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Effect of Scar Compaction on the Therapeutic Efficacy of Anisotropic Reinforcement Following Myocardial Infarction in the Dog

Samantha A. Clarke, BS

Department of Biomedical Engineering, University of Virginia

Norman C. Goodman, BS

Center for Comparative Medicine, University of Virginia

Gorav Ailawadi, MD

Departments of Biomedical Engineering and Surgery, Robert M. Berne Cardiovascular Research Center, University of Virginia

Jeffrey W. Holmes, MD, PhD

Departments of Biomedical Engineering and Medicine, Robert M. Berne Cardiovascular Research Center, University of Virginia

Abstract

Cardiac restraint devices have been used following myocardial infarction (MI) to limit left ventricular (LV) dilation, although isotropic restraints have not been shown to improve post-MI LV function. We have previously shown that anisotropic reinforcement of acute infarcts dramatically improves LV function. This study examined the effects of chronic, anisotropic infarct restraint on LV function and remodeling. Hemodynamics, infarct scar structure, and LV volumes were measured in 28 infarcted dogs (14 reinforced, 14 control). Longitudinal restraint reduced 48hr LV volumes, but no differences in LV volume, function, or infarct scar structure were observed after 8 weeks of healing. All scars underwent substantial compaction during healing; we hypothesize that compaction negated the effects of restraint therapy by mechanically unloading the restraint device. Our results lend support to the concept of adjustable restraint devices, and suggest that scar compaction may explain some of the variability in published studies of local infarct restraint.

Keywords

myocardial infarction; infarct restraint; scar compaction; left ventricular remodeling

Corresponding Author Phone: (434) 243-6321, holmes@virginia.edu.

Compliance with Ethical Standard & Disclosures

All institutional and national guidelines for the care and use of laboratory animals were followed and approved by the University of Virginia Animal Care and Use Committee. The authors have no conflicts of interest to report.

Introduction

A heart attack, or myocardial infarction (MI), occurs when blood flow to an area of the myocardium is interrupted, causing immediate loss of muscle contraction and eventual myocyte necrosis in the unperfused region. Every year, there are almost 1 million new or recurrent MIs in the United States alone [1]. Due to temporal improvements in the administration of acute therapies, mortality rates due to acute MI have dramatically declined over the past several decades (10.4% to 6.3%, 1990-2006) [1, 2]. As a result, there are more patients living with a previous MI now than ever before. Unfortunately, ventricles containing large regions of infarct scar often undergo pathological remodeling in the long term, characterized by ventricular dilation and progression toward heart failure [3, 4]. Therefore, in addition to minimizing both initial muscle loss and acute functional depression, preserving heart function chronically and preventing the development of post-MI heart failure are increasingly urgent clinical goals.

Mechanical restraint has been explored as a way to improve post-MI function by minimizing abnormal motion of the passive infarct during systole. Numerous studies have investigated the effects of infarct reinforcement with a material that is isotropic, or equally stiff in all directions. These studies of both global (whole-heart or whole-ventricle) and local post-MI reinforcement have consistently found that isotropic mechanical restraint can be effective at reducing pathologic left ventricular (LV) dilation [5]. However, the majority of these studies also fail to indicate a direct improvement in ventricular function with isotropic restraint. Isotropically stiffening an infarct scar improves systolic function by limiting bulging, but impairs diastolic function by reducing filling, leading to minimal net improvement in pumping [6]. In a finite element model of an infarcted ventricle, our group previously predicted that anisotropic reinforcement of an acute infarct (selectively stiffening the infarct in one direction) can improve LV function by reducing systolic dysfunction while preserving diastolic filling [7]. In an *in vivo* study, we then showed that local, anisotropic reinforcement of an acute infarct using an epicardial patch dramatically improved LV function following a large MI [8]. However, the chronic effects of local, anisotropic restraint on LV function and remodeling are unknown. The goal of this study was to use the canine model of permanent ligation MI to test the effects of local, anisotropic infarct reinforcement on chronic LV function, remodeling, and progression to heart failure.

