Dissociation between cardiomyocyte function and remodeling with β-adrenergic receptor blockade in isolated canine mitral regurgitation

Betty Pat,1 Cheryl Killingsworth,1 Thomas Denney,3 Junying Zheng,1 Pamela Powell,1 Michael Tillson,4 A. Ray Dillon,4 and Louis J. Dell'Italia1,2

1Center for Heart Failure Research, Department of Medicine, University of Alabama at Birmingham, and 2Birmingham Department of Veteran Affairs, Birmingham; and 3Department of Electrical Engineering and 4College of Veterinary Medicine, Auburn University, Auburn, Alabama

Submitted 17 July 2008; accepted in final form 6 October 2008

Pat B, Killingsworth C, Denney T, Zheng J, Powell P, Tillson M, Dillon AR, Dell'Italia LJ. Dissociation between cardiomyocyte function and remodeling with β-adrenergic receptor blockade in isolated canine mitral regurgitation. Am J Physiol Heart Circ Physiol 295: H2321–H2327, 2008. First published October 10, 2008; doi:10.1152/ajpheart.00746.2008.—The low-pressure volume overload of isolated mitral regurgitation (MR) is associated with increased adrenergic drive, left ventricular (LV) dilatation, and loss of interstitial collagen. We tested the hypothesis that β1-adrenergic receptor blockade (β1-RB) would attenuate LV remodeling after 4 mo of MR in the dog. β1-RB did not attenuate collagen loss or the increase in LV mass in MR dogs. Using MRI and three-dimensional (3-D) analysis, there was a 70% increase in the LV end-diastolic (LVED) volume-to-LV mass ratio, a 23% decrease in LVED midwall circumferential curvature, and a >50% increase in LVESD 3-radius/wall thickness in MR dogs that was not attenuated by β1-RB. However, β1-RB caused a significant increase in LVED length from the base to apex compared with untreated MR dogs. This was associated with an increase in isolated cardiomyocyte length (171 ± 5 μm, P < 0.05) compared with normal (156 ± 3 μm) and MR (165 ± 4 μm) dogs. Isolated cardiomyocyte fractional shortening was significantly depressed in MR dogs compared with normal dogs (3.73 ± 0.31 vs. 5.02 ± 0.26%, P < 0.05) and normalized with β1-RB (4.73 ± 0.48%). In addition, stimulation with the β-adrenergic receptor agonist isoproterenol (25 nM) increased cardiomyocyte fractional shortening by 215% (P < 0.05) in β1-RB dogs compared with normal (56%) and MR (50%) dogs. In summary, β1-RB improved LV cardiomyocyte function and β-adrenergic receptor responsiveness despite further cell elongation. The failure to attenuate LV remodeling associated with MR could be due to a failure to improve ultrastructural changes in extracellular matrix organization.

heart failure; volume overload

ISOLATED MITRAL REGURGITATION (MR) is characterized by initial left ventricular (LV) dilation and augmented stroke volume that is mediated by the Starling mechanism and facilitated by regurgitation into the low-pressure left atrium. Increased sympathetic drive follows the initial recruitment of preload reserve in the early phase of MR in the human (8) and dog (4, 9, 17). Using the microdialysis technique in healthy open-chest canine, we have shown that there is compartmentalized norepinephrine release into the LV interstitial fluid during electrical stimulation of the stellate ganglion (4, 17). We have reported that catecholamine release into the cardiac interstitial fluid in response to ANG II and stellate ganglion stimulation is enhanced in 4-wk MR compared with normals and, moreover, that the interstitial fluid catecholamine release was normalized after chronic treatment with extended release metoprolol succinate (4), a relatively selective β1-adrenergic receptor blocker (β1-RB) (17). In addition, β1-RB with atenolol, initiated 3 mo after the induction of MR in the dog, improved LV contractile function and cardiomyocyte function (18). Whether these cardiomyocytes maintain their β-receptor responsiveness to isoproterenol (Iso) is still unknown and may determine LV function after surgical valve repair or replacement.

