Myofilament dysfunction contributes to impaired myocardial contraction in the infarct border zone

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**METHODS**

The Animal Studies Subcommittee of San Francisco Veterans Affairs Medical Center approved all procedures.

**Surgical model.** Male adult Dorset sheep (n = 13) underwent anteroapical MI as previously described (20). Briefly, the left anterior descending and second diagonal coronary arteries were permanently ligated at a point 40% of the distance from the apex to base, which resulted in a large transmural apical infarct. A subset of five sheep was treated with the broad-spectrum matrix metalloproteinase (MMP) inhibitor doxycycline (10 mg/kg bid via PORT-A-CATH) at the time of the MI and continuing for 2 wk after MI. Sheep were euthanized 2 wk after MI. Hearts were excised and arrested in diastole by immersion in ice-cold cardioplegic solution (Plegisol, Hospira, Lake Forest, IL; 1 liter plus 10 ml of 8.4% NaHCO₃ to achieve pH 7.8 at room temperature), and 1 liter of cold Plegisol solution was given retrograde into the aortic root. A transmural strip of the LV free wall myocardium (~2 × 6 cm) was excised extending from the apical infarct toward the base. The border zone myocardium was defined as the region immediately adjacent to the infarct (0–1 cm from the infarct edge). The remote zone myocardium was defined as the region 5 cm

AFTER A MYOCARDIAL INFARCTION (MI), there is a border zone adjacent to the infarct that remains normally perfused with blood (14). However, the nonischemic border zone myocardium is hypocontractile, as assessed by finite element-based calculations of myocardial contractility generated from MRI measurements of left ventricular (LV) volume and strain (24).

Border zone dysfunction is a very significant clinical issue. First, post-MI border zone dysfunction contributes to impaired pump function. Second, post-MI cardiac remodeling involves progressive expansion of the border zone, leading to further diminution of pump function and ultimately to heart failure (14). Third, our recent study (27) has suggested that improvement of border zone function could lead to improvement of pump function. Thus, the post-MI border zone may represent a novel therapeutic target for inhibiting dysfunctional ventricular remodeling. Multiple mechanisms have been implicated in the impaired contraction of the border zone myocardium. Acute changes in border zone geometry (decreased curvature and wall thinning) cause increased myocardial wall stress in the border zone (13), which may lead to a chronic and intrinsic deficit in border zone function (8). At the cellular level, mitochondrial dysfunction and energy insufficiency are associated with contractile dysfunction of the border zone myocardium (11). Moreover, decreased Ca²⁺ transients and decreased myocyte contraction are associated with border zone dysfunction (17).

In heart failure, defective myofilament force development is thought to play a critical role in the impaired myocardial function of the failing heart (2, 9). Therefore, the goals of this study were to test the hypothesis that impaired myofilament force development plays a role in border zone dysfunction after an anteroapical infarct and to investigate the mechanism involved.

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from the infarct edge. Results were compared with data from anatomically comparable regions (apical and basal) of myocardium from nonoperated control sheep (n = 7).

**Demembranated myocardial preparations.** Thin slices of the epicardial myocardium were pinned onto a silicone substrate and immersed in demembranating (skinning) solution containing 1% Triton X-100 (Sigma-Aldrich) for 24 h at 4°C (26). Visually discrete cardiac muscle bundles running in the plane of the slice were dissected free. For post-MI hearts, the width and thickness of skinned preparations from the border zone (133 ± 10 and 120 ± 10 μm) were not statistically different from skinned preparations from the remote zone (146 ± 8 and 120 ± 7 μm, n = 6, P > 0.05). However, the cross-sectional area was statistically increased by 25% in skinned preparations from the border zone (0.0139 ± 0.0014 mm²) versus the remote zone (0.0111 ± 0.0017 mm², n = 6, P > 0.05), possibly reflecting myocardial hypertrophy. The dimensions of skinned preparations in the remote zone did not differ from skinned preparations from control hearts.

