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Controlled delivery of fibroblast growth factor-1 and neuregulin-1 from biodegradable microparticles promotes cardiac repair in a rat myocardial infarction model through activation of endogenous regeneration

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ABSTRACT

Acidic fibroblast growth factor (FGF1) and neuregulin-1 (NRG1) are growth factors involved in cardiac development and regeneration. Microparticles (MPs) mediate cytokine sustained release, and can be utilized to overcome issues related to the limited therapeutic protein stability during systemic administration. We sought to examine whether the administration of microparticles (MPs) containing FGF1 and NRG1 could promote cardiac regeneration in a myocardial infarction (MI) rat model. We investigated the possible underlying mechanisms contributing to the beneficial effects of this therapy, especially those linked to endogenous regeneration. FGF1- and NRG1-loaded MPs were prepared using a multiple emulsion solvent evaporation technique. Seventy-three female Sprague–Dawley rats underwent permanent left anterior descending coronary artery occlusion, and MPs were intramyocardially injected in the peri-infarcted zone four days later. Cardiac function, heart tissue remodeling, revascularization, apoptosis, cardiomyocyte proliferation, and stem cell homing were evaluated one week and three months after treatment. MPs were shown to efficiently encapsulate FGF1 and NRG1, releasing the bioactive proteins in a sustained manner. Three months after treatment, a statistically significant improvement in cardiac function was detected in rats treated with growth factor-loaded MPs (FGF1, NRG1, or FGF1/NRG1). The therapy led to inhibition of cardiac remodeling with smaller infarct size, a lower fibrosis degree and induction of tissue revascularization. Cardiomyocyte proliferation and progenitor cell recruitment were detected. Our data support the therapeutic benefit of NRG1 and FGF1 when combined with protein delivery systems for cardiac regeneration. This approach could be scaled up for use in pre-clinical and clinical studies.

1. Introduction

Ischemic heart disease is the leading cause of morbidity and mortality worldwide [1]. Thus, there has been great interest in novel therapeutic options, such as gene (reviewed in [2]) and stem cell therapy (reviewed in [3]), or even direct administration of pro-angiogenic cytokines [4]. In the case of growth factor-based therapy, although preclinical studies and initial clinical trials had suggested beneficial effects [5,6], double-blinded clinical trials with large cohorts of patients failed to validate the efficacy [7–9]. These negative findings may have resulted from issues related to growth factor selection, monotherapy instead of combinatorial therapy, and/or timing of growth factor delivery. Moreover, the therapeutic benefit of directly administered growth factors can be limited by the short circulating half-life and high instability of these proteins after injection. In this context, new strategies involving injectable biocompatible and biodegradable microparticles (MPs), which mediate sustained release of cytokines, might offer valuable approaches for overcoming these limitations [10].

Poly(lactic-*co*-glycolic acid) (PLGA) is a biopolymer that is FDA-approved for use as a drug delivery platform due to its excellent biocompatibility, high safety profile, and suitable biodegradation [11]. PLGA MPs were already shown to be useful for growth factor delivery [12,13]. Moreover, we demonstrated the efficacy of treating infarcted hearts with PLGA MPs loaded with vascular endothelial growth factor (VEGF), which induced neovascularization and reduced cardiac remodeling after myocardial infarction (MI) in rats [14]. Indeed, many

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pre-clinical and clinical studies aimed at repairing an infarcted heart tissue have explored pro-angiogenic cytokine administration as a means to promote tissue revascularization.

In addition to mediators of angiogenesis, the list of potential therapeutics for cardiac regeneration has continued to grow, and the use of factors involved in cardiac development, stem cell homing, cardiac differentiation/proliferation, or direct cardioprotection could lead to novel approaches for repairing a damaged heart (reviewed in [4]). In this regard, in vitro studies have shown that adult cardiomyocytes do not proliferate under resting conditions, but may divide in response to extracellular mitogens, such as periostin [15], acidic fibroblast growth factor (FGF1) [16], and neuregulin-1 (NRG1) [17]. These findings have supported a new paradigm, which suggests that the heart might be capable of repair and regrowth in response to extracellular mitogens. Consistent with this idea, it is known that FGF1 regulates cardiac remodeling by exerting a protective and proliferative effect after MI [18,19]. On the other hand, neuregulins play crucial roles in the adult cardiovascular system by inducing structural organization of sarcomeres, cell integrity, cell–cell adhesion [20], cell survival [21,22] and angiogenesis [23]. In fact, several studies using animal models of heart failure have demonstrated the therapeutic benefits of neuregulins, which improved cardiac performance, attenuated disease markers, and prolonged animal survival [24,25]. Furthermore, phase I and II clinical trials for chronic heart failure in humans confirmed the favorable effects mediated by neuregulins [26,27], highlighting the therapeutic potential of these growth factors in cardiac repair.

In this study, we have examined the efficacy of novel MP-based delivery of NRG1 and FGF1 in a rat model of MI. Notably, the use of MPs prevented issues related to growth factor stability, facilitating sustained treatment in the damaged tissues. As a result, we observed significant improvement in cardiac function upon MP-mediated delivery of these factors to infarcted hearts. Finally, we investigated the underlying mechanisms contributing to this positive effect, especially those linked to endogenous regenerative capacity.