Chronic MR is characterized by eccentric remodeling caused by extensive LV dilatation, wall thinning, and cardiomyocyte elongation. In the aforementioned studies, there was no accompanying geometric analysis to determine if β1-RB had an effect on LV eccentric remodeling. Thus, although β1-RB prevents excessive adrenergic stimulation of the myocardium, there is little evidence to suggest that it improves LV chamber remodeling during chronic MR.

The extent to which the LV remodels during MR may be associated with changes in the extracellular matrix (ECM). We have shown that after 4 wk of MR, there is a significant decrease in endomysial collagen in both the LV endocardium and epicardium (17). Treatment with β1-RB significantly attenuated the loss of epicardial collagen but did not affect endocardial collagen after 4 wk of MR (17). Therefore, we tested the hypothesis that long-term administration of a β1-RB (long-acting metoprolol succinate, 100 mg, twice daily) started within 24 h of MR induction would attenuate LV remodeling and improve function by preventing the loss of endocardial ECM after 4 mo of MR in the dog.

MATERIALS AND METHODS

Experimental procedures. Mitral valve regurgitation was induced at Auburn University College of Veterinary Medicine in 15 conditioned mongrel dogs of either sex (19–26 kg) by chordal rupture using a fluoroscopic guided catheterization method previously described in our laboratory (2, 11, 16, 17). Eleven dogs survived to 4 mo. We collected hemodynamic data and performed MRI and three-dimensional (3-D) analysis in all dogs at baseline (n = 11) and after 4 mo of MR using a Picker Vista 1.0-T magnet. Dogs were randomly assigned to one of two groups: 1) 4 mo of MR (n = 5, 3 male dogs and 2 female dogs); and 2) 4 mo of MR treated with β1-RB (extended release metoprolol succinate, 100 mg po, twice daily; n = 6, 4 male dogs and 2 female dogs). All drugs were

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
started 24 h after MR induction. Ten normal dogs were killed for tissue morphometry, isolated cardiomyocyte experiments, and collagen analysis. Dogs were transferred to the University of Alabama at Birmingham for euthanization. Drugs were withheld on the day of the terminal experiments. This study was approved by the Animal Services Committees at the University of Alabama at Birmingham and Auburn University College of Veterinary Medicine.

**Terminal experiment: instrumentation.** Animals were maintained at a deep plane of general anesthesia using 1–2% isofluorane in 100% oxygen as previously described (17). The heart was arrested with KCl, quickly excised, and placed in ice-cold Krebs solution [containing (in mM) 118.4 NaCl, 27.1 NaHCO₃, 1.0 KH₂PO₄, 1.2 MgSO₄·7H₂O, 11.1 glucose, and 11.3 HEPES], and the coronaries were flushed with the same solution. The LV was sectioned and either perfusion fixed with 3% paraformaldehyde and/or frozen in OCT for immunohistochemistry. Additionally, a wedge of the LV was cannulated for cardiomyocyte isolation.

**Isolated cardiomyocyte experiments.** LV wedges were cannulated at larger-brancheat arteries at the base that typically extended toward the apex and then perfused with warm (36–37°C) Ca²⁺-free Krebs solution [containing (in mM) 120.4 NaCl, 14.7 KCl, 0.6 KH₂PO₄, 0.6 Na₂HPO₄, 1.2 MgSO₄·7H₂O, 4.6 NaHCO₃, 10 Na-HEPES, 30 taurine, and 5.5 mM glucose] gassed with 100% O₂ for 5–7 min. Cardiomyocytes were isolated from the tissue by recirculating perfusion buffer supplemented with 2 mg/ml collagenase type II (Invitrogen, Carlsbad, CA) for 10–20 min.

