TEMPORAL EVOLUTION OF MYOCARDIAL PERFUSION, VIABILITY AND FUNCTION AFTER INTRAMYOCARDIAL TRANSFER OF PLASMID DNA GENE EXPRESSING TWO ISOFORMS OF HEPATOCYTE GROWTH FACTOR

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INTRODUCTION: Coronary angioplasty or bypass surgery is routinely applied to restore flow to ischemic myocardium. Nevertheless, many patients with end stage coronary artery disease continue to suffer from disabling angina. This problem has increased interest in alternative revascularization strategies such as angiogenic growth factors, genes or stem cells.

PURPOSE: To determine the temporal evolution of myocardial perfusion, viability and LV function after intramyocardial transfer of plasmid DNA gene (HGF gene) expressing two isoforms of human hepatocyte growth factor (HGF) using MR imaging, histochemical and histopathological staining.

MATERIALS AND METHODS: The HGF gene was injected intramyocardially 1 hr after reperfusion of acute myocardial infarction (2hr occlusion), for the purpose of evaluating this strategy as a therapeutic approach for protection from LV remodeling. MR imaging was performed at 3 days and 7-8 weeks on a 1.5-T MR clinical scanner (Philips Medical Systems). First pass perfusion, delayed contrast enhancement and cine MR imaging was used in the evaluation. At 7-8 weeks the hearts were sliced and stained with the histochemical stain (TTC) to delineate scar tissue. Histopathological stains were utilized to quantify vascular density and myocardial viability. Sections (5μm) were stained with Masson's trichrome and isolectin B_4 stains. Capillary (<15μ) and arteriole (>15-100μ) were counted (vessels/mm^2) in sampled regions. Student's t-test was implemented for statistical analysis.

RESULTS: The peak signal intensity (SI), extents of hypoenhanced ischemic myocardium and max upslope data in the two groups were not significantly different at 3 days. At 7-8 weeks the extent of hypoenhanced ischemic myocardium was smaller, peak signal intensity was higher and maximum upslope data was steeper in treated animals compared to controls (**Fig. 1**). The extent of hyperenhanced scar was significantly larger in control (13.2 ± 1.6% LV) compared with HGF treated (7.0 ± 0.5% LV) animals at 7-8 weeks. TTC analysis also showed that the extent of scar tissue significantly larger in control (12.0 ± 1.7%) compared with treated (6.6 ± 0.7% LV, *P* = 0.04) animals (**Fig. 2**). There was no significant difference between the extent of hyperenhanced scar on MRI and TTC (*P*=0.32). The infarction tended to be non-transmural with a residual thicker wall in treated compared with control animals on MR images. Control animals showed a decline in ejection fraction (41.2±0.7% to 36.9±1.0%, *P*=0.01), while treated animals showed increased ejection fraction (40.3±1.3% to 45.7±1.8%, *P*=0.001). Quantitative analysis of vascular density in control animals revealed clear gradients in density between remote (422±20 capillaries/mm^2), peri-infarcted myocardium (145±7/mm^2) and scar (119±17/mm^2). Control animals showed sharp borders separating viable myocardium from scar. In contradistinction, treated animals showed irregular scars with collagen interdigitation with viable myocardium (peninsulas/islands) in the peri-infarcted region and scar (**Fig. 3**). The capillary density in peri-infarcted myocardium (245±47 capillaries/mm^2, *P*=0.03) and scar (218±41, *P*=0.02) in treated animals was significantly greater than in control animals.