**Solutions.** Slices were stored at −20°C (for up to several weeks before study) in a solution that was a 1:1 mixture of glycerol and relaxing solution containing (in mM) 20 EGTA, 7.05 MgCl₂, 6.31 Na₂ATP, 10 creatine phosphate, and 80 N,N-bis(2-hydroxyethyl)3,5,a-minoethane sulfonic acid, with pH adjusted to 7.1 with KOH and ionic strength adjusted to 200 mM with KCl.

In addition to relaxing solution, we used a preactivating solution in which Ca²⁺ buffering was reduced by replacing 19.5 mM EGTA with hexamethylenediamine-N,N,N',N'-tetraacetate (Fluka). We also used an activating solution containing 20 mM Ca²⁺-EGTA. All solutions contained 1% (vol/vol) Protease Inhibitor Cocktail P-8340 (Roche) using polypropylene pellet pestles in microcentrifuge tubes while in liquid nitrogen. Tissue homogenates were mixed with 4× loading buffer [Tris·HCl (pH 6.8), SDS, glycerol, bromophenol blue, DTT, and 2-mercaptoethanol], and equal volumes were loaded onto 4–20% SDS-PAGE gels (Coomassie blue (Fisher) and ultrapure water, and scanned at 302 nm using ChemiDoc (Bio-Rad) and quantitated with ImageJ software.

**In vitro contractions.** Skinned myocardial preparations were cut to a length of ~1.5 mm and attached with aluminum T-clips to a force transducer and computer-controlled motor (Permeabilized Fiber Test System, model 1400A, Aurora Scientific) and bathed in relaxing solution. Muscle sarcomeres were observed using a 40× objective, and sarcomere lengths were assessed using a video-based system (model 900B, Aurora Scientific). Muscle length was adjusted to set the muscle sarcomere length to 2.1 μm. Skinned preparations were briefly transitioned to preactivating solution and then to different solutions of various Ca²⁺ concentrations (achieved by mixing relaxing solution and activating solution to obtain intermediate Ca²⁺ levels) (26).

At the highest Ca²⁺ concentration (38 μM), the following measurements were made: maximum isometric force development (Fₘₐₓ), isotonic contractions (using rapid decreases of muscle length to control the muscle force at a steady submaximal level), and force redevelopment kinetics immediately after mechanical disruption of cross-bridges. In high Ca²⁺ concentration rigor solutions (absence of ATP), muscle stiffness (a measure of cross-bridge attachment) was assessed using rapid (400 Hz) low-temperature muscle length oscillations (0.5% muscle length). Data were pooled from two to four preparations obtained per region per heart.

**Histology.** Freshly isolated samples from the border zone and remote zone myocardium were fixed in phosphate-buffered 4% paraformaldehyde (Fisher Scientific, Fair Lawn, NJ) at 4°C for at least 24 h. Paraffin-embedded 5-μm-thick sections were stained with hematoxylin and eosin and picrosirius red, as previously described (5). Images of stained cross-sections (2 images per region per animal) were analyzed using ImageJ (National Institutes of Health, Bethesda, MD). Measurement of myocyte areas was performed only using cells containing visible nuclei to ensure measurement in a region near the center of the long axis of the cell. Collagen content in picrosirius red-stained sections was quantified using ImageJ thresholding analysis for collagen-positive areas.

**Electron microscopy.** Myocardial samples were immobilized by pinning, fixed overnight in buffered 4% glutaraldehyde at 4°C, rinsed, osmicated, dehydrated, and embedded in EPON. Ultrathin transverse sections were stained with lead citrate-uranyl acetate using standard methodology. Sections from the border and remote zones were quantitated using ImageJ analysis of the fraction of the cell area occupied by myofilibrils.

