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Targeted Delivery of VEGF after a Myocardial Infarction Reduces Collagen Deposition and Improves Cardiac Function

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Abstract

The development of adjunctive therapies which attenuate adverse remodeling and improve LV function post myocardial infarction (MI) is of significant clinical interest. Previously, we have shown that targeted delivery of therapeutic vascular endothelial growth factor (VEGF) to the infarct border zone significantly increases vascular perfusion and results in improvements in LV function. In this study, we tested the hypothesis that improvements in cardiac function observed with this novel targeted drug delivery system strongly correlate with reductions in collagen deposition in the scar tissue after an MI. Rats received anti-P-selectin conjugated immunoliposomes containing VEGF immediately post-MI. Over 4 weeks, evolutionary changes in LV geometry and function were correlated with collagen deposition and infarct size quantified by Gomori's trichrome and picrosirius red staining. Targeted VEGF treated hearts showed a 37% decrease in collagen deposition in the anterior wall, as well as significant improvements in LV filling pressures. Multi-regression analysis showed that the extent of collagen deposition post MI can be predicted by a linear combination of normalized LV mass and ejection fraction. Targeted delivery of VEGF post-MI results in significant decreases in collagen deposition and adverse remodeling. Improvements in cardiac function in this model are related to degree of collagen deposition and extent of scar formation.

Keywords

Pro-angiogenic compounds; Diastolic function; Cardiac remodeling

INTRODUCTION

During an acute myocardial infarction (MI), the loss of coronary blood flow due to prolonged coronary artery occlusion leads to death of myocardial tissue and a brisk inflammatory response. During this process, invasion of the infarct zone by inflammatory cells leads to collagen degradation and ultimately neovascularization and reconstruction of

the collagen network. Failure to adequately restore blood flow can result in the development of an ischemic cardiomyopathy, associated with adverse cardiac remodeling and reduced long-term survival.¹⁰ An essential component to the development of adverse post infarction remodeling is the degree of infarct expansion. In this regard, the extent of collagen damage is a strong predictor of the degree of infarct expansion (for a review see Ref. 5) and development of non-invasive methodologies for predicting the extent of collagen deposition post-MI is of significant clinical interest.

Therefore, the association of adverse remodeling with increased mortality makes attenuation of the adverse remodeling and collagen deposition a desirable therapeutic target for any potential adjunctive post infarction therapy.

Over the years, there has been great interest in adjunctive therapies which can alter the pathophysiologic processes that lead to infarct expansion.^{10,13} To date, only a few interventions exist to promote favorable remodeling after an MI. These well-known pharmacological interventions include Angiotensin-Converting Enzyme (ACE) inhibitors, angiotensin receptor blockers, and beta blockers, which achieve their benefits by attenuating the remodeling process.^{2,15} Development of an acutely administered adjunctive therapy which limits adverse cardiac remodeling and collagen build up could significantly improve clinical outcomes by reducing infarct expansion.

Previously, we have shown that endothelial cell adhesion molecules, such as P-selectin, are significantly up-regulated in the MI region and that this up-regulation provides a potential avenue for selectively targeting drugs to infarcted tissue.²¹ Using immunoliposomes decorated with a mAb to P-selectin, our laboratory has quantified the biodistribution and efficacy of this novel drug carrier system. We have demonstrated that anti-P-selectin conjugated immunoliposomes delivered post MI, result in a 140 and 180% increase in selective adhesion to the infarct border zone at 1 or 4 h post MI, respectively²¹ correlating with the peak up-regulation of P-selectin. Furthermore, we have shown that post-MI targeted delivery of low doses of VEGF containing anti-P-selectin conjugated liposomes results in significant increases in the number of anatomical and perfused vessels in the MI region of treated animals and that this increase may correspond to improvements in cardiac function.²⁰ Collectively these findings support the concept that drug therapy can be preferentially delivered to the infarct border zone using selected inflammatory markers as a target.

