Residual Strain in Ischemic Ventricular Myocardium

Structural remodeling during acute myocardial infarction affects ventricular wall stress and strain. To see whether acute myocardial infarction alters residual stress and strain in the left ventricle (LV), we measured opening angles in rat hearts after 30 minutes of left coronary artery occlusion. The mean opening angle in 18 ischemic hearts (51 ± 20 deg) was significantly greater than in five sham-operated controls (29 ± 11 deg, P < 0.05). To determine whether these alterations in residual strain may be associated with strain softening caused by systolic overstretch of the noncontracting ischemic tissue, we also measured opening angles in isolated hearts that had been passively inflated to high LV pressures (120 mmHg). The mean opening angle of the strain-softened hearts was not significantly different from the sham-operated hearts (34 ± 27 deg, P = 0.74). Mean collagen area fractions in the myocardium were not significantly different between ischemic hearts (0.027 ± 0.014) and the nonischemic group (0.022 ± 0.011). Although there were significant differences in opening angles measured with ischemia, they do not appear to be a result of altered extracellular collagen content or softening associated with overstretch. Thus, there is a significant change in residual strain associated with acute ischemia that may be related to changes in collagen fiber structure, myocyte structure, or metabolic state.

Introduction

Acute myocardial ischemia is associated with characteristic regional alterations in ventricular wall stress, strain, and myocardial material properties [13]. There are substantial alterations in diastolic mechanics in the ischemic region that result from myocardial edema and cell swelling [21], contracture and necrosis, and restructuring of the extracellular matrix (ECM) [15, 24] associated with collagen degradation [13, 28] and disruption during systolic bulging [7, 8].

To understand the interactions between normally perfused and ischemic tissue, transmural distributions of ventricular wall stress and strain are needed [9]. However, residual stress exists in the unloaded resting ventricular wall [18] and is known to affect the distribution of stress and strain [10], and sarcomere length [22]. Many authors have postulated that regional coronary blood flow and perfusion are affected by regional myocardial stress and strain [1, 4, 5, 17, 27]. Residual stress could change transmural stress distributions [10] and hence potentially affect regional myocardial perfusion. It is also known that ventricular wall stress is a primary determinant of myocardial oxygen demand and hence the risk and extent of ischemic injury [13, 23]. Although the mechanisms of residual stress are unknown, alterations in myocardial residual strain have been reported in mutant mice that overexpress type I collagen [19], suggesting that the extracellular matrix might be involved.

Previous studies have shown both decreased and increased diastolic myocardial stiffness early after coronary occlusion [11, 26]. Forrester et al. [8] reported that overstretch or “systolic bulging” of noncontracting ischemic tissue during systole contributes to diastolic dysfunction during ischemia. Recently, Emery et al. [7] reported that passive ventricular inflation to loads exceeding normal physiologic levels causes significant myocardial softening without changing the unloaded left ventricular volume and described this behavior as “strain softening.” Johnson and Beatty [12] proposed a general structural model for strain softening and described a molecular mechanism by which clusters of cross-linked long-chain molecules become partially unlinked under loading. Emery and colleagues [7] proposed that this property may be associated with ischemic diastolic dysfunction, but whether softening affects the zero-stress state has not been investigated.

It has been previously shown that acute myocardial ischemia and infarction are accompanied by an early loss of myocardial collagen and disruption of the normal organization [24, 29]. Brief episodes of acute ischemia can lead to rapid activation of collagenases (which are already bound to the substrate but inactivated [6]) and degradation of matrix as reported by Sato et al. [24]. Abnormal mechanics in the ischemic myocardium could also contribute to matrix disruption [6]. The early events following myocardial infarction appear to involve degradation of collagen before collagen synthesis is adequately stimulated to patch the necrotic area. Remodeling of the myocardial extracellular matrix postinfarction eventually leads to reparative scars composed of collagen.

Therefore, the objective of this study was to examine whether left ventricular residual strain is altered following acute myocardial ischemia and to investigate whether changes in residual strain may be associated with changes in the myocardial collagen matrix or acute passive over-stretching of the myocardium.