Methods

A canine model of non-reperfused MI was used to test the effects of chronic longitudinal reinforcement applied on the epicardial surface [9]. Studies were powered to detect a 50% reduction in dilation at 8 weeks, an effect size similar to that of angiotensin converting enzyme inhibitor treatment in this animal model [10]. 48 male mongrel dogs weighing 25-33kg were subjected to a left thoracotomy and MI was induced by permanent ligation of the left anterior descending coronary artery (LAD), producing a large infarct on the anterior wall and apex of the LV. In patch animals, a longitudinally inextensible patch made of knitted polyester and bovine collagen (HEMASHIELD knitted double velour 2×3in cardiovascular patch, Maquet, Getinge Group) was sutured to the epicardial surface over the infarct at the end of the 45 minute openchest ischemic period (Fig. 1a). The patch was

modified to include six parallel, longitudinal slits cut through the center and was applied under longitudinal tension, which we previously showed induces a 10% decrease in longitudinal segment lengths throughout the cardiac cycle without altering circumferential segment lengths (Fig. 1b).

48 hours after MI induction, animals underwent a cardiac magnetic resonance (MR) scan to evaluate acute myocardial viability and baseline ventricular geometry. Cine MR was used to measure LV cavity volumes and late-gadolinium enhancement to measure infarct size [11, 12]. The same imaging protocol was repeated again 8 weeks post-MI. Images were manually contoured in Segment (v1.9 R2178, <http://segment.heiberg.se>) [13] and analyzed in MATLAB (R2011b). Changes in LV cavity, muscle, and scar volumes were calculated from short axis (SA) image stacks according to Simpson's rule, with fractional slice volume adjustments at the base and apex based on long axis views. Muscle volume was calculated by subtracting infarct volume from LV wall volume. Scar compaction dimensions were quantified from enhanced SA images; changes in the circumferential and radial directions were averaged across slices, and longitudinal extent was determined by the number of SA slices containing scar.

Hemodynamic data were collected during a terminal study following the final MR scan. The chest was opened via midline sternotomy and dogs were instrumented with a Millar conductance catheter (Millar Instruments, Houston, TX) to measure LV pressure and volume and an ultrasonic flow probe (A20, Transonic Systems, Ithaca, NY) was placed around the aorta to measure aortic flow. LV preload was varied by temporary occlusion of the inferior vena cava in order to collect data across a range of filling pressures. Dobutamine (Db) was administered intravenously (5 μ g/kg/min) to evaluate the ability of the viable myocardium to respond to β -adrenergic stimulation [3, 14, 15]. At the completion of the study, animals were euthanized and hearts were immediately excised and perfused with arrest solution. Hemodynamic responses from this study were compared to the previously unpublished, pre-infarction data for a group of 9 animals collected by Fomovsky et al using a similar surgical procedure [8].

LV pressure, LV volume, and aortic flow traces from the terminal study were exported as text files and analyzed in MATLAB. For each occlusion run, end diastolic (ED) and end systolic (ES) points were selected manually from the pressure recording. The flow velocity signal was integrated from ED to ED to determine the stroke volume (SV) of each beat (ignoring retrograde flow), and cardiac output (CO) was calculated as the product of stroke volume and instantaneous heart rate. Function curves were generated by plotting SV and CO against end diastolic pressure (EDP), and for each parameter linear interpolation of the log transformed relationship [SV or CO vs. $\ln(\text{EDP})$] was used to calculate SV and CO at a matched EDP of 12mmHg. Average heart rate, the maximum rate of LV pressure generation (max dP/dt), and end systolic elastance (E_{es} , slope of the end systolic pressure-volume relationship) were calculated to compare contractility between baseline and β -adrenergic stimulated runs.

