limited to salvage the damaged heart after myocardial infarction (MI), cardiac regeneration therapy has emerged as an attractive treatment option for severe MI. However, the ideal method of cardiac regeneration therapy has not been established.

Cardiac regeneration therapy consists of cell-based or molecular-based therapy. Each therapy possesses advantages and disadvantages. Cell-based therapy can directly replenish the damaged heart. However, successful cell transplantation requires optimizing the best cell type and site for engraftment, overcoming limitations to cell migration and tissue integration, and occasionally needing to control immunologic reactivity [1]. Therapeutic approaches for cell-based therapy involve bone marrow-derived mononuclear cells and their subsets such as mesenchymal stem/

Abbreviations: GHM, gelatin hydrogel microsphere; MI, myocardial infarction; MSCs, mesenchymal stem cells; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; miRs, microRNAs; GA, glutaraldehyde; DDA, double-distilled water; LAD, left anterior descending; LV, left ventricular; LVDd, left ventricular end-diastolic diameter; LVDs, left ventricular end-systolic diameter; FS, fractional shortening; BrdU, bromodeoxyuridine; DAPI, 4′,6-diamidino-2-phenylindole.

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stromal cells (MSCs), endothelial progenitor cells as well as adipose tissue-derived MSCs, cardiac tissue-derived stem cells, and cell combinations. Clinical trials employing these cells have demonstrated that cellular therapy is feasible and safe, but overall clinical efficacy in patients with MI is modest [2,3]. Therefore, cell-based therapy is still challenging and further innovations are needed to be established as a common clinical practice.

On the other hand, molecular-based therapy has mainly focused on therapeutic angiogenesis, because angiogenesis is a key to success for cardiac regeneration after MI, Protein delivery or gene transfer of angiogenic growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) has demonstrated improvement in tissue perfusion and morphological and angiographic evidence of new vessel formation in various instances of animal models [4,5]. Such reproducible and credible successes in early animal studies have prompted clinical trials employing angiogenic growth factors. Unfortunately, in spite of promising early data coming from many pioneering clinical trials, recent larger and better-designed clinical trials provided disappointing results. An appreciable number of randomized controlled trials using VEGF and FGF proteins have shown far from optimal results [6]. Similarly, several phase I and phase II clinical trials using angiogenic gene transfer in patients with coronary artery disease have provided limited evidence regarding the efficacy and long-term sustainability of therapeutic effect, although generally supporting the safety and tolerability of angiogenic gene transfer [7]. Such limited success of molecular-based therapy using angiogenic gene transfer for cardiac regeneration may be attributed to the fact that tissue repair is not a simple process. Understanding cells, the signals that they respond to, and the keys to appropriate survival and tissue formation are orders of magnitude more complicated than understanding the pathways targeted by most genes. Thus, it is anticipated that targeting any single genes is difficult to confer appreciably beneficial outcomes for regeneration of the heart after MI.

MicroRNAs (miRs) are small non-coding RNAs with short ~22 nucleotide RNA sequences that bind to complementary sequences in the 3′ UTR of multiple target mRNAs, usually resulting in their silencing or translational repression. Each miR inhibits the function of hundreds of target RNAs, thereby integrating and fine-tuning the network of cellular processes involved in plethora of biological events including cardiovascular development and angiogenesis [8,9]. The ability of single miRs to modulate multiple gene expression makes them an attractive tool in cardiac regeneration therapy.