The intracellular Ca²⁺ concentration was measured with the fluorescent indicator fluo3-AM (Molecular Probes, Eugene, OR). Cardiomyocytes were incubated at 37°C in the dark for 30 min in normal HEFES-buffered solution consisting of (in mM) 126 NaCl, 11 l-dextrose, 4.4 KCl, 1.0 MgCl₂, 1.0 CaCl₂, and 24 HEPES with pH adjusted to 7.4 and 5 μm fluo3-AM added. Fluo3-AM-loaded cardiomyocytes were placed in a glass-bottom, temperature-controlled bath (37°C) that was mounted on the stage of an inverted microscope (Eclipse TE300/200, Nikon, Lewisville, TX), as previously described (5). Cells were continuously bathed at a rate of 1–2 ml/min with the HEFES-buffered bathing solution containing 0.5 mM probenicid to help retard fluo3-AM transport from the cells. Cardiomyocytes were allowed to equilibrate for at least 5 min before stimulation. Fluorescence emission (530 nm) was measured with the Axoclamp 2B amplifier system (Axon Instruments, Foster City, CA), digitized with a 12-bit analog-to-digital converter (Axon Instruments), and recorded with a computer using pCLAMP 9 software (Axon Instruments).

**Geometric analysis: MRI.** Endocardial and epicardial contours were manually traced on end-diastolic (ED) and end-systolic (ES) images. Contours were traced to exclude papillary muscles. Contour data were then transformed to a coordinate system aligned along the long axis of the LV and converted to a prolate spheroidal coordinate system as previously described (19). The prolate spheroidal coordinate system has one radial coordinate (r) and two angular coordinates (ϕ, θ). Prolate spheroidal coordinates were used because surfaces of constant λ are ellipsoids, which more closely approximate the shape of the LV wall than cylinders or spheres. Cubic B-spline surfaces, λendocard(μ, θ) and λepicard(μ, θ), were fitted to the λ-coordinates of the endocardial and epicardial contours for each time frame. Each surface used 12 control points in the circumferential (θ) direction and 10 control points in the longitudinal direction (μ). The control points of each surface were computed to minimize the following error function (ε):

\[
ε = \sum_{i} \left[ \lambda_i^2 - \lambda_{i,0}^2 \right] + γS(λ),
\]

where γ is a weight set to 0.1, and S(λ) is a smoothing function. The first term in the error function is the squared difference between the contour points, λ_i, and the corresponding surface points, λ_i,0. The second term is smoothing function S(λ), which penalizes the bending energy of the surface as follows:

\[
S(λ) = \int_0^T \left[ \frac{\partial^2 λ}{\partial r^2} + \frac{1}{r} \frac{\partial λ}{\partial r} \right]^2 + \left( \frac{\partial λ}{\partial θ} \right)^2 \, dΩ
\]

where Ω is the domain of the surface. Surface curvatures were computed using standard formulas (7) at the wall segments as previously defined (1) (excluding the apex). 3-D wall thickness (T) was computed at the same segments by measuring the 3-D distance from a point on the epicardial surface to the closest point on the endocardial surface along a line perpendicular to the epicardial surface. The radius of curvature-to-wall thickness ratio (R/T) was computed using the reciprocal of the product of the endocardial circumferential curvature (κ) and T. ES wall stress was computed according to the following formula (3):

\[
\text{Wall stress} = 0.133 \frac{P}{2\kappa T \left(1 + \frac{T}{2r}\right)}
\]

where P is LVES blood pressure.

**Table 1. LV morphometry and function hemodynamics in normal, MR, and MR + BB dogs**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>MR</th>
<th>MR + BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, kg</td>
<td>21.2±0.7</td>
<td>21.1±1.2</td>
<td>21.5±1.4</td>
</tr>
<tr>
<td>LV/body weight mass, g/kg</td>
<td>3.9±0.2</td>
<td>4.9±0.3*</td>
<td>5.5±0.3*</td>
</tr>
<tr>
<td>Right ventricle/body weight mass, g/kg</td>
<td>1.6±0.1</td>
<td>1.7±0.1*</td>
<td>2.0±0.1*</td>
</tr>
<tr>
<td>Sample size, n</td>
<td>10</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