A number of studies have shown that restoration of adequate blood flow to the ischemic myocardium limits the amount of damage after an infarction.²² Studies have shown that delivery of an adequate therapeutic dose of pro-angiogenic compounds, such as vascular endothelial growth factor (VEGF), to the infarct border zone could initiate cardioprotective effects,¹¹ as well as induce angiogenesis in diseased cardiac tissue.¹² The transient VEGF exposure is probably responsible for the limited clinical success with this molecule to date, as VEGF needs to be present for relatively long time periods to prevent regression of the newly formed vessels.²⁶ Targeted VEGF therapy, in which the VEGF compound is encapsulated within a drug vehicle and therefore protected from plasma degradation, may limit the amount of myocardial damage after an infarct and subsequently reduce adverse cardiac remodeling associated with MI. However, declines in LV function may also be related to the extent of collagen damage.^{3,5} Therefore, we have studied the effects of targeted VEGF therapy on left ventricular geometry and cardiac function and tested the hypothesis that improvements in cardiac function observed with this novel targeted drug delivery system strongly correlate with reductions in collagen build up in the scar tissue after an MI.

MATERIALS AND METHODS

Rodent Model of Myocardial Infarction

Six-week old male Sprague-Dawley rats (150–200 g) were obtained from Harlan Laboratories (Indianapolis, Indiana) for these studies. All rats were housed 3 animals per cage, received 12 h light/dark cycle and were fed standard rat chow. Anesthesia was induced using Isoflurane (4% induction, 2% maintenance) and the animals received ventilatory support. Upon adequate anesthesia, a left thoracotomy was performed, the heart was rapidly exteriorized, and ligation of the left anterior descending coronary artery was performed using a 6.0 silk suture. Upon completion of the procedure, the chest was closed and the lungs re-inflated using positive end-expiratory pressure. The animals were allowed to recover at ambient room temperature and were available for additional studies 24 h after surgery.

Experimental Protocol

Animals that underwent MI surgery were either treated with VEGF immunoliposomes (targeted VEGF, $n = 6$) or left untreated (UMI, $n = 6$). A separate group of normal animals without MI or treatment (sham, $n = 3$) were also followed for comparison. Upon completion of the surgical procedure, all animals in the treated group received targeted delivery of VEGF-encapsulated immunoliposomes via tail vein injection. Serial echocardiographic measurements were obtained over period of 28 days after surgery. All procedures conformed to the guidelines of the NIH Guide to the Care and Use of Laboratory Animals.

Preparation of VEGF Immunoliposomes Conjugated to Anti-P-Selectin

Previously, we have shown that the up-regulation of cell adhesion molecules, specifically P-selectin which is maximally up-regulated at 1–4 h after infarction, on the surface of microvasculature in the infarct border zone can be used to selectively deliver drug carrying particles to this tissue site.²¹ Similar to methodologies developed for selectively targeting drugs to tumors, our liposomal drug delivery vehicle (Fig. 1a) targets adhesion molecules that are known to be upregulated in the tissue of interest (Fig. 1b). Preparation of anti-P-selectin immunoliposomes containing VEGF has been described previously.²⁰ Briefly, liposomes were composed of 50 mol% hydrogenated soy L- α -phosphatidylcholine (HSPC), 45 mol% cholesterol, 3 mol% 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[(polyethylene glycol)2000] (DSPE-PEG2000) and 2 mol% DSPE-PEG-maleimide (Avanti, Alabaster, AL). Lipids were dried under vacuum overnight, and then rehydrated in 37 °C deionized water and extruded (Lipex, Vancouver, BC, Canada) 10 times with a 0.2 micron filter (Nucleopore) yielding a liposome diameter of 180 ± 13 nm.