Histology was performed to detect effects of the patch on remaining viable muscle in the remote myocardium. Remote tissue was harvested from the posterior LV midwall and fixed

in 10% formalin. In each animal, tissue blocks were embedded in paraffin in perpendicular orientations and 7 μ m sections were cut from each to measure myocyte cross-sectional area (CSA) and length. Ten sections per animal cut parallel and five cut perpendicular to the long axes of the cells were stained with 5 μ g/ml wheat germ agglutinin conjugated to Alexa Fluor 555 to fluorescently label myocyte cell membranes and intercalated discs [16]. Slides were imaged on a Nikon Eclipse TE2000-E confocal microscope using 543nm excitation and a 590/50nm emission filter. Short and long axis images were taken using 60x and 20x magnification, respectively. Analysis of myocyte CSA was automated in ImageJ using a custom macro script and analysis of myocyte lengths was done by manually tracing cells in MATLAB.

To examine the collagen structure of the infarct, scar blocks were cut from the anterior midwall of the LV, from the center of the scar in control animals and from underneath the center of the patch in patch animals. One scar block per animal was fixed in 10% formalin, embedded in paraffin and sectioned parallel to the epicardial plane. 7 μ m sections were cut from epicardium to endocardium, and five sections per animal – one section at each transmural depth of 0%, 25%, 50%, 75% and 100% – were selected for comparison across animals. Sections were stained with Picrosirius Red using a slightly modified protocol to eliminate cytoplasmic staining of any remaining myocytes [17, 18]. A 1.5 x 1.5 mm grid was overlaid on each slide to divide each into smaller, non-overlapping imaging fields, and ten fields were chosen at random for imaging and analysis. Imaging and analysis of infarct collagen structure was performed using methods that harness the optical birefringence of collagen fibers [19]. All infarct scar imaging was performed on an Olympus BX51 microscope, which was modified for imaging with circular instead of linear polarized light to make fibers in all orientations appear equally bright, while non-birefringent materials (such as muscle) appear dark [20]. All infarct imaging was done at 10x magnification and captured with a Sony XCD-X710 CCD camera. For each field, the same image was captured twice: once under polarized light and once under bright-field illumination. The two images were then digitally subtracted and thresholded to isolate collagen pixels. The collagen area fraction of each imaging field was calculated as the ratio of collagen pixels to tissue pixels. Analysis of collagen fiber alignment was done in MatFiber, a custom MATLAB script adapted from the Fiber3 software developed by Karlon et al. [21, 22]. Wall thickness was determined by the number of sections spanning the wall.

Results

48 animals underwent LAD ligation surgery. Animals whose infarcts were too small to cause significant LV dilation (<20% LV) were excluded from analysis (n=3). An additional 16 animals did not survive the 8 week study period and 1 animal was excluded due to instrumentation error, leading to final group sizes of n=14 for both patch and control. By design, these group sizes were powered to detect a similar reduction in post-MI LV dilation as observed with post-MI ACE-inhibitor treatment in dogs [10]. An apparent trend towards higher survival in the patch group was not statistically significant (Fig. 2). Importantly, 48 hour infarct size was similar in both groups (33.8 $\pm$ 5.6% patch, 31.4 $\pm$ 6.8% control, P=NS), ensuring that any differences observed between groups were not due to differences in the initial infarct size (Table 1).

Average 48 hour end diastolic volume (EDV) was $72.8 \pm 17.6 \text{ cm}^3$ in the patch group vs. $92.5 \pm 20.5 \text{ cm}^3$ in control ($P < 0.05$), and average end systolic volume (ESV) was $55.3 \pm 14.4 \text{ cm}^3$ in the patch group, $73.3 \pm 15.2 \text{ cm}^3$ in control ($P < 0.05$) (Fig. 3a,b). However, by 8 weeks, differences in volumes between the two groups were no longer significant, suggesting that the previously reported acute benefits of anisotropic reinforcement are either not sufficient to mitigate LV remodeling or are not sustained over the 8 week period of the study. Ejection fraction significantly increased during healing in both groups ($p < 0.001$), and increases in both groups were similar (48hr control = 20.4 ± 6.8 , 48hr patch = 23.8 ± 7.9 , 8wk control = 43.2 ± 8.6 , 8wk patch = 45.0 ± 4.0). LV muscle volume increased significantly between 48 hours and 8 weeks in both groups ($p < 0.001$) (Fig. 3c). Myocardial volume was significantly greater in control animals than in patch animals at both time points ($p < 0.05$). Histological measurements of remote myocyte dimensions revealed a trend toward longer, thinner myocytes in both groups compared to normal myocytes harvested from a single non-infarcted animal (Fig. 3d). However, no differences in final myocyte dimensions were observed between patch and control animals.

