Cardiac specific Plasmid-VEGF Increases myocardial angiogenesis, Perfusion and Infarct Resorption

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Introduction. Optimal influence of gene therapy is dependent on efficient delivery, localized site of action and state of target myocardium. The purpose of this study was to determine the effects of intramyocardially injected cardiac-specific and hypoxia–inducible VEGF expression gene on perfusion, infarct resorption and angiogenesis.

Materials and Methods. The myosin light chain (MLC) 2v promoter is a contractile protein and abundant in slow-twitch skeletal and cardiac muscles. The promoter was cloned between nine copies of Epo HRE and human VEGF165 cDNA in an AAV vector to generate AAV-VEGF. The promoter was used to derive genes for a human VEGF isoform, $VEGF_{165}$ and LacZ. Approximately 10^{11} copies of the AAV-VEGF vector mixed with 10^{10} copies of AAV-LacZ. A total of 10^{11} copies of AAV-VEGF were injected directly into swine myocardium 1h after reperfusion. Myocardial infarction was produced in 12 pigs by occluding LAD coronary artery for 2h/reperfusion. At 3 days and 8 weeks control (n=6) and plasmid-VEGF treated pigs (n=6) were imaged (1.5T MR scanner, Philips Medical Systems, The Netherlands). Gd-DOTA (0.1 mmol/kg bolus, Guerbet Group, France) first pass perfusion (SR-GRE: TR/TE: 3/1.5ms) and delayed contrast enhancement (IR-GRE: TR/TE: 4.4/2.1ms, TI: 270 to 325ms) were performed to assess regional perfusion, area at risk (AAR) and infarct size. Tissue samples were stained with special dyes.

Results.

First pass Perfusion. For both plasmid-VEGF and control animals, at the time when normal myocardium demonstrated peak signal enhancement on first-pass MR contrast media, AAR demonstrated hypoenhancement. At 3 days the increases in signal intensity of remote myocardium and AAR during first-pass was not significantly different between the groups, as reflected by the maximum upslope of the first pass curve (170±17ms in treated versus 167±23ms control animals, P=ns). At 8 weeks after infarction, infarcts in animals treated with plasmid-VEGF showed greater enhancement during the first-pass of contrast media than control animals, suggesting better perfusion and/or increasing blood volume. The maximum upslope of the first pass curve at 8 weeks in plasmid-VEGF treated animals was significantly steeper as compared with control animals (225±46ms versus 166±16ms, P<0.05) (Fig. 1).

Myocardial Infarcts. At 3 days, the extent of hyperenhanced region was comparable between treated (19±2% of LV mass) and control animals (18±2, p=NS unpaired t-test). However, at 8 weeks the extent of hyperenhanced region was smaller in treated (10±3% LV mass) than control animals (15±2% LV mass, p<0.01). The difference in infarct size suggest that the resorption of necrotic tissue was greater in treated than control animals. The true infarct size on TTC was also smaller in treated (10±1% LV mass, p<0.01) than control animals (14±3% LV mass). Infarct sizes defined by TTC and MR were identical for the intra-group.

Angiogenesis. The new vessels were thin-walled at 8 weeks after therapy and located in scar tissue and peri-infarcted myocardium. The sites of injection were recognized by the presence of abundant hemosiderin. The biotinylated isolectin B4 stain localized vascular endothelial cells with brown reaction product. The normal remote myocardium showed numerous and uniformly distributed microvessels coursing in parallel with the myocytes. Sparse remodeled blood vessels (thick walled and small lumen), coursed in the scar tissue in control animals. The number of thick walled arteries was significantly smaller in treated (8±1 vessels/mm^2) compared with control animals (17±1 vessels/mm^2). The ratio of the vessel diameter to lumen diameter was 3.3±0.1 in treated and 8.2±0.5 in control animals. Furthermore, plasmid treated animals showed greater numbers of new thin-walled vessels (38±2 vessels/mm^2) and capillaries 1065±78 capillaries/mm^2 than control animals (6±2 vessels/mm^2) and 576±50 capillaries/mm^2, respectively (Fig. 2).