**Myosin Western blot analysis.** After cold arrest, myocardial samples were dissected in cold cardioplegia solution and then snap frozen (~15 min after explan). Equal volumes of tissue from infarct border and remote zones were obtained using disks of tissue (3 mm diameter × 50 μm thick) cored from frozen sections. Samples were homogenized using polypropylene pellet pestles in microcentrifuge tubes while in liquid nitrogen. Tissue homogenates were mixed with 4× loading buffer [Tris·HCl (pH 6.8), SDS, glycerol, bromophenol blue, DTT, and 2-mercaptoethanol], and equal volumes were loaded onto 4–20% SDS-PAGE gels (Coomassie blue (Bio-Rad), CA) and electrophoresed at 75–80 V for 3–4 h at room temperature. Separated proteins were transferred to nitrocellulose membranes at 250 mA for 1 h at 4°C, blocked, and probed with NOQ7.5.4D mouse anti-β-myosin heavy chain (Sigma) at 1:20,000 (19). Secondary antibody conjugated with horseradish peroxidase was used in conjunction with an ECL substrate (Thermo Scientific) to detect bands. Bands were imaged using ChemiDoc (Bio-Rad) and quantitated with ImageJ software.

**Phosphorylation experiments.** Equal volumes of frozen tissue samples (5 disks of tissue, 3 mm diameter × 50 μm thick, see above) from the infarct border and remote zones as well as anatomically equivalent regions from nonoperated controls were homogenized in RIPA buffer [50 mmol/l Tris, 150 mmol/l NaCl, 0.1% SDS, 0.5% Na deoxycholate, 1% Triton X-100, Phosphatase Inhibitor Cocktail 2 (Sigma), Phosphatase Inhibitor Cocktail 3 (Sigma), and one Complete Mini Protease Inhibitor Cocktail Tablet per 10 ml (no. 1183613001, Roche)] using polypropylene pellet pestles in microcentrifuge tubes while in liquid nitrogen. Tissue homogenates were mixed with 4× loading buffer [Tris·HCl (pH 6.8), SDS, glycerol, bromophenol blue, DTT, and 2-mercaptoethanol]. Equal volumes of homogenate were loaded onto 4–20% SDS-PAGE gels (Coomassie blue (Bio-Rad) and electrophoresed at 100 V for 3 h at room temperature. Gels were processed as follows: fixed in 50% methanol and 10% acetic acid, washed three times with 100 ml ultrapure water for 10 min, stained in 60 ml Pro-Q Diamond (P33330, Molecular Probes, Invitrogen) for 90 min at room temperature, destained three times for 30 min each in 100 ml Pro-Q destain solution (P33311, Molecular Probes, Invitrogen) and ultrapure water, and scanned at 302 nm using a ChemiDoc (Bio-Rad) ultraviolet transilluminator. To control for protein loading, gels were stained with Coomassie blue (Fisher GelCode Blue 24590) for 1 h at room temperature, destained in ultrapure water for 1 h at room temperature, and imaged in ChemiDoc (Bio-Rad). Images were analyzed with ImageJ software. The integrated optical density of the Pro-Q diamond stain was divided by the optical density of the Coomassie blue stain.

To identify myosin essential light chain (ELC), in-gel Western blot analysis was performed using gels after Pro-Q diamond staining and using monoclonal antibody ab680 (Abcam, Cambridge, MA) at 1:2,000 concentration. Secondary antibody conjugated with a fluorescent dye with infrared excitation spectra was used for detection. One-color infrared in-gel Western blots were scanned (Odyssey Infrared Imaging System, LI-COR Biosciences).

**Immunohistochemical detection of MMP-2 isoforms.** For localization of the c-Myc-tagged MMP-2 transgene, paraformaldehyde-fixed ventricular sections were incubated with murine monoclonal anti-c-Myc of the c-Myc-tagged MMP-2 transgene, paraformaldehyde-fixed ventricular sections were incubated with murine monoclonal anti-c-Myc (9E11, Abcam) followed by the MOM kit (Vector, Burlingame, CA) and development with VIP purple substrate (Vector) as previously described (3). Digital images were acquired at 1,000 dpi. For each animal, 2 random windows of 100 × 100 pixels were analyzed for density of immunohistochemical staining using ImageJ (National Institutes of Health).