Analysis of changes in infarct size over time revealed a potential mechanism underlying the lack of sustained benefit: surprisingly dramatic scar compaction sufficient to completely reverse the effects of anisotropic reinforcement. Infarct volume decreased by more than 50% in both patch and control animals (Fig. 4a). In both groups, the majority of compaction occurred in the circumferential direction; however, longitudinal compaction was greater than 10% on average (Fig 4b), sufficient to reverse the dimension changes originally induced by reinforcement (Fig. 1b). No significant differences were identified between groups, indicating that surgical reinforcement did not affect the degree or direction of scar compaction.

Scar collagen structure at 8 weeks was also similar between groups. Similar transmural alignment of collagen fibers, collagen area fraction, and infarct wall thickness were observed in patch and control scars ($p = \text{NS}$ for all patch v. control comparisons at each depth) (Fig. 5). In control animals, the transmural variation in mean collagen fiber angle mimics that of native myocardium (Fig. 5a), while strength of alignment was lowest at the epicardium but then fairly constant through the wall (Fig. 5b). Collagen alignment in patch animals was similar, although we observed a slight trend toward higher alignment at the midwall and lower alignment at the epi- and endocardial surfaces (Fig. 5b). Below the epicardial surface at 25% transmural depth, a slight trend toward stronger circumferential alignment in patch animals was observed, although no differences were sustained transmurally (Fig. 5a). The total collagen content of patch and control scars was also similar, as indicated by similarities in both collagen area fraction and infarct wall thickness (Fig. 5c,d).

Hemodynamics data were measured at the terminal 8 week study and used to compare LV function (Fig. 6). Baseline function in both groups was compared to data from a group of open chest, uninjured control animals studied previously[8], and Db was used to measure the ability of the myocytes to respond to β -adrenergic stimulation. At baseline, SV and CO (interpolated at a matched EDP of 12mmHg) were similar across groups. In uninjured animals, heart rate, Ees, and max dPdt all significantly increased in response to Db

treatment. In both infarct groups, Db stimulation produced a significant increase in max dPdt but had no effect on Ees, and an increase in heart rate was observed in control but not patch animals. Control animals also exhibited an increase in CO following β -adrenergic stimulation, although no other groups showed changes in either CO or SV with Db administration.

Discussion

We conducted a chronic, large-animal trial of anisotropic reinforcement, a strategy previously shown to dramatically improve acute LV function following a large anteroapical infarct [8]. At 48 hours, patch animals had lower EDV and ESV on MRI, confirming that anisotropic reinforcement altered LV mechanics acutely. However, by 8 weeks there were no significant differences in patch animal survival, LV function, or LV remodeling compared to untreated controls. Our scar remodeling data suggest that compaction of the infarct scar in the epicardial plane over the 8-week study may have reversed the effect of the treatment by relieving the initial tension in the patch. Upon harvest, patches were found to be strongly adhered to the epicardium with visible wrinkling in the longitudinal direction, further corroborating our hypothesis that the patches were mechanically unloaded by the end of the study. These results were surprising, because the infarct healing literature typically focuses on infarct expansion – circumferential and longitudinal dilation plus radial thinning – rather than scar compaction. However, a recent review of studies that actually reported infarct dimensions suggests that expansion and compaction occur with similar frequency [23]. Accordingly, different therapeutic approaches may have very different efficacy in different animal models or even in different patients. We would expect surgical restraint – as in our study – to be most effective for infarcts that would normally expand if untreated. This might explain why studies that applied isotropic patches locally to prevent infarct expansion have produced more variable effects on LV remodeling than those that employed global restraint with cardiac support devices [5].