Methods

A total of 47 rats were used in this study, in three groups: ischemic, sham-operated, and strain-softened. All animal studies were performed according to American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines.
for the use of animals in research, and protocols were approved by the University of California, San Diego, Animal Subjects Committee.

Thirty-seven male Sprague-Dawley rats weighing 259–425 g were anesthetized with sodium pentobarbital (100 mg/kg), intubated, and ventilated with air. The heart was exposed by a median sternotomy and the pericardium resected. A stitch, using 5-0 polypropylene suture and a C-1 tapered needle, was passed under the left coronary artery near its origin and the vessel was occluded with a small snare. After 30 minutes of ischemia, the vena cavae were ligated, the aorta was clamped, and the heart was arrested via an injection of 1 ml of cold heparinized arrest solution (0.0225 g/10 ml KCl, 100 units/10 ml heparin) through the left ventricle apex, taking care to avoid overloading the left ventricle. The heart was then excised, rinsed, weighed, and the aorta cannulated to allow a brief 1-min perfusion to flush the coronary circulation with room-temperature cardioplegic buffer.

Afterward, India ink was injected retrograde through the aorta to stain the nonischemic region of the ventricles. A circumferential section, 1–2 mm thick, was cut from the equatorial region of the left ventricle [22] and immersed in cold Krebs solution (68 mM NaCl, 60 mM KCl, 36 mM NaHCO3, 2.0 mM MgCl2, 1.4 mM NaSO4, 11 mM dextrose, 30 mM 2,3-butanediol monoxime (BDM), and 10,000 Units/L heparin). The ring was photographed so that the geometry of the stress-free state could be measured. Figure 1 shows how the myocardial rings were cut and the relationship to the at-risk area.

Five sham-operated control hearts were obtained using the same procedure, except the aorta was not ligated and no ischemia was induced. After 30 minutes, the heart was arrested with an injection of heparinized arrest solution through the left ventricle apex. In some studies, video capturing (BW Cohu camera, Data Translation capture board, and a Macintosh IIci), instead of still photography, was used to record the geometry of the ventricular cross sections.

Strain Softening. To determine the possible effects of strain softening due to myocardial overstretch, 5 male Sprague-Dawley rats weighing 288–333 g were isolated, arrested, and prepared as the sham-operated animals. The hearts were mounted on an aortic cannula and a balloon was inserted into the left ventricle and inflated three times to 10 mmHg followed by three loading cycles to a peak pressure of 120 mmHg to simulate globally the effects of overstretch in noncontracting ischemic tissue during systole. Afterward, the heart was removed and the opening angles were measured.

Opening Angle Measurements. The geometry of the stress-free state was measured from the images recorded after the tissue had been cut radically to relieve residual stress. The ring sprang open into an arc characterized by measuring the “opening angle,” which is related to the residual strain present in the LV wall [18]. The angle formed was defined by the midpoint of the cut ends relative to the center of the LV (Fig. 1). The center of the LV was defined as the midpoint of the line that bisected both a straight line between the cut edges and the LV endocardial perimeter between the edges. It was measured using image processing software (NIH Image 1.52) from digital images scanned from the photographs or grabbed from the video camera [22].

Collagen Area Fraction. In a subgroup of 5 ischemic and 5 sham-operated hearts, tissue was fixed in formalin (10 percent formalin in phosphate buffered saline), embedded in paraffin, sectioned, and stained with picrosirius red (Sirius red F3BA Roboz, Washington, DC, in saturated picric acid). A semi-automated method, which has been described in detail previously, was used to quantify the collagen area fraction based on enhanced birefringence seen with picrosirius-polarization microscopy [14, 15]. Briefly, the sample was transilluminated with polarized light and the transmitted light viewed through an analyzer polarized at 90 deg to the incident plane (cross-polarization microscopy) using a 40X objective (Olympus 0.95NA). The axes of cross-polarization were initially rotated until the autobirefringence of the muscle was extinguished. Views near edges of tissue or blood vessels were avoided. Since the perimysial collagen fibers are wavy, sections of the collagen that were similar in orientation to the myofibrils were therefore also extinguished in this view. As described previously, a composite view was constructed from two images by digital image processing (NIH Image 1.52). One image was obtained with the polarizers in this initial orientation and the other with them rotated by 45 deg. The 24-bit 640 × 480 pixel RGB color images were captured with a Framegrabber board (Data Translation Model DT2255) on a microcomputer (Apple Macintosh Quadra 900) using a color video camera (Sony DVC-151). The RGB-coded images were converted to a hue-saturation-brightness representation and the brightness planes were thresholded to exclude background birefringence as described previously [14, 15]. A logical union of the binary images then gave a total count of pixels representing collagen. Finally, this count was normalized by the total tissue area in the image of the same view so that nontissue pixels were excluded. Twenty views were taken for each wall location. Collagen area fractions were measured at epicardial, midwall, and endocardial locations in the ischemic area of the tissue for the ischemic group and at
epicardial, midwall, and endocardial locations of the tissue for the control group.