**Statistical analysis.** Data are presented as mean ± SE. Statistical comparison used unpaired or paired Student’s t-tests and two-way ANOVA.
ANOVA with repeated measures followed by the Bonferroni’s post hoc test to compare between groups. The significance level was set at P < 0.05.

RESULTS

Reduced myofilament force in the infarct border zone. Figure 1A shows the relationship between force development versus bath Ca²⁺ concentration. Over a range of submaximal and maximal bath Ca²⁺ levels, there was impaired myofilament force development in the border zone myocardium versus remote zone myocardium. Ca²⁺-activated Fₘₐₓ for the border zone myocardium (58 ± 2 mN/mm²) was reduced by 31% compared with Fₘₐₓ for the remote zone myocardium (85 ± 1 mN/mm², n = 6/group, P < 0.0001). Fₘₐₓ of the remote zone myocardium was reduced by 6% compared with Fₘₐₓ of an anatomically equivalent region in nonoperated control hearts (91 ± 1 mN/mm², n = 7/group, P < 0.03).

Myofilament Ca²⁺ sensitivity (EC₅₀, Ca²⁺ level at 50% Fₘₐₓ) of the border zone myocardium (1.32 ± 0.08 μM) was not significantly different from EC₅₀ of the remote zone myocardium (1.33 ± 0.06 μM, n = 5/group, P = 0.85). These EC₅₀ values were not different from values from anatomically equivalent regions of nonoperated control hearts.

The spatial gradient of border zone dysfunction. Myocardial samples were obtained from the border zone myocardium at various distances from the apical infarct. Figure 1B shows that there was a significant linear relationship between force development and distance from the infarct. In contrast, there was no significant relationship of force development versus distance for comparable anatomic locations of noninfarcted hearts. Figure 1B shows that myofilament force was significantly depressed over a fairly broad region extending for several centimeters from the apical infarct. Moreover, the degree of injury was directly related to proximity to the infarct.

Border zone dysfunction was not due to changes in myocardial structure. Loss of contractile material within the border zone could lead to a decrease in force development per unit area of myocardium. To assess this possibility, we quantified 1) replacement fibrosis, 2) myofibril content, and 3) myosin abundance. All three parameters were not different between the border zone versus remote zone myocardium.

Figure 2A shows representative histological cross-sections of the border zone and remote zone myocardium. In gross appearance, the border zone myocardium exhibited larger cell size, but picrosirius red staining for collagen appeared similar in the border zone and remote zone myocardium. Image analysis (Fig. 2B) found that the abundance of collagen per area of myocardium was not significantly different between the border zone versus remote zone myocardium.

Image analysis was also used to quantitate myocyte cross-sectional areas. The results shown in Fig. 2C confirmed the presence of appreciable myocyte hypertrophy in the border zone relative to the remote zone or relative to samples from control noninfarcted hearts. Cell area in the border zone was increased ~70% relative to the remote zone.

Combining force per area data with myocyte areas allows calculation of the force developed per myocyte. Interestingly, the calculated force per myocyte in the border zone was not different from that of the remote zone (Fig. 2D) or for comparable anatomic locations in noninfarcted hearts (P > 0.05).

Myocardial ultrastructure was studied using electron micrographs of cross-sections of the remote zone and border zone myocardium (Fig. 3A). Image analysis showed that there was no difference between remote zone and border zones in the area fraction of myocardium that was occupied by myofibrils (Fig. 3B). Moreover, Western blot analysis of the abundance of the contractile protein myosin (Fig. 3C) showed that myosin abundance was not different between the remote zone and border zone myocardium. Together, these results suggest that the reduction in force per unit area...
of border zone myocardium versus remote zone myocardium was not due to greater fibrosis per area, fewer myofibrils per area, or decreased myosin abundance in the border zone myocardium. Instead, these results indicate that the content of contractile material was not reduced in the border zone and therefore suggest that the contractile mechanism in the border zone was dysfunctional.

