Microparticle-Mediated Delivery of Growth Factors Promotes Cardiac Repair in Infarcted Rats

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ABSTRACT SUMMARY

In this work, we evaluated the therapeutic potential of growth factors (GF) encapsulated in Poly (lactic-co-glycolic acid) (PLGA) microparticles (MP) to repair the myocardium after a myocardial infarction (MI). MP with a diameter of 5 µm were found to be compatible for intramyocardial administration in terms of injectability and tissue response. In an experimental rat model of MI, PLGA-MP efficiently delivered growth factors in infarcted hearts, promoting myocardial regeneration by distinct mechanisms of cardiac repair.

INTRODUCTION

Growth factor (GF)-based therapy has emerged as a promising strategy to treat patients with MI [1]. Acidic fibroblast growth factor (FGF-1) and neuregulin-1 (NRG-1) are growth factors involved in cardiac development and regeneration. However, 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. To circumvent these limitations, we have investigated the administration of PLGA microparticles containing FGF-1 and NRG-1 in a rat model of MI.

EXPERIMENTAL METHODS

PLGA microparticles containing FGF-1 or NRG-1 were separately prepared through a solvent extraction/evaporation method using the Total Recirculation One Machine System (TROMS) [2]. Particle size distribution of the microparticles was measured by laser diffractometry. Encapsulation efficiency and in vitro release of factors from MPs was quantified by western blot. Cumulative release kinetics was conducted to determine the in vitro growth factor release profiles from the microparticles. For bioactivity, cell growth–promoting activity was detected by MTS assay after stimulation of HL-1 cardiomyocyte-cell line proliferation by microencapsulated growth factors. Next, female Sprague-Dawley rats (Harlan-IBERICA, Spain) underwent permanent occlusion of the left anterior descending coronary artery (LAD). Four days post-MI, rats were divided in four groups to receive FGF1-MP or NRG1-MP or a combination of microparticles loaded with the same doses of FGF-1 and NRG-1 (FGF1/NRG1-MP) or control non-loaded microparticles (NL-MP). Three months after treatment, echocardiography was performed. Animals were sacrificed and their hearts collected for subsequent morphometric and histological studies. Cardiac function, tissue remodeling, revascularization, cardiomyocyte proliferation, and stem cell homing were evaluated. Prior to these experiments, biodegradation and tissue reaction were studied in a group of infarcted rats injected with fluorescence-labeled PLGA microparticles containing rhodamine B isothiocyanate (growth factor free).

RESULTS AND DISCUSSION

MPs with a diameter of 5 µm were the most compatible with intramyocardial administration. No signs of physiological disturbances such as fibrillation upon
microparticles injection and no adverse cellular reactions were observed. MPs were present in the heart tissue for up to 3 months and no particle migration toward other solid organs was observed, demonstrating good myocardial retention. These findings encouraged preparation of growth factor-loaded MPs. Characterization results are summarized in Table 1 and Fig. 1. MPs released the bioactive factors in a sustained manner for up to 28 days.

Table 1. Characterization of growth factor-loaded microparticles

<table>
<thead>
<tr>
<th>Mean size (µm)</th>
<th>Yield (%)</th>
<th>FGF-1 entrapment efficiency (%)</th>
<th>NRG-1 entrapment efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.8 – 5.4</td>
<td>&gt; 90</td>
<td>87.4 ± 2.3</td>
<td>65.5 ± 5.1</td>
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Three months after treatment, a cardiac function improvement was detected in the rats treated with FGF1-MP (16.7 ± 4.9%, P<0.05), NRG1-MP (18.0 ± 5.7%, P<0.05) or FGF1/NRG1-MP (13.0 ± 1.9%, P<0.05) in comparison with the non loaded-MP control group (1.1 ± 3.6%). From cardiac repair mechanisms standpoint, growth factor-loaded MP therapy led to inhibition of cardiac remodeling with smaller infarct size, a lower fibrosis degree and induction of tissue revascularization.

Also, we observed a significant increase in the number of Ki67+ cardiomyocytes in the infarcted and peri-infarcted zones following treatment with NRG1-MP compared with NL-MP (Fig. 2). Importantly, recruitment of c-Kit+ progenitor cells towards the ischemic myocardium under stimulation of NRG-1 delivered from the MP was also detected.

Figure 1. Encapsulation efficiency by western blot. Duplicate bands correspond to microparticle-extracted FGF-1 (A) or NRG-1 (B) using DMSO. The blot signals were quantified by densitometry by performing a growth factor standard curve (20, 40, 60, 80 and 120 ng) in order to determine the quantity of encapsulated FGF-1 or NRG-1.

Figure 2. Representative image of a proliferating Ki67+ adult cardiomyocyte (pink nucleus) in the NRG1-MP group, indicating cardiomyocyte proliferation. Myocytes were stained by a cardiac troponin T (cTnT) antibody (green). Nuclei were stained by DAPI (blue).

CONCLUSION
PLGA-MP efficiently delivered FGF-1 and NRG-1 in infarcted hearts, promoting myocardial regeneration by distinct mechanisms of cardiac repair after MI.

REFERENCES

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