Immunoliposomes were conjugated to IgG_{2a}mAb RMP-1 to rat P-selectin²⁴ by incubating the thiolated antibody with the liposomes overnight at 4 °C to attach to the maleimide group on the DSPE-PEG2000 component of the liposomes. The unconjugated antibody was separated from the liposomes by ultracentrifugation, then the immunoliposomes were freeze-dried under vacuum overnight and rehydrated in a Tris buffer (pH 7.4) solution containing 100 μ g of human VEGF_{165A} (Genentech Inc, San Francisco, CA) at 37 °C and vortexed until vesicles were fully reformed and then incubated at room temperature for 1 h. Free VEGF (not encapsulated) was separated from the immunoliposomes with a Hi Trap Heparin HP Column (GE Healthcare, Piscataway, NJ). Immunoliposomes were then resuspended to a working 10 mM lipid concentration.

Transthoracic Echocardiographic Studies

Upon recovery from surgery, an echocardiogram was performed to assess regional wall motion, systolic and diastolic function. The presence of a myocardial infarction was detected

by evidence of elevated ST segments and the appearance of a Q wave on EKG and regional wall motion abnormality on echo. Transthoracic echocardiograms were performed after semiconscious sedation was administered intraperitoneally using Xylazine (10 mg/kg) and Ketamine (50 mg/kg). After anesthesia, each animal had their chests shaved and was placed in the supine position for imaging. Imaging began 5 min after the administration of sedation to allow the animal to stabilize. A Philips Sonos 5500 machine and a multi-frequency transducer set at 12 MHZ was used for two-dimensional imaging. Imaging was performed using a depth of 2 cm. Echocardiograms analyzed by different operators were shown to be highly reproducible using an intraclass correlation, obtained using a two-way mixed effect model, to assess consistency of measurements.¹⁴ Pulse wave Doppler imaging was performed using a sample volume of 6 mm and a paper speed of 100 mm/s.

Regional wall motion assessment was used to give a non-invasive evaluation of left ventricular function and internal chamber dimensions. The left ventricular end-diastolic (LVEDD) and end-systolic dimensions (LVESD), as well as anterior (AW) and posterior wall (PW) thicknesses were measured. All measurements were made using the leading-edge method. A large MI was defined as a significant increase in the LVEDD greater than two standard deviations above the normal chamber size. LV mass was calculated using the cubed formula equation⁴ and normalized to body weight. The left ventricular ejection fraction and fractional shortening was calculated using the LV internal dimension.¹⁷ The fractional shortening was calculated using the following equation:

$$\text{Fractional shortening} = \frac{\text{LVEDD (mm)} - \text{LVESD (mm)} \times 100\%}{\text{LVEDD (mm)}}$$

The peak early filling wave velocity (*E* wave) was measured with the Doppler sample volume at the mitral valve leaflet tips. Pulsed Tissue Doppler imaging from the septal mitral annulus was performed in the apical four chamber view to measure the early myocardial relaxation (*E'*) velocity. The ratio of the peak *E* wave velocity to *E'* velocity (*E/E'*) was calculated, and a value of greater than 15 was considered abnormal and reflects an increase in left ventricular filling pressures. The left ventricular end-diastolic pressure (LVEDP) was calculated using the following equation¹⁶:

$$\text{LVEDP (mm HG)} = 0.987 \frac{E}{E'} - 4.229$$

Gomori's Trichrome Staining and Analysis

Gomori's trichrome stain (Richard Allen Scientific) is a one-step stain used to differentiate collagen (blue) from muscle tissue (red). Cross-sections of fresh frozen heart tissue sliced 9 µm thick were obtained on polylysine coated slides. Heart sections were fixed with Bouin's solution at 56 °C for 1 h, and then stained according to the manufacturer's directions. Stained heart sections were dehydrated using various grades of alcohol and xylenes, and then mounted with glass slides. Images were obtained using a Nikon Digital Sight color camera and Nikon Element software. A full mosaic image was created using Adobe Photoshop CS software. Image J was used to measure the length of the blue infarct scar, normalized to the circumference of the heart, and the area of the collagen region, normalized to the total area of the heart (Fig. 2).