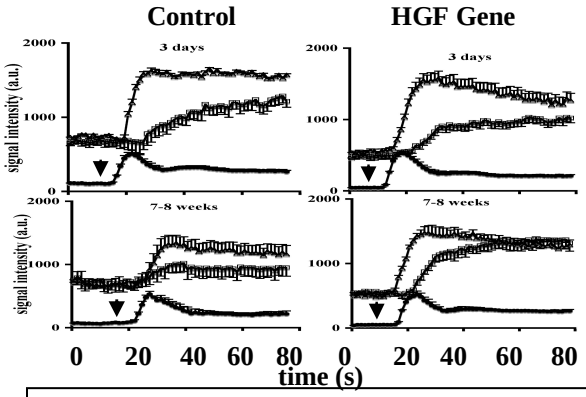


Fig. 1. First pass MR perfusion of Gd-DOTA (0.1 mmol/kg) at 3 days showed no difference in enhancement between control (n=8) and HGF gene treated (n=8) animals. At 7-8 weeks after infarction treated, but not control, animals showed improvement in perfusion. At 3 days, max upslope (s^{-1}) was 188±38 in control and 207±44 in treated animals, and at 7-8 weeks 225±19 and 314±26 P=0.002, respectively. ∇ represents contrast injection. Blood pool SI is 1/10 of actual SI.

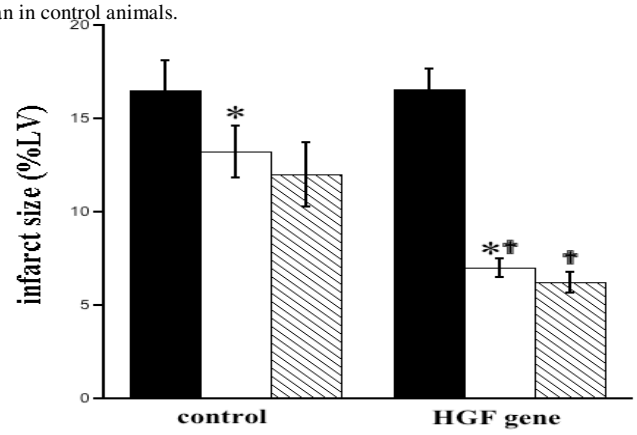
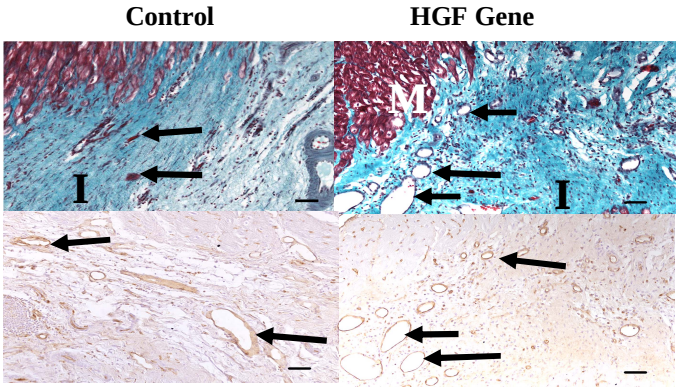


Fig. 2. Histogram shows temporal evolution in infarction size in control (left) and HGF gene treated (right) animals. The decline in infarction size was significantly greater in treated than control animals. Black bars = MR enhanced region at 3 days, white bars = MR enhanced region at 7-8 weeks, and striped bars = TTC true infarction at postmortem. *P< 0.03 compared to 3 days, †P<0.04 compared to control animals.

Fig. 3. Microscopic findings showed at the edge of infarction in control animals (left) few sparse thin-walled vessels (arrows), thick-walled vessels (arrowheads) and degenerated myocytes are shown (top left). The few vessels in the peri-infarction region are localized by the brown reaction product of the lectin stain (bottom left). In contrast, a treated HGF heart (right) showed an irregular healed infarct (I) borders (top right) with viable peninsulas/islands. The edge of the scar contains numerous thin-walled blood vessels (arrows). Top panels—Masson trichrome stain. Bottom panels—isolectin B_4. Calibration bars = 45μm.



CONCLUSION: Our study is the first to demonstrate the biological effects of coexpression of HGF and dHGF in myocardial infarction. The beneficial effect of this gene in infarcted myocardium lies in the formation of new blood vessels and peninsulas/islands of viable cardiomyocytes. This MR study provides comprehensive assessment of myocardial perfusion, viability and function after intramyocardial transfer of gene therapy.

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