**Cross-bridge kinetics were not altered in the border zone myocardium.** We measured two indexes of cross-bridge kinetics in border zone myocardium. We assessed the force-velocity properties of isotonically contracting muscle (Fig. 4A). The force-velocity curves for border and remote zone samples were almost identical and did not differ from samples from anatomically equivalent regions in nonoperated control hearts. Accordingly, the maximum velocity of muscle shortening extrapolated at zero load was similar among the border and remote zone myocardium and myocardium from nonoperated control hearts.

A second kinetic assessment of cross-bridge function was the rate constant of force redevelopment (k_tr). Cross-bridges were mechanically disrupted by a rapid muscle length release/restretch maneuver, and the rate constant of subsequent force redevelopment was determined (4). Figure 4B shows that there were no differences in k_tr values for border versus remote zones or versus comparable regions from control hearts.

**Rigor stiffness was lower in the border zone myocardium and normalized by doxycycline.** In the absence of ATP (rigor), cross-bridge formation is maximal as cross-bridges are locked into an attached state. Thus, the stiffness of cardiac muscle in rigor has been used as an indicator of cross-bridge formation (6). Figure 5A shows that rigor stiffness of the border zone myocardium was significantly reduced by 35% compared with that of the remote zone myocardium. This finding suggests that the border zone myocardium may have fewer cross-bridges that are capable forming compared with the remote zone myocardium, which may contribute to impaired contraction of the border zone myocardium.

Some animals were treated for 2 wk post-MI with doxycycline, an inhibitor of MMPs. Figure 5A shows that doxycycline treatment significantly increased the rigor stiffness of the border zone myocardium.

Consistent with doxycycline having a beneficial effect on cross-bridge function, force development in the border zone was increased by 27% by 2 wk of doxycycline treatment (Fig. 5B). Interestingly, doxycycline also increased force development in the remote zone myocardium by 15%.
Decreased phosphorylation of ELC in the border zone myocardium. We performed a phosphoprotein screen of muscle proteins using a Pro-Q Diamond fluorescent assay to stain for phosphoproteins in acrylamide gels. Figure 6 shows the results of Pro-Q diamond staining analysis performed using a 4–20% gel with samples from the remote zone and border zone myocardium and from anatomically comparable regions of nonoperated control hearts. The border zone myocardium had a significant decrease in intensity of a phosphoprotein band positioned ~20 kDa, close to the position expected for the ELC subunit of the myosin molecule. Figure 6B shows the results of in-gel Western blot analysis using a fluorescent antibody to myosin ELC (same gel as shown in Fig. 6A). The ELC antibody colocalized with the 20-kDa phosphoprotein band, consistent with this band representing ELC. Phosphorylated ELC was quantitated and normalized to the total ELC level, which was assessed using Coomassie blue staining (Fig. 6C). The border zone myocardium had a significant reduction in the level of phosphorylated ELC/total ELC (41 ± 10%, n = 4, P < 0.05). However, for animals treated with doxycycline for 2 wk after MI, the level of phosphorylated ELC was not reduced (Fig. 6D).

Elevated MMP-2 in the border zone myocardium. Figure 7 shows representative histological sections with immunohistochemical staining for MMP-2 and summary data quantitating immunohistochemical staining for MMP-2. Compared with the noninfarcted control myocardium, 2 wk after MI,
MMP-2 was significantly elevated in myocytes in the border zone myocardium. Previously, we found that increased MMP-2 activity leads to further increases in MMP-2 levels (3). Therefore, inhibition of MMP-2 activity with doxycycline was expected to inhibit the rise of MMP-2 in the border zone. Consistent with this, Fig. 7 shows that with doxycycline treatment, MMP-2 was not appreciably elevated in the border zone myocardium.

Fig. 5. Muscle stiffness was decreased in the border zone myocardium in rigor. A: rigor stiffness was significantly reduced in border versus remote zone samples ($n = 5$/group, *$P = 0.011$). With doxycycline (Doxy) treatment for 2 wk after MI, there was significantly greater stiffness in the border zone myocardium ($n = 5$/group, *$P = 0.012$). B: Doxy significantly increased force development in the border zone myocardium ($n = 6$/group, **$P < 0.001$).