All results are expressed as mean ± S.D. Analysis of variance was used for comparison between groups. Differences were regarded as significant when \( P < 0.05 \).

**Results**

In the ischemic group, twelve of the animals died from acute ventricular failure before the 30-min period of ischemia was over. Seven of the hearts were excluded because we were unable to confirm from the India ink injection that the equatorial region of the ventricular wall was ischemic. Data were analyzed for the 18 remaining animals in this group. All animals from the sham-operated and strain-softened groups were included in the analysis. The mean opening angles for the three groups are shown in Fig. 2. The mean opening angle in the ischemic rat hearts (51 ± 20 deg, \( n = 18 \)) was significantly greater (\( P = 0.027 \)) than in the sham-operated controls (29 ± 11 deg, \( n = 5 \)). The mean opening angle of the strain softened group was (34 ± 27 deg, \( n = 5 \)).

In the ischemic hearts, the area not stained by India ink ranged from 41–85 percent of the LV short-axis cross-sectional area as measured from the images of the ventricular slices (mean 64 ± 14 percent). Figure 3 shows the relationship between the opening angles in the ischemic group and the sham group versus ischemic area. No significant correlation was found for this relationship (\( P = 0.15 \), \( r^2 = 0.13 \)). However, when the regression was repeated for only the ischemic group, a weak inverse correlation (\( r^2 = 0.56 \)) that was statistically significant (\( P = 0.04 \)) was found. Since this paradoxical observation suggests the possibility of a nonlinear relationship between opening angle and ischemic area, these linear regressions should be interpreted cautiously and thus are not plotted in Fig. 3.

Similar to earlier results reported by Emery et al. [7], we observed in the strain-softened group a substantial increase in LV chamber compliance following inflation to high cavity pressure. The change in left ventricular volume at 10 mmHg filling pressure (normalized by the unloaded volume at 0 mm Hg) increased by 310 ± 22 percent (\( P < 0.001 \)) after the ventricle had been inflated to a peak pressure of 120 mmHg. Figure 4 compares collagen area fractions in the ischemic and nonischemic groups with regard to transmural location. No significant differences in collagen content were found between the two groups of hearts with respect to group (\( P > 0.18 \)) or wall location (\( P > 0.16 \)). Also, there was no significant difference in variations with wall location between groups (\( P > 0.35 \)).

**Discussion**

The purpose of the present study was to determine whether residual strain is altered in acutely ischemic myocardium and whether collagen alterations or strain softening may be associated with these changes. We found that opening angles were significantly higher in ischemic hearts than in sham-operated or strain-softened hearts, suggesting that acute coronary artery occlusion may significantly increase residual stress and strain in the left ventricle. However, these changes do not appear to be associated with overstretch of the ischemic region due to systolic bulging, because acute strain softening did not alter the opening angle. Nor can we attribute the increased residual strain to changes in myocardial collagen content since no significant change in histological area fraction were observed.

A possible mechanism of the increased residual strain with coronary occlusion group is ischemic contracture. Some reports have indicated that opening angles tend to increase with time after cardiac arrest and these changes can be delayed with agents that block crossbridge interactions and deny rigor [22]. Edema
is another consequence of acute ischemia [20, 21], that is known to alter passive myocardial mechanics and may also affect residual strain. However ischemia is also associated with myocyte disruption and cell death [3, 28]. These may also have contributed to the changes in residual strain.