Considering what is known about the process of infarct scar formation, we were also surprised to find that mechanical reinforcement did not alter the collagen structure of the resulting scar. In the weeks following an MI, fibroblasts infiltrate the unperfused region and begin to deposit and remodel collagen to eventually form a stiff, noncontractile scar [24]. The recruitment and activity of these cells are governed by an array of biochemical and biophysical cues, including mechanical stretch [25, 26]. *In vitro*, fibroblasts can respond to uniaxial stretch by depositing new collagen fibers parallel to the strain cue, or by rotating existing fibers toward alignment with the stretch direction [27, 28]. *In vivo*, our group has shown that the strain pattern in an infarct is a crucial determinant of the scar structure that forms, and can explain differences in scar collagen arrangement across different infarct locations and animal models [29, 30]. Specifically, in infarcts subject to uniaxial in-plane stretching we have observed collagen alignment in the direction of greatest stretch, while infarcts that undergo equibiaxial stretching form scars with randomly oriented collagen fibers [22, 29]. Therefore, we would expect reinforcement therapies that dramatically alter deformation and stretching in an acute infarct to change the collagen structure of the resulting scar. This surprising result may offer some clues to the timing of the mechanical unloading of the patch by compaction: since most of the collagen deposition occurs in weeks

1-4 following infarction in the dog [31], substantial unloading may have already occurred by this point in healing. Consistent with this timeline, Theroux et al. reported a 10% reduction in ED circumferential segment lengths measured by sonomicrometry at 2 weeks and a 21% reduction at 3 weeks in healing infarcts in dogs [32]. An alternate potential explanation is that the mechanical effects of the patch are confined to the outer part of the infarct (where we saw some qualitative but non-significant differences in structure); however, this explanation seems less likely given that we previously measured large acute changes in mechanics at the midwall in response to patch application (Figure 1).

Given previous reports of attenuation of LV dilation with both global and local infarct restraint approaches, we were also surprised to find no differences in final LV cavity volumes between our patch and control groups. Patch animals did have significantly smaller LV volumes at 48 hours post-MI, but differences in cavity volumes between groups had faded by 8 weeks. In accordance with the cavity volumes, final LV mass, heart mass, and remote myocyte dimension measurements all indicated similar degrees of myocardial remodeling. Surprisingly, myocardial volume calculated from MRI indicated significantly greater muscle volume in control animals at both 48 hour and 8 week time points. However, since 48 hours is too soon to observe differences in myocardial volume due to LV hypertrophy, we suspect that the apparent difference in wall volume arises from an inability to detect differences in acute radial thinning in the border zone due to the transmural resolution of the MR images. It is possible that the apparent difference in 8 week muscle volumes stems from a similar phenomenon, since differences in final myocardial volume on MRI conflicted with direct measurements of final LV mass and remote myocyte size. Although we used myocyte size data from a single, un-infarcted animal as a basis for comparison to our patch and control groups, our un-infarcted cell length and CSA values matched well with previously reported values in dogs. Using fixed, paraffin-embedded sections, Gerdes & Kasten and Komamura et al. measured average myocyte CSAs of $279\mu\text{m}^2$ (heart mass = 141g) and $179\mu\text{m}^2$ (heart mass = 198g), respectively, and Volders et al. measured an average length of $140\mu\text{m}$ (heart mass = 222g) in isolated myocytes [33–35]. However, substantial variability in reported myocyte dimensions between studies using different preparation and measurement techniques led us to compare our patch and control values to data that were generated using the exact same experimental and analysis procedures.