MMP-2 was significantly elevated in myocytes in the border zone myocardium. Previously, we found that increased MMP-2 activity leads to further increases in MMP-2 levels (3). Therefore, inhibition of MMP-2 activity with doxycycline was expected to inhibit the rise of MMP-2 in the border zone. Consistent with this, Fig. 7 shows that with doxycycline treatment, MMP-2 was not appreciably elevated in the border zone myocardium.

Fig. 6. Decreased phosphorylation of essential light chain (ELC) in the border zone myocardium. A: Pro-Q Diamond assay showing decreased intensity of the 20-kDa band in the border zone myocardium. B: in-gel Western blot analysis showing that fluorescent antibody to ELC colocalizes with the 20-kDa band. C: representative image of phosphorylated (p-)ELC detected in the Pro-Q Diamond assay and total ELC detected by Coomassie blue staining of the same gel. D: pooled data summary showing that p-ELC relative to total ELC was significantly reduced in the border zone myocardium ($n = 4$/group, *$P < 0.05$). For animals treated with Doxy for 2 wk after MI, p-ELC was not reduced.
DISCUSSION

The major finding of this study was that in sheep hearts after apical infarction, myofilament force development was appreciably reduced in the noninfarcted border zone adjacent to the infarct compared with myocardium remote from the infarct. The hypocontractile border zone was wide, extending from the infarct to close to the base of the heart. Our results suggest that impaired border zone function in the intact heart arises from a defect in myofilament function at the cross-bridge level. Treatment with doxycycline diminished the defect in myofilament function, potentially through inhibition of MMP-2.

Decreased myofilament force in the border zone. For sheep hearts subjected to anteroapical infarcts, a previous in vivo study (8) used long- and short-axis magnetic resonance images to create a finite element model of the LV. Optimized contractility parameters suggested considerably reduced force in the infarct border zone versus the remote zone myocardium. Consistent with this, the present study found reduced myofilament force in the border zone myocardium. Moreover, the magnitude of the myofilament force reduction was similar to that estimated using finite-element methods (8).

The border zone region with reduced myofilament force was relatively wide, extending for several centimeters from the infarct toward the base. The reduction of myofilament force in the border zone was greatest immediately adjacent to the infarct. The reduction of border zone force was attenuated linearly with distance from the infarct. This gradient of force reduction is consistent with a recent mathematical model of border zone dysfunction that predicted a 3-cm-wide area of dysfunction where there was a linear relationship between contractility and distance from the infarct (18). The presence of such a large region of dysfunctional myocardium could have a considerable impact on pump function in the post-MI heart.

In the border zone myocardium, there was both cellular hypertrophy and a low force per unit area of myocardium. The cell hypertrophy balanced the reduction of myocardial force such that the calculated force per cell in the border zone myocardium was unchanged relative to that in the remote zone myocardium. Thus, the degree of cell hypertrophy may be regulated to achieve a set point for force generation per cell.

Although the calculated force per cell was not reduced in the border zone versus remote zone myocardium, myocardial force development was reduced when considered on the basis of force per unit area of myocardium. Despite the presence of cellular hypertrophy in the border zone, wall thickness is not increased. Indeed, wall thickness close to the infarct is reduced and is tapered toward the infarct. In the intact heart, both the reduction in wall thickness and the decreased force generation per unit area of myocardium contribute to the impaired contraction of the border zone myocardium close to the infarct.

For the remote zone myocardium, there was a 6% reduction in myofilament force compared with control uninjured hearts. The postinfarct heart likely has increased β-adrenergic activation to counter a lack of function in the infarct region, along with impaired function of the border zone. Acute β-activation augments contraction, but sustained β-activation is injurious (1) and may contribute to the impaired myofilament force in the remote zone.