**Baseline hemodynamics**

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>MR</th>
<th>MR + BB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>128±11</td>
<td>110±6</td>
<td>122±16</td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td>4.8±0.4</td>
<td>3.2±0.3*</td>
<td>4.0±0.3</td>
</tr>
<tr>
<td>LV end-diastolic volume, ml</td>
<td>38±4</td>
<td>69±9*</td>
<td>81±13*</td>
</tr>
<tr>
<td>LV end-systolic volume, ml</td>
<td>21±3</td>
<td>35±8</td>
<td>44±9*</td>
</tr>
<tr>
<td>LV ejection fraction, %</td>
<td>45±3</td>
<td>51±5</td>
<td>47±3</td>
</tr>
<tr>
<td>LV end-diastolic pressure, mmHg</td>
<td>12±2</td>
<td>24±3*</td>
<td>16±2</td>
</tr>
<tr>
<td>LV end-systolic pressure, mmHg</td>
<td>108±2</td>
<td>107±3</td>
<td>101±5</td>
</tr>
<tr>
<td>LV +dP/dt, mmHg/s</td>
<td>2.649±175</td>
<td>2.423±297</td>
<td>1.826±214*</td>
</tr>
<tr>
<td>LV -dP/dt, mmHg/s</td>
<td>-2.360±205</td>
<td>-2.470±112</td>
<td>-1.664±135*</td>
</tr>
<tr>
<td>Systemic vascular resistance, dyne·cm⁻²·s⁻³</td>
<td>1.593±243</td>
<td>2.143±312</td>
<td>1.729±58</td>
</tr>
<tr>
<td>Pulmonary artery mean pressure, mmHg</td>
<td>14±1</td>
<td>12±2</td>
<td>16±1</td>
</tr>
<tr>
<td>Pulmonary artery wedge pressure, mmHg</td>
<td>5±1</td>
<td>5±1</td>
<td>9±1*</td>
</tr>
<tr>
<td>Pulmonary vascular resistance, dyne·cm⁻²·s⁻³</td>
<td>164±20</td>
<td>179±37</td>
<td>157±17</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of dogs. LV, left ventricular; MR, mitral regurgitation; BB, β₁-adrenergic receptor blockade. *P < 0.05 vs. normal dogs; †P < 0.05 vs. MR dogs.
Collagen analysis. Heart wedges were removed, perfusion fixed in 3% paraformaldehyde, and then switched to 70% ethanol. Paraffin-embedded sections (3 μm) from long-axis and short-axis epicardial and endocardial sections through the mid-LV were stained with picric acid sirius red F3BA. Interstitial collagen was identified by light microscopy at high power (×40 objective, ×1,600 total magnification), and the percent collagen volume was quantified with a digital-based image-analyzer system (Image-Pro Plus version 6.0, Media Cybernetics, Bethesda, MD) and with the aid of a 540-nm (green) filter to provide grayscale contrast of the collagen with the background. The percent collagen volume of 30–40 randomly selected fields was blindly evaluated in each section, and the mean value was calculated for each animal.

Myocyte cross-sectional area. Formalin-fixed frozen LV midendocardial sections were laminin stained for cardiomyocyte cross-sectional area (CSA) analysis. Briefly, 5-μm sections were subjected to proteinase K (20 μg/ml, Roche, Indianapolis, IN) enzymatic antigen retrieval for 30 min at 37°C and blocked with 5% goat serum-1% bovine serum in PBS for 1 h at room temperature. Sections were incubated with rabbit α-laminin (1:200, Abcam, Cambridge, MA) overnight at 4°C, incubated with FITC-conjugated goat anti-rabbit IgG secondary antibody (Molecular Probes, 1:400) for 1 h at RT, and then coverslipped with 4',6-diamidino-2-phenylindole hard set mounting medium (Vector Labs, Burlingame, CA). Fluorescent images in the cross section were analyzed using a digital-based image-analyzer system to count the myocyte number per square area (in μm²) of tissue to determine the CSA. A minimum of four areas was measured for each animal.

Statistics. Data are presented as means ± SE. Statistical interpretation was performed on all groups by one-way ANOVA and the Holm-Sidak post hoc t-test for significance. For MRI data, 4-mo values in untreated MR or β1-RB-treated MR dogs were compared with their respective baseline values before MR induction using a paired Student’s t-test. P < 0.05 was considered to be significant.