The results of this study lend support to the potential of restraint devices that adjust to account for scar and ventricular remodeling. One group has examined the efficacy of adjustable, global (restraining one or both ventricles) restraint devices. In a study of post-MI LV restraint in sheep, Ghanta et al. observed that the restraint level (selected to be optimum at the time of implantation) decreased over time as the LV reverse remodeled, to the point where there was no longer any restraint pressure being applied to the LV at the final time point [36]. In a follow-up study exploring the effects of adjustable restraint during healing, the same group explored a dynamic approach in which restraint pressure was maintained constant during healing and found enhanced reductions in LV volumes compared to static restraint [37]. Another group used a local device to examine the effects of optimized, static infarct restraint on LV remodeling and found that local reinforcement led to smaller LV volumes but no improvement in SV [38]. Although the optimal degree of local restraint was

held constant, a similar approach could potentially be used to examine the effects of adjustable, local restraint during scar remodeling.

It is worth noting that all measurements of scar compaction in this study were made from MR images acquired at end diastole in a pressurized LV. Changes in scar dimensions measured in a loaded configuration could simultaneously reflect changes in cavity pressure, scar mechanical properties, and/or scar dimensions. As an example, imagine two identical, unloaded ventricles with the same size scar, but one scar is much stiffer than the other. When both ventricles are inflated to the same cavity pressure, the stiffer scar will not deform or stretch as much as the soft scar, making it appear smaller (circumferentially and longitudinally) and thicker (radially) than the soft scar. Due to stiffening of the scar during healing, our scar compaction data therefore likely overestimate the amount of in-plane (circumferential and longitudinal) compaction and underestimate radial compaction compared to the ideal case of making serial measurements of scar dimensions in an unloaded LV. In addition, changes measured from MRI are often, as in this study, unaccompanied by simultaneous measurement of LV pressure, so differences in loading during measurement of scar dimensions are also unknown. Although changes in unloaded scar dimensions in this study are unknown, the efficacy of our patch therapy hinges on its ability to generate longitudinal infarct *in vivo*, so we focused on changes in scar dimensions in the loaded state.

The results of the current study also highlight the importance of developing computational models that can accurately predict not only the acute effect of a therapy on LV mechanics, but also its long-term effects on scar and LV remodeling. In order to predict the chronic effects of acute infarct reinforcement, such models will need to better represent the dynamic interplay between scar remodeling, ventricular remodeling, and degree of restraint. Such models will not only be useful for predicting the chronic effects or the optimal timing of potential therapies, but could also be used to design and optimize dynamic approaches.

In summary, the present study explored the use of longitudinal infarct restraint as a chronic, post-MI therapy. None of the acute functional benefits of patch application observed in a previous study were sustained over 8 weeks, nor were any differences identified in LV remodeling or scar structure. Examination of the infarct dimensions during healing suggested that our local restraint device was mechanically unloaded over the course of the study by surprisingly prominent inplane scar compaction. The results of the present study highlight the need for computational models that can predict infarct and ventricular remodeling and suggest that local, dynamic restraint therapies may offer enhanced benefit over static devices.

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Abbreviations

MI	Myocardial infarction
LV	Left ventricle
LAD	Left anterior descending coronary artery
MR	Magnetic resonance
SA	Short axis
Db	Dobutamine
CSA	Cross sectional area
ED	End diastole
ES	End systole
SV	Stroke volume
CO	Cardiac output
EDP	End diastolic pressure
Max	dPdt Maximum rate of LV pressure generation
Ees	End systolic elastance
EDV	End diastolic volume
ESV	End systolic volume

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Fig. 1.

Selective longitudinal infarct reinforcement. **a** Diagram of anisotropic patch sewn onto epicardial surface to generate longitudinal reinforcement, reproduced from Fomovsky 2010[39]. Anterior infarct generated by permanent LAD ligation is shaded in gray. **b** Changes in longitudinal (solid lines) and circumferential (dotted lines) anterior segment lengths between pre-infarction baseline (red), 45min of ischemia (gray) and longitudinal patch application (black). Both segments are actively contracting at baseline and become passive following ligation, but only longitudinal segment lengths are affected by anisotropic reinforcement, as evidenced by a reduction in segment length throughout the entire cardiac cycle. Pressure-segment length data are previously published data from Fomovsky 2012[8]

Fig. 2.