Picrosirius Red Staining and Analysis

Picrosirius Red (Polysciences, Inc.) stains collagen bright red when viewed under polarized light. Picrosirius red was used to determine collagen deposition in the infarcted anterior wall.²⁵ Briefly, 9 μ m sections of fresh frozen heart tissue were obtained on polylysine coated slide. Heart sections were fixed with formalin (4% formaldehyde) for 10 min, and then stained according to the manufacturer's directions. Stained heart sections were dehydrated using various grades of alcohol and xylenes, and then mounted with glass slides. Images were obtained under linearly polarized light using a Nikon Digital Sight color camera and Nikon Element software. A full mosaic image was created using Adobe Photoshop CS software. Image J was used to measure the mean grey value of collagen through the anterior wall of the section. RGB channels were separated, and separate histograms of the red and green channels were obtained and analyzed for mean grey value (intensity). The ratio of green to red intensity was used to determine the ratio of thin fiber to collagen (green) to thick fiber collagen (red) in the anterior wall of the left ventricle.

Statistical Analysis

All results are expressed as the mean value $\pm$ SEM. One Way Repeated Measures ANOVA was used to test for the improvements in cardiac function over time within a treatment group. Differences between groups were analyzed using a two-way analysis of variance (ANOVA). The intraclass correlation was used to assess reproducibility between measurements made by two separate observers. Multi-regression analysis was used to determine whether the degree of hypertrophy and of LV functional impairment are good predictors of collagen deposition (as determined by trichrome staining) in our model. In all cases, statistical significance was defined as $p < 0.05$.

RESULTS

Changes in Left Ventricular Geometry and Systolic Function

Serial changes in LV internal dimensions and systolic function over 4 weeks following the induction of MI are depicted in Fig. 3. There were no significant differences in the left ventricular end diastolic diameter (LVEDD), left ventricular end systolic diameter (LVESD) or fractional shortening between the untreated (UMI) and targeted VEGF treatment groups at 1 day after MI. Likewise, both MI groups exhibited a significant difference in geometric measurements at 1 day post-MI compared to the sham (no MI) group. At 4 weeks post-MI, both the left ventricular end diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD) significantly increased over the course of the study among both MI groups. At 28 days after infarction, animals treated with targeted VEGF therapy exhibited a significant reduction in LVESD, as well as a significant increase in fractional shortening. These changes in LV chamber size were associated with progressive reductions in anterior wall (AW) thickness. At 4 weeks post-MI, both MI groups exhibited a trend toward AW wall thinning, although the UMI group experienced a stronger trend toward AW thinning at 28 days, compared to the targeted VEGF treated group (data not shown). These findings indicate that targeted delivery of VEGF to the infarct border zone results in improvements in LV morphology and cardiac function. A representative image illustrating the changes in LV morphology is shown in Fig. 4. Previously we have shown that neither administration of blank immunoliposomes (no VEGF), high dose systemic administration of VEGF, or IgG-conjugated VEGF immunoliposomes (non-targeted) resulted in significant improvements in LV systolic function or dilation.²² Echocardiograms analyzed by different operators were shown to be highly reproducible with an intraclass correlation coefficient of $r = 0.998$, and confidence intervals of 0.997 to 0.999, $p < 0.001$.