RESULTS

LV hemodynamics. LV hemodynamics are shown in Table 1. Body weight did not differ among groups. LV mass (normalized to body weight) at the time of death was significantly increased in MR dogs and was not attenuated by β1-RB. Right ventricular mass (normalized to body weight) was increased in β1-RB-treated dogs compared with normal dogs (P < 0.05). The mean heart rate was not significantly different among all groups under general anesthesia at the time of death. Cardiac output was significantly depressed in MR dogs yet was maintained at near-normal levels with β1-RB.

LVED volume (LVEDV) was significantly elevated in both untreated and β1-RB-treated MR dogs consistent with a volume load (P = 0.01). LVES volume (LVESV) was significantly increased in β1-RB-treated MR dogs (P = 0.05). LVED pressure was significantly elevated after 4 mo of MR compared with normal dogs (24 ± 3 vs. 12 ± 2 mmHg, P = 0.001) and slightly decreased with β1-RB (16 ± 2 mmHg). LVES pressure and ejection fraction were unaltered by MR or β1-RB. Both peak positive and peak negative dP/dt were unchanged by MR but significantly decreased below normal in β1-RB-treated MR dogs (P < 0.05). Pulmonary arterial wedge pressure was significantly elevated in MR dogs treated with β1-RB compared with normal and untreated MR dogs (P < 0.05). LV pulmonary artery mean pressure and systemic or pulmonary vascular resistance were not different among all groups.

It should be noted that a total of 15 dogs had MR induced. Of these, there were nine untreated dogs and six dogs treated with β1-RB. All six β1-RB-treated dogs survived to 4 mo. Of the nine untreated dogs, only five dogs survived to 4 mo.
LV remodeling by MRI. LV remodeling by MRI is shown in Figs. 1 and 2. LVEDV normalized to the LV mass ratio (LVEDV/mass) was significantly increased after 4 mo in MR dogs compared with baseline and did not improve with /H9252 1-RB (Fig. 1A). LVED length from the base to apex did not change after 4 mo in untreated MR dogs; however, there was a 35% increase in MR dogs treated with /H9252 1-RB (P = 0.021; Fig. 1B). LVESV measured by MRI (Fig. 2A) did not change in untreated MR dogs but was significantly elevated in /H9252 1-RB-treated dogs compared with baseline values (P = 0.037). There were no differences in LVES length in all MR dogs compared with their respective baselines (Fig. 2B).

LV remodeling by MRI. LV remodeling by MRI is shown in Figs. 1 and 2. LVEDV normalized to the LV mass ratio (LVEDV/mass) was significantly increased after 4 mo in MR dogs compared with baseline and did not improve with /H9252 1-RB (Fig. 1A). LVED length from the base to apex did not change after 4 mo in untreated MR dogs; however, there was a 35% increase in MR dogs treated with /H9252 1-RB (P = 0.021; Fig. 1B). LVESV measured by MRI (Fig. 2A) did not change in untreated MR dogs but was significantly elevated in /H9252 1-RB-treated dogs compared with baseline values (P = 0.037). There were no differences in LVES length in all MR dogs compared with their respective baselines (Fig. 2B).

Fig. 2. LV end-systolic (LVES) remodeling by MRI at baseline and after 4 mo of MR. 3-D MRI indexes of LV remodeling at end systole in dogs before MR induction (baseline) and after 4 mo of MR or MR + BB treatment are shown. A: LVES volume (LVESV). B: LVES long-axis length from the base to apex. C: LVES circumferential curvature. D: LVES circumferential radius-to-wall thickness ratio. Data obtained after 4 mo of MR or MR + BB were compared with their respective baseline values. Values are means ± SE. *P < 0.05 vs. the corresponding baseline measurements.