2. Materials and methods

All animal procedures were approved by the University of Navarra Institutional Committee on Care and Use of Laboratory Animals as well as the European Community Council Directive Ref. 86/609/EEC. An expanded Methods section is available in the Supplementary Material.

2.1. Materials

Recombinant human FGF1 and NRG1 were supplied from ImmunoTools GmbH (Friesoythe, Germany). PLGA with a monomer ratio (lactic acid/glycolic acid) of 50:50 Resomer® RG 503H (M_w: 34 kDa) was provided by Boehringer-Ingelheim (Ingelheim, Germany). Polyethylene glycol (PEG; M_w: 400), human serum albumin (HSA), bovine serum albumin (BSA), dimethylsulfoxide (DMSO) and sodium azide were provided by Sigma-Aldrich (Barcelona, Spain). Dichloromethane and acetone were obtained from Panreac Quimica S.A. (Barcelona, Spain). Poly(vinyl alcohol) (PVA) 88% hydrolyzed (M_w: 125,000) was obtained from Polysciences, Inc. (Warington, USA). Murine HL-1 cardiomyocyte-cell line (kindly donated by Dr. Claycomb, Louisiana State University Medical Center, USA) was used in the in vitro assays. Claycomb medium was provided by SAFC Biosciences (Lenexa, KS, USA) and 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H tetrazolium (MTS) was purchased from Promega (Madison, USA). Rabbit polyclonal anti-human FGF-1 antibody (ab9588) was supplied by Abcam (Cambridge, UK) and horseradish-peroxidase conjugated donkey anti-rabbit (NA934V) were purchased from GE Healthcare. Goat polyclonal anti-human NRG-1 antibody (sc-1793) and horseradish-peroxidase-conjugated donkey anti-goat IgG (sc-2020) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). ECL™ anti-rat IgG horseradish peroxidase-linked whole antibody was from Amersham Biosciences (Buckinghamshire, UK). Monoclonal anti-alpha smooth muscle actin-Cy3 (C6198) was provided by Sigma (St. Louis, MO, USA), anti-caveolin-1 and rat anti mouse CD45 (550539) from BD Pharmingen (Heidelberg, Germany). Rabbit polyclonal anti-human c-Kit antibody (A4502) was supplied from Dako (Carpinteria, CA, USA), monoclonal anti-human Ki-67 antibody (RM9106) was purchased from Thermo Fisher Scientific (Fremont, CA, USA) and mouse monoclonal cardiac troponin I antibody (ab19615) was obtained from Abcam (Cambridge, UK). DAPI nucleic acid stain was supplied from Molecular Probes-Invitrogen (Carlsbad, CA, USA) and TOPRO-3 was from Molecular Probes.

2.2. Preparation and characterization of MPs containing FGF1 and NRG1

FGF1- and NRG1-loaded PLGA MPs were prepared through a solvent extraction/evaporation method using the Total Recirculation One Machine System (TROMS) [14]. Particle size and size distribution were measured by laser diffractometry. Cytokine encapsulation efficiency and in vitro release from MPs was quantified by western blot. The bioactivity of MP-released proteins was evaluated in vitro by determining HL-1 cardiomyocyte proliferative capacity following growth factor treatment.

2.3. MI model and intramyocardial administration of MPs

Seventy-three female Sprague–Dawley rats underwent permanent left anterior descending coronary artery occlusion to induce MI. Among the surviving animals ($n = 57$), only those with a left ventricular ejection fraction (LVEF) below 50% ($n = 46$) at 2 days post-MI were included in the study. Four days post-MI, rats were divided into four groups, and the chests were reopened. Two milligrams of FGF1-loaded MPs (FGF1-MP; 1740 ng of FGF1), NRG1-loaded MPs (NRG1-MP; 1300 ng of NRG1), a mixture of MPs loaded with FGF1 and NRG1 (FGF1/NRG1-MP; loaded with the same doses), or control non-loaded MPs (NL-MP) were injected with a 29-gauge needle into four regions surrounding the border of the infarct. At 1 week ($n = 6$ rats) and 3 months ($n = 40$ rats) post-injection the animals were sacrificed.

2.4. Morphometric and histological studies

The heart function was assessed 3 months post-treatment. In addition, heart tissue remodeling, revascularization, cardiac proliferation, and endogenous stem cells were investigated. All results obtained from the growth factor-treated groups were compared to the NL-MP-injected control group.

2.5. Statistical analysis

Results are expressed as mean ± SEM. Statistics were calculated using Prism 5.0 software (Graphpad Software Inc., San Diego, CA, USA). P values <0.05 were considered significant.

3. Results

3.1. FGF1 and NRG1 induce adult cardiomyocyte proliferation and survival in vitro

The effect of FGF1 and/or NRG1 on adult cardiomyocyte proliferation and apoptosis was studied in vitro. The treatment of HL-1 cardiomyocytes with different doses of FGF1 or NRG1 (alone or in combination) led to a statistically significant increase in cell proliferation (Fig. 1A) ($P < 0.01$). HL-1 cell apoptosis could be induced by hypoxia and serum deprivation, and addition of both FGF1 and NRG1 resulted in a statistically significant decrease in the apoptotic phenotype (Fig. 1B and C).