Changes in Left Ventricular Diastolic Function

Intracardiac hemodynamics and diastolic function were assessed using Pulsed wave and Tissue Doppler imaging (Table 1). A significant difference was observed in the progression of diastolic dysfunction between the two MI groups over 4 weeks after MI, compared to the sham group, suggesting the presence of impairment in LV relaxation for all MI groups. Early filling (E velocity) values showed a stronger trend toward greater increase at 28 days in the UMI group vs. the targeted VEGF treated group. Likewise, after 28 days values for E' showed a significantly greater decline in the UMI compared to the targeted VEGF group. UMI animals exhibited the greatest increase in the E/E' ratio, indicating an abnormal increase in left atrial pressure associated with impaired LV relaxation. LVEDP was shown to be significantly greater in the UMI group, compared to the targeted VEGF treated group at 28 days post-MI. Collectively, these data show that myocardial relaxation is impaired in the UMI, and is attenuated in the animals receiving targeted VEGF therapy. The relatively higher E' and lower E/E' ratio seen in the targeted VEGF group suggest that diastolic function is favorably altered with targeted VEGF therapy. Please note that changes observed in the E/E' ratio in the sham group during the course of study likely reflect changes in myocardial interstitial edema created from sham operation, as the ratio declines closer to normal by day 28.

Collagen Deposition and its Correlation with Functional Impairment

Gomori's trichrome staining revealed a thick, collagenous scar (stained blue) along the anterior wall of the left ventricle in both targeted VEGF treated and untreated animals. The scar length, as a percentage of the total circumference of the heart was calculated, and was significantly shorter in hearts treated with targeted VEGF than untreated MI hearts (Fig. 5b). Likewise, the area in the anterior wall which was composed of the collagen scar tissue was significantly smaller in hearts treated with targeted VEGF therapy, compared to untreated MIs (Fig. 5c).

Collagen deposition through the anterior wall was quantified using linearly polarized images of picrosirius red stained hearts. Under linearly polarized light, thin collagen fibers appear green, and thick collagen fibers appear red. In general, untreated MI hearts had a trend of greater deposition of thick fiber (52.48 ± 8.3 vs. 35.9 ± 2.1) and thin fiber (20.1 ± 3.5 vs. 17.4 ± 2.3) collagen deposition compared to VEGF treated animals respectively. Picrosirius red pixel intensity quantification of hearts treated with targeted VEGF therapy revealed significantly less collagen deposition (37% decrease) in the anterior wall (Fig. 6b) and a 27% increase in the ratio of thin to thick collagen fibers, compared to untreated MI hearts (Fig. 6c). The increase in the ratio of thin to thick collagen fibers in VEGF treated hearts reflects a reduction in the amount of immature collagen deposition in the infarct region. Overall, among VEGF treated animals, there is less early (immature) and late (mature) collagen deposition at 4 weeks.

Changes in collagen deposition (as measured by scar length) were strongly associated with several parameters of adverse cardiac remodeling and LV function. For example, there was a strong correlation between fractional shortening and scar length, as measured by trichrome staining, in both treated and untreated MI animals (Fig. 7a). Furthermore, multi-regression analysis was used to estimate the degree by which the extent of hypertrophy and LV functional impairment predicts collagen deposition (as determined by trichrome staining) in our model. As shown in Fig. 7b, the extent of collagen deposition post MI can be well predicted by a function describing a linear combination of normalized LV mass and ejection fraction. Again, consistent with the data presented in Fig. 6, the targeted VEGF treated group show a lower level of collagen deposition as measured by trichrome staining. Other combinations of measures of cardiac LV geometry (e.g. relative wall thickness) and cardiac

function (e.g. fractional shortening) can also predict the extent of collagen deposition post MI albeit with a lower correlation coefficient (data not shown). This finding indicates that a combination of non-invasive measures of cardiac geometry and function may be used as a predictor of the degree of collagen deposition and the extent of infarct scar.