The miR-17-92 cluster has been considered as a therapeutic target for angiogenesis. The miR-17-92 cluster is composed of miR-17, miR-18a, miR-19a/b, miR-20a, and miR-92a. It was among the first miRs that were linked to tumor angiogenesis [10]. Overexpression of the entire miR-17-92 cluster in myc-induced tumors increased angiogenesis by a paracrine mechanism [11]. Interestingly, miR-92a has been found as a negative regulator of endothelial function and angiogenesis. Bonauer et al. [12] demonstrated that miR-92a is increased in the heart after MI, and systemic administration of antagomir-92a augmented neovascularization and repaired the infarcted tissue in mice. Moreover, the efficacy of anti-miR-92a therapy may not be confined to angiogenesis but is also attributed to cell-protective and anti-inflammatory effects [13]. Such pleiotropic salutary effects of anti-miR-92a on cardiac regeneration have prompted the investigators to develop a delivery system to optimally antagonize miR-92a in the post-infarction myocardium.

A critical issue in systemic administration of pro-angiogenic proteins or genes is unwanted angiogenesis in off-target organs such as cancer tissues and eyes with diabetic retinopathy. Systemic administration also requires a large quantity of drugs. Local delivery of pro-angiogenic agents overcomes such drawbacks. Indeed, catheter-based delivery of locked nucleic acid-modified antisense miR-92a was found to be more effective than systemic delivery in protecting the pig heart after MI [13]. However, proteins, plasmids or oligonucleotides are readily degraded or dissipated after local delivery to the target tissue. Therefore, the therapeutic efficacy of bolus injection of antagomir-92a into the heart may be limited. To achieve long-lasting delivery of antagomir-92a into the infarcted myocardium, we developed a biodegradable gelatin hydrogel microsphere (GHM) sheet that is slowly degraded and release impregnated plasmid DNA or proteins over 14 days [14]. We attempted to use the GHM sheet impregnated with antagomir-92a in the rat with acute MI, because mir-92a increases on the day 1 after MI, peaked on the day 2 and then gradually decreases over 14 days [12]. Therefore, the efficacy of the GHM sheet was thought to be maximally brought out by applying it to the heart immediately after MI. In this acute MI model, we investigated whether controlled local delivery of antagomir-92a to the heart increases angiogenesis, enhances cardiomyogenesis, and improves left ventricular (LV) function after MI.

2. Methods

2.1. Preparation of GHM-impregnated sheet

Antagomir-92a and its scramble sequence antagomir-control were synthesized by Greiner Bio-One (Frickenhausen, Germany) as described previously [12]. The GHM sheet used in the present study was prepared as described previously [15]. Briefly, GHMs were prepared by chemical cross-linking of aqueous gelatin solution with glutaraldehyde (GA). Gelatin with an isoelectric point of 5.0 was prepared as an acidic gelatin. After we mixed 2.5 mmol of aqueous GA solution (Wako Pure Chemical Industries, Osaka, Japan) with aqueous gelatin solution (5 wt%) preheated at 40 °C, the mixed aqueous solution was cast into balance dishes and left for 12 h at 4 °C to allow for chemical cross-linking of gelatin. The resulting GHM sheets were placed in 100 mmol/L aqueous glycine solution and then agitated at 37 °C for 1 h to block the residual aldehyde groups of unreacted GA. Cross-linked GHM sheets were thoroughly washed with double-distilled water (DDW), freeze-dried, and sterilized with ethylene oxide gas. GHM sheets were then trimmed in approximately 10 × 10-mm squares and 0.7-mm thick. Next, DDW containing 1 mg of antagomir-92a or antagomir-control was added drop-wise onto freeze-dried GHM sheets under sterile conditions and then left for 1 h to allow the GHM to be impregnated. The RNA-free, empty GHM sheets were prepared by the same method as described above.

2.2. Animal preparation

Male Sprague–Dawley rats weighing 250–300 g were used in the present study. All animals were handled in accordance with the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of the Care and Use of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised in 1996. The study was approved by the Animal Care Committee of Kansai Medical University (Moriguchi, Japan).

2.3. Surgical procedures

The rats were anesthetized with xylazine (5 mg/kg, s.c.) and ketamine (100 mg/kg, i.m.), and placed on a temperature-controlled surgical table. The trachea of rats was cannulated with a polyethylene tube connected to a respirator (Shinano,