Although it has been previously shown that a significant increase in LV diastolic stiffness occurs several days after acute myocardial infarction due to cellular infiltration, fibrosis, and edema, studies also show that passive myocardial stiffness decreases significantly after coronary occlusion [8]. A small part of the increased passive compliance during ischemia may be explained by a loss in coronary vascular tone in the ischemic region [13] and most investigators have concluded that the passive material properties of the myocardium are altered, however, the cause of this effect remains unclear.

Collagen degradation in the heart may play an important role in the pathophysiology of myocardial dysfunction following ischemic injury [25]. It has previously been postulated that extracellular degradation of collagen seen in acute ischemia involves activation of latent collagenases and may be sufficient to impair the mechanical properties of the myocardium. Although the experimental ischemia was acute and brief, there was reason to expect that collagen degradation may have been observed. Sato et al. [24] found fibrillar collagen damage within 40 minutes of ischemia produced by occlusion of the left anterior descending coronary artery in porcine myocardium. Similar early collagen damage has been found in a canine model of coronary reperfusion [29, 30]. However, in this study, no decrease in mean collagen area fraction was found with ischemia.

In the heart, it is possible that changes occur in the collagen architecture that could alter ventricular stiffness without affecting the total myocardial collagen content. For example, an increased number of fibers with smaller diameter would reduce their contributions to diastolic stiffness [16]. Conversely, there may be changes in collagen content that do not affect myocardial stiffness. Acute ischemia has been shown to lead to damage of the cardiac collagen extracellular matrix [24, 30]; however, this does not mean that the collagen area fractions would be decreased due to the resulting disarray. It is also possible that the duration of ischemia may have been too short or our sample size too small to detect histologically significant collagen degradation in the ischemic region. The fact that we did not reperfuse after coronary ligation may have contributed to not finding an observable loss of collagen. In the other acute models in which significant degradation was reported, ischemia and reperfusion were involved.

Since there is thought to be a close structural coupling between the coronary microvasculature and the surrounding myocytes [3], perfusion may affect the regional shape and tissue volume of the myocardium. Perfusion has also been previously shown to produce a significant increase in myocardial tissue volume that is greatest at the endocardium in the unloaded ventricle [17]. In terms of mechanics, the increase in volume of the myocardium may increase the strain energy and hence the wall stress. Since myocardial elasticity is nonlinear, this would cause a stiffening effect [17]. Coronary perfusion produces slight global, but larger local changes in the mechanical behavior of the myocardium during loading [17].

The rat model of left coronary artery ligation is a common one for myocardial ischemia and infarction. However, since the coronary anatomy is difficult to visualize in the rat, a wide variation in the size of the ischemic zone is common. This probably explains the high acute mortality in the ischemic group and the incidence of cases where no ischemic area could be discerned post-mortem. In the remaining animals there was also a high variation in the size of the ischemic zone, but we found no significant correlation between the size of the ischemic zone and either the opening angle or the collagen area fraction, when ischemic and nonischemic hearts were considered. However, while the opening angle increased with ischemia, the magnitude of the opening angle in the ischemic group actually had a slight but significant inverse correlation with the size of the area at risk. The greater structural and mechanical heterogeneity of the LV wall in the ischemic heart compared with control may have contribute to these findings. We postulated that this paradoxical result might be the result of a variation with ischemic area in the transmural uniformity of the area at risk. There was no apparent trend between the ratio of endocardial to epicardial ischemic area and the size of the area at risk. Therefore, we measured the polar moment of inertia of the ischemic zone in a subset of hearts, but there was no correlation with opening angle (P = 0.23, r² = 0.40). These findings do not rule out the importance of nonhomogeneity in this experimental model. They reflect the comparatively small sample size, given the large variability in opening angle that is typically observed. While our sample size was large enough to detect the significant effects of ischemia on residual strain, it was not sufficient to elucidate the influence of factors such as ischemic zone shape, location and area. These factors as well as the possible effects of myocardial infarction as well as ischemia should be investigated in future studies.

In summary, significant alterations in residual strain due to acute coronary artery occlusion may have important functional consequences for the mechanics of ischemic myocardium. However, this change appears to be unrelated to changes in collagen area fraction or myocardial overstretch.

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References