No targeted VEGF treated animals died or suffered from observable symptoms due to VEGF treatment during the course of the study. An independent pathological examination of tissue indicated that targeted VEGF therapy resulted in no pathological changes in any of the major organs.²³

DISCUSSION

The myocardium is dependent on the availability of oxygen-rich blood for its function and survival, especially in the setting of an acute coronary occlusion. During an acute MI, restoration of coronary flow limits the extent of myocardial damage.^{13,18} The current therapeutic approach to this condition involves the aggressive restoration of blood flow to the infarct region largely through achieving patency of the infarct-related artery. While restoration of flow in the infarct related artery is the standard of care, clinical investigations have shown that close to 25% of patients who achieve patency of the infarct related artery still do not achieve adequate myocardial perfusion.⁸ Studies have shown that the delivery of oxygen-rich blood to the myocardium is not only dependent on epicardial artery flow but also on the integrity of the microcirculation and collateral vessels.⁷ Under conditions where significant regions of microvascular dysfunction occur, even in the presence of adequate epicardial flow, progressive LV dilation, diastolic dysfunction and the development of heart failure have been reported.¹ These findings suggest that methods used to preserve and improve microvascular structure and function could have an important impact on LV remodeling.

Maintenance of microvascular integrity through targeted delivery of pro-angiogenic compounds, such as VEGF, may lead to preservation of myocardial function by reducing LV dilatation and improving diastolic filling dynamics.²² Although large systemic doses (1–30 µg/kg animal weight) of VEGF have been shown to improve vascular density in the MI region, they have failed to significantly improve cardiac function.⁶ This may be due to the very short half-life (several minutes) of VEGF in plasma.¹⁹ The half-life of VEGF in circulation may be extended by encapsulation in immunoliposome, where VEGF is protected from immediate degradation. Recently we have shown that a single low dose (0.12 µg/kg body weight) of VEGF targeted via anti-P-selectin conjugated immunoliposomes to the infarcted heart leads to vascular growth and increases vascular perfusion by 74%²⁰ which is a major cause of attenuation in the functional impairment observed post MI.²⁰ However, the extent of collagen damage, which ultimately correlates with the degree of infarct expansion, may also be correlated with cardiac function. The purpose of the current study is to quantify effects of targeted VEGF therapy on left ventricular geometry and cardiac function and to determine if improvements in cardiac function observed with this novel targeted drug delivery system strongly correlate with reductions in collagen build up in the scar tissue after an MI.

In this study, we observed attenuation in both LV chamber dilation and progression toward a less spherical geometry among the targeted VEGF group. These favorable changes in LV geometry were accompanied by improvements in fractional shortening in this group. In addition, noninvasive assessment of cardiac filling pressures showed improvement in the targeted VEGF group, with the lowest LVEDP and E/E' ratio occurring in the VEGF treated group at 28 days. In contrast to the UMI and EI groups, the targeted VEGF group displayed relative preservation of anterior wall thickness at 28 days. Collectively, these findings

suggest that the targeted delivery of VEGF to the infarct zone attenuated the LV remodeling process with relative preservation of systolic function when compared to the other experimental groups.

While other measures of collagen deposition exist, such as up-regulation of fibronectin, we chose to evaluate overall collagen content and deposition and correlate it with changes in LV morphology. Utilizing the complementary techniques of Trichome and Picrosirius staining, we were able to further analyze the extent and type of collagen deposition. We observed improvements in LV geometry and function in VEGF treated animals, accompanied by reductions in scar length, overall collagen scar area, and reductions in both immature and mature collagen types in the anterior wall among the VEGF treated compared to untreated animals. These findings support our contention of more favorable remodeling linked to an overall decrease in collagen deposition with our intervention. Furthermore, we have shown a strong correlation between collagen deposition and the loss of systolic function in our model. This finding indicates that a combination of measures of cardiac geometry and function may be used as a noninvasive, indirect predictor of the extent of collagen deposition and the extent of infarct scar. This combined with our previous studies suggest that the degree of microvascular integrity and collagen deposition may both contribute to the functional impairment post MI. Our data supports the conclusion that the targeted delivery of even a single dose of anti-P-selectin conjugated immunoliposomes containing VEGF promotes favorable cardiac remodeling.