Mechanism of decreased myofilament force in the border zone. We found that decreased force per unit area of border zone myocardium was not due to loss of contractile material involving greater tissue fibrosis, fewer myofibrils per area, or decreased myosin abundance relative to the remote zone myocardium. Moreover, border zone dysfunction did not involve altered cross-bridge kinetics as quantified by measures of force redevelopment kinetics ($k_{tr}$) and isotonic shortening velocity. $k_{tr}$ reflects the sum of the apparent rate constants for cross-bridge attachment and detachment (4). Furthermore, the cross-bridge dissociation rate is a determinant of the maximum velocity of unloaded shortening (12). Our results suggest that
altered cross-bridge attachment and detachment kinetics do not play a role in the decreased force development of the border zone myocardium.

Our findings do suggest that cross-bridge function was impaired in the border zone myocardium. For the border zone myocardium, the stiffness in rigor was appreciably reduced compared with the remote zone myocardium, suggesting fewer cross-bridges were capable of forming in the border zone versus remote zone myocardium. In the border zone versus remote zone myocardium, the reduction in rigor stiffness (35%) was similar to the reduction in myofilament force development (31%). Thus, the reduction in myofilament force development in the border zone myocardium may be due to a reduction in the number of myosin cross-bridges that bind to actin. Consistent with a role for impaired cross-bridge formation as a mediator of border zone dysfunction, both rigor stiffness and myofilament force development in the border zone myocardium were increased in parallel by the use of doxycycline as a therapeutic intervention.

We found that ELC phosphorylation was appreciably reduced by 41% in the border zone versus remote zone. The role of ELC phosphorylation in contraction remains unclear. A recent study (21) has reported that a small (~10 amino acid) COOH-terminal truncation of ELC containing a phosphorylation site causes markedly reduced force development. Thus, COOH-terminal truncation of ELC with removal of the phosphorylation site could contribute to border zone dysfunction. The cellular protease MMP-2 is elevated in ischemic cardiomyopathy (10). Moreover, intracellular MMP-2 can damage the myofilaments, including a 10-amino acid COOH-terminal truncation of ELC, and cause reduced force generation (23, 26). We found that the MMP inhibitor doxycycline prevented the decrease in ELC phosphorylation level in the border zone. Moreover, protection of ELC phosphorylation was associated with improved cross-bridge formation and force development. Nevertheless, doxycycline can mediate multiple protective effects, including oxygen radical scavenging and anti-inflammatory effects, which may have played a role (7).

Limitations. Phosphorylation/dephosphorylation of myofilament proteins can take place rapidly. Since several of these reactions depend on mechanical activity; once the heart is arrested, the levels of phosphorylation can change. A recent study (25) found that the contractile protein phosphorylation level changed up to 15%/h. In the present study, samples were not immediately snap frozen, which may have limited the detection of myofilament protein phosphorylation status.

The average force per cell was calculated but not directly measured in this study. The actual force per cell might be heterogeneous among cells, with some cells having low forces and other cells having high forces.

To detect myosin ELC, we used a commercial monoclonal antibody (ab680, Abcam) that reacts to ELC from the mouse, rat, rabbit, cow, human, and pig. Recent studies (15, 16, 22) have shown that detection of ELC with ab680 is distinct from detection of myosin regulatory light chain with a myosin regulatory light chain-specific antibody. However, it remains possible that in sheep ab680 could be less specific for ELC.

Further study is required to determine the impact of this model on other sarcomeric phosphoproteins and to determine whether reduced ELC phosphorylation in the border zone involves proteolytic breakdown.

Conclusions. After MI, there is a broad region adjacent to the infarct in which myofilament force development is impaired. The presence of such a broad region with myofilament dysfunction could contribute to impaired pump function post-MI. Decreased myofilament force development per unit area of myocardium in the border zone adjacent to an infarct is not due to histological structural changes within the myocardium, loss of myosin, or alteration in cross-bridge kinetics. Decreased myofilament force in the border zone is associated with impaired cross-bridge formation, possibly related to decreased phosphorylation of ELC. Therapies designed to improve border zone contractility, such as MMP-2 inhibition, may be a novel approach to improve pump function for patients with failing hearts post-MI (27).

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

REFERENCES