8 week survival curves in animals initially randomized to patch application or untreated control group. An apparent trend towards higher survival in the patch group was not statistically significant ($p=0.3$ by logrank test)

Fig. 3.

Left ventricular remodeling. **a** End diastolic and **b** end systolic volumes were significantly smaller in patch animals at 48 hours post-MI ($p < 0.05$ EDV, $p < 0.01$ ESV), although differences in cavity volumes faded by 8 weeks. **c** LV muscle volume (estimated from MRI as LV wall volume – infarct volume) was significantly greater in control animals at both time points ($p < 0.05$). **d** Remote myocyte measurements from both groups suggested that myocytes were longer and thinner than those from an uninfarcted control animal (white bars), consistent with the observed changes in EDV. * $p < 0.05$ patch v. control (unpaired t-test), † $p < 0.05$, †† $p < 0.001$ compared to 48 hour value (paired t-test)

Fig. 4.

Infarct remodeling. **a** Infarct volume decreased significantly in both groups over the course of the study period. **b** Quantification of scar compaction in each dimension revealed that scars compacted by an overall average of 39% circumferentially and 14% longitudinally; significant compaction in the radial direction was observed only in the patch group.

† $p < 0.01$, †† $p < 0.001$ compared to 48 hour value (paired t-test). $p = \text{NS}$ for all comparisons between patch and control (unpaired t-test)

Fig. 5.

Scar Structure. **a** Mean angle and **b** mean vector length (indicating strength of alignment, where 0=randomly oriented and 1=perfectly aligned) of collagen fibers measured at 5 transmural depths. In control animals, the mean orientation of collagen fibers mimics the transmural distribution of muscle fiber angles, and strength of alignment is highest at the midwall. $p=NS$ between patch and control for mean angle and mean vector length at every transmural depth (one-sample t-test with Bonferroni correction of the weighted circumferential component against a mean of 0). **c** Collagen area fraction at each depth (paired t-test with Bonferroni correction at each depth) and **d** infarct wall thickness (paired t-test) were also similar between groups, leading to no differences in total scar collagen volume fraction

Fig. 6.

Hemodynamics at 8-week terminal study compared to previously unpublished data a group of 9 non-infarcted animals. **a** Heart rate, **b** end systolic elastance, and **c** maximum rate of LV pressure generation at baseline (gray bars) were all significantly decreased in both patch and control groups compared to non-infarcted. A significant response to Db (black bars) was measured in all three indices in non-infarcted animals, but was only observed in the max dPdt of infarcted animals and in the heart rate of control animals. **d** Cardiac output (interpolated at a matched EDP of 12mmHg) was maintained across experimental groups, and only control animals exhibited an increase in CO with Db treatment. **e** Stroke volume (interpolated at a matched EDP of 12mmHg) was also similar among groups and did not change with Db treatment. *p<0.01, compared to pre-Db value (paired t-test with Bonferroni correction)

Table 1

Summary of Experimental Groups

	Patch	Control
Final Group Size	14	14
Surgery Body Mass (kg)	29.3 ± 2.1	29.8 ± 1.3
48hr Infarct Size (%LV)	33.8 ± 5.4	31.4 ± 6.8
Final Body Mass (kg)	30.7 ± 2.0	30.3 ± 2.0
Final LV Mass (g)	117.9 ± 25.5	126.0 ± 27.6
Final Heart Mass (g)	171.9 ± 17.3	183.2 ± 18.8

Initial body mass at surgery was similar in both groups. Importantly, initial infarct size did not differ between the two groups, suggesting that any differences observed between groups were an effect of the treatment and not due to differences in the initial degree of dysfunction. Body mass, heart mass, and LV mass were all similar between groups at the completion of the study (p=NS for all comparisons by unpaired t-test)