Creation of a suitable microenvironment, particularly a functioning microvasculature, may be an important adjunctive requirement for favorably altering the remodeling process.²² Administration of targeted VEGF immediately following a myocardial infarction may ultimately be a clinically relevant model, since microvascular dysfunction has been recognized as a consequence of prolonged coronary occlusion. Targeted VEGF therapy may also offer additional advantages post MI either directly preserving the microvascular integrity or reducing collagen deposition.²²

The approach used in this study has the advantage of providing a method for limiting the well-known side-effects of systemic VEGF administration⁹ offering a more directed approach to drug delivery. This novel approach of using a targeted drug delivery method to transport VEGF to the infarct zone could potentially lead to the development of a novel adjunctive therapeutic intervention for post infarction. Administration of targeted VEGF at either 4 or 24 h post-infarction demonstrated no significant difference in the outcome of fractional shortening compared to administration of targeted VEGF immediately post MI (data not shown). Nevertheless, further studies are needed to clarify what doses and on what time scale angiogenic compounds should be targeted to post MI tissue to maximize the effectiveness of these treatments.

In conclusion, targeted drug delivery to diseased cardiac tissue provides a safer and more effective mean of regenerating and maintaining vasculature and reducing the extent of infarct expansion which occurs after MI. The benefits of this technique include improvements in systolic and diastolic function as well as attenuation in adverse remodeling. Further studies are needed to optimize this novel delivery method to maximize the favorable changes in LV geometry and function post MI.

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FIGURE 1.

(a) Immunoliposomes may be used as a drug vehicle for targeting pro-angiogenic drugs to the infarcted heart. Anti-P-selectin is attached to the maleimide group on the surface of the liposome, and the drug (VEGF) is encapsulated within the immunoliposome. (b) Drug vehicles may be targeted to the region of interest via upregulated adhesion molecules in the border zone of the MI. In our model, P-selectin is upregulated after 1–4 h at the infarct site post-MI. Once in the circulation, anti-P-selectin conjugated immunoliposomes containing VEGF will complex with upregulated P-selectin at the infarct border zone for targeted and local delivery of the drug.

FIGURE 2.

(a) The length of the collagen scar was calculated by measuring the length of the scar tissue that penetrates through the entire thickness of the tissue, and normalizing it to the entire perimeter of the heart. (b) The area of the scar tissue was calculated by normalizing the area of the collagen scar (blue) to the entire area of the heart section (red and blue), not including the papillary muscles.

FIGURE 3.

Serial changes in left ventricular geometry and systolic function for the 3 experimental groups indicate that the extent of left ventricular end diastolic diameter (LVEDD) and left ventricular end systolic diameter (LVESD) changes are reduced in the targeted VEGF treatment group. (a) Serial changes in LV fractional shortening for 4 weeks after MI. (b) Left ventricular end diastolic diameter (LVEDD) vs. Time. (c) Left ventricular end systolic diameter (LVESD) vs. Time. Untreated MI ($n = 6$), targeted VEGF treated MI ($n = 6$), sham ($n = 3$). Significant improvement in LV systolic function occurred among the targeted VEGF treatment group ($*p < 0.05$).

FIGURE 4.

Parasternal short axis images taken at the mid ventricular region taken from normal (a), untreated MI (b) and VEGF treated (c) rats respectively. Left ventricular internal dimensions are represented by adjacent labels. These images demonstrate left ventricular chamber enlargement and septal wall thinning among the untreated infarct group. Among the VEGF group, improvement in anterior wall systolic thickening and function is noted.

FIGURE 5.

(a) Trichrome staining of targeted VEGF treated ($n = 6$) and untreated ($n = 6$) MI rat hearts distinguishes collagen scar tissue (blue) from muscle tissue (red). (b) Collagen scar length as a percentage of the heart circumference. Hearts treated with targeted VEGF therapy show a significant decrease in the length of the infarcted tissue. (c) Hearts treated with targeted VEGF therapy showed a decrease in the total cross-sectional area that is composed of scar tissue; collagen scar area as a percentage of total area (excludes papillary muscles). * $p < 0.05$ for targeted VEGF treated vs. untreated MI.