Conclusion. AAV-VEGF gene improves myocardial perfusion by 86%. This gene increased vascular density (angiogenesis by 86% and arteriogenesis by 533%). Finally, AAV-VEGF gene enhanced infarct resorption by 32%. MRI is sensitive enough in demonstrating the effects of local gene therapies in infarcted myocardium.

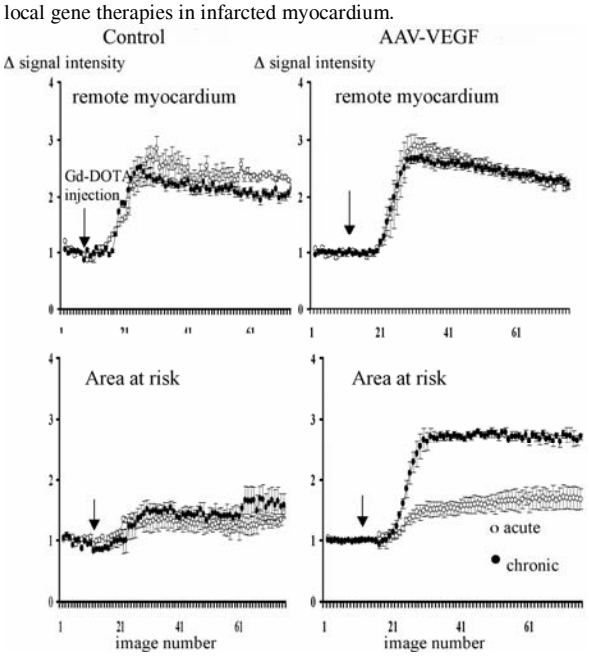

Figure 1. Comparison of the percent changes in signal intensities of normal and area at risk at 3 days and 8 weeks after infarction in control (n=6) and AAV-VEGF treated (n=6) animals. Note that the difference reached significant level (*P*=0.001) in treated compared to control animals (bottom right). Maximum upslope of the curves in AAR were 225±19ms in control and 166±16ms in treated animals (*P*=0.038).

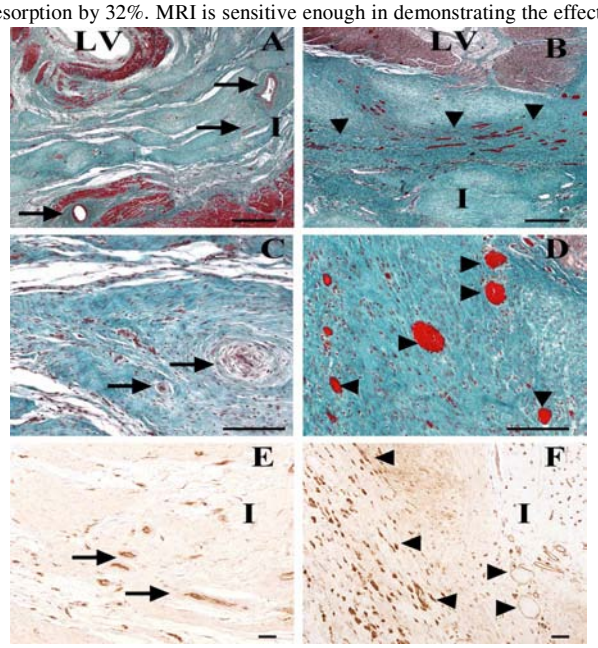

Figure 2. Representative infarcts from control (A, C, E) and AAV-VEGF treated (B, D, F) animals are shown. At low magnification (top panels), the infarct from the control animal (panel A) shows no appreciable neovascularization, while that from the AAV-treated animal (panel B) contains numerous vessels (arrowheads). At high magnification (C, D), control infarct has a few scattered thick-walled vessels (arrows), while treated infarct contains numerous thin-walled vessels (arrowheads) filled with blood. Lectin localizes all vessels by brown reaction product (E, F). Sparse vessels in the infarct from control animal (panel E) and numerous vessels in AAV-VEGF treated infarct (panel F) were demonstrated by the lectin stain.