FIGURE 6.

(a) Left ventricle anterior wall stained with picrosirius red staining and viewed under polarized light reveal collagen deposition in targeted VEGF treated ($n = 6$) and untreated ($n = 6$) MI hearts. (b) Picrosirius red pixel intensity for anterior walls of untreated MI tissue, targeted VEGF treated MI tissue, and non-infarcted tissue. Hearts that underwent MI surgery exhibit significantly greater collagen deposition compared to non-infarcted heart tissue. Hearts treated with targeted VEGF therapy showed a 37% decrease in the amount of collagen deposition compared to untreated MI hearts, $*p < 0.05$ for targeted VEGF treated vs. untreated MI. (c) The ratio of green to red pixel intensity in the anterior wall of sections stained with Picrosirius red and analyzed under linearly polarized light. There is a trend towards a 27% increase in the ratio of thin to thick collagen fibers in targeted VEGF treated hearts compared to untreated samples.

FIGURE 7.

The contribution of the extent of collagen deposition to LV function. (a) Scar length, as determined by Gomori's trichrome stain, correlates with fractional shortening at 28 days after infarction in untreated and targeted VEGF treated MIs, $r = 0.75$. (b) Multi-regression analysis indicates that a linear combination of ejection fraction (EF) and LV mass normalized to body weight (LM) is a strong predictor of scar length (SL). Solid line is the model prediction and the dashed lines show the 95% confidence interval. ($SL = 23.6 - 24.6EF + 7.2LM$, $r = 0.81$).

TABLE 1

Diastolic filling at baseline and 28 days.

Variables	E velocity (m/s)	E' velocity (m/s)	E/E' ratio	LVEDP	Heart rate (BPM)
Day 1					
UMI (n = 6)	0.575 ± 0.03	0.023 ± 0.002	26.66 ± 2.76	22.22 ± 3.04	331.6 ± 12.2
Targeted VEGF treated (n = 6)	0.725 ± 0.07	0.023 ± 0.003	33.16 ± 3.70	28.31 ± 4.08	303.5 ± 7.9
No MI (n = 3)	0.844 ± 0.05	0.030 ± 0.001	27.59 ± 1.93	23.00 ± 1.90	306.5 ± 14.9
Day 7					
UMI (n = 6)	0.643 ± 0.09	0.025 ± 0.004	34.34 ± 13.24	34.08 ± 13.76	271.1 ± 9.7
Targeted VEGF treated (n = 6)	0.771 ± 0.05	0.025 ± 0.001	31.58 ± 2.54	26.69 ± 2.76	263.8 ± 14.0
No MI (n = 3)	0.841 ± 0.03	0.036 ± 0.001	23.53 ± 1.79	18.99 ± 1.77	277.4 ± 13.7
Day 28					
UMI (n = 6)	0.909 ± 0.05	0.019 ± 0.001	49.13 ± 2.48	45.08 ± 2.51	243.4 ± 4.2
Targeted VEGF treated (n = 6)	0.820 ± 0.03	0.027 ± 0.001 *	31.58 ± 3.20 *	27.95 ± 3.34 *	261.1 ± 12.1
No MI (n = 3)	0.769 ± 0.08	0.041 ± 0.003	19.06 ± 2.71	14.58 ± 2.68	255.5 ± 15.4

Evolutionary changes in LV diastolic parameters over the course of the study are presented. E wave: early transmitral velocity, A wave: late transmitral velocity, E' velocity: early tissue Doppler diastolic velocity. LVEDP: Left ventricular end-diastolic pressure. All values are expressed as mean ± SEM.

* $p < 0.05$. UMI animals exhibited the greatest increase in the E/E' ratio, indicating an abnormal increase in left atrial pressure associated with impaired LV relaxation compared to animals receiving targeted VEGF therapy.