Fig. 3. Representative MRI images of a normal dog versus a 4-mo MR dog at end diastole and end systole. A and B: ED (A) and ES (B) views of the LV in a normal dog. Note the absence of an MR jet at end systole. The LV is cone shaped, and the left atrium is not enlarged. C and D: ED (C) and ES (D) views of the LV 4 mo after the induction of MR. There is remodeling of the LV, which is dilated and more spherical, and the left atrium is also enlarged. *MR jet at end systole.
There was a decrease in circumferential curvature in all MR dogs at both end diastole and end systole (Figs. 1C and 2C). LV R/T, a 3-D index of eccentricity, was increased significantly at end diastole (P = 0.014) in untreated MR dogs (Figs. 1D and 2D). This was clearly demonstrated by the results shown in Fig. 3, which shows an increase in diameter of the left atrium can be seen at end systole in Fig. 3D, confirming MR in these dogs. These changes in geometry were not altered by β1-RB.

**Isolated cardiomyocyte function.** Isolated cardiomyocyte function is shown in Table 2. There was an increase in LV cardiomyocyte CSA in MR dogs, which was normalized with β1-RB (P < 0.05). MR caused an elongation of LV cardiomyocytes (165 ± 4.0 μm), which was enhanced by β1-RB (171 ± 4.6 μm, P < 0.05) compared with normal cells (156 ± 3.3 μm).

LV cardiomyocyte fractional shortening was depressed to 3.73 ± 0.31% (P < 0.05) in MR and restored to normal (5.02 ± 0.26%) with β1-RB (4.73 ± 0.48%). MR also caused a significant decrease in intracellular Ca²⁺ transients compared with normal cells (P < 0.05), which was normalized by β1-RB.

**Responses of LV cardiomyocytes to Iso.** Responses of LV cardiomyocytes to Iso are shown in Fig. 4. To determine the β-receptor responsiveness of LV cardiomyocytes, cells from all three groups were isolated and exposed to the β-receptor agonist Iso (25 nM) in vitro. Results are presented as percent changes from baseline without Iso stimulation. Iso induced an ~50% increase in fractional shortening in normal and MR cardiomyocytes (Fig. 4A). However, cardiomyocytes from MR + β1-RB-treated hearts were hyperresponsive to Iso compared with normal and MR LV cardiomyocytes (>200%, P < 0.05).

Iso induced a 10% increase in Ca²⁺ transients in normal cardiomyocytes, which did not differ in MR (7.5 ± 4%) or β1-RB-treated (13 ± 3%) groups (Fig. 4B). The effect of β1-RB on the ECM. The effect of β1-RB on the ECM is shown in Fig. 5. In the present study, 4 mo of MR caused a significant decrease in endocardial collagen compared with normal dogs (2.25 ± 0.22% vs. 3.15 ± 0.24%, P = 0.02), and this remained significantly depressed in MR dogs treated with β1-RB (1.72 ± 0.25%, P < 0.01). Interstitial epicardial collagen was significantly decreased in MR versus normal dogs (2.18 ± 0.23% vs. 3.38 ± 0.25%, P = 0.008) but was partially preserved with β1-RB (2.73 ± 0.34%).

**DISCUSSION**

In the present study, we demonstrate that long-term administration of a β1-RB preserves cardiomyocyte function and markedly enhances the response to Iso but does not attenuate adverse cardiomyocyte or LV remodeling after 4 mo of MR. This may be attributed to the failure of β1-RB to preserve the interstitial collagen that connects individual cardiomyocytes, contributing to myocyte slippage, thereby causing further cardiomyocyte and LV chamber elongation.

Currently, there is no single or multiple drug therapy available for the treatment of chronic MR that prevents adverse LV remodeling and prolongs the time for surgical intervention. Various classes of drugs have been trialed in a canine model...
of MR, yet their efficacy in preventing the deleterious effects of volume overload is limited (10). Despite elevated levels of ANG II and angiotensin-converting enzyme in the MR dog, we have shown that neither angiotensin-converting enzyme inhibition nor ANG II type 1 receptor blockade improved LV remodeling and function or attenuated cardiomyocyte elongation (2, 11).

There is a strong impetus for the use of β-receptor blockers in the treatment of MR (15) given that there is increased sympathetic drive in both the human (8) and dog (4, 9, 17). Although initial sympathetic drive is a necessary compensatory mechanism in MR, long-term activation may have a cytotoxic effect on cardiomyocytes. Indeed, in the present study, β1-RB started immediately after MR induction and continued for 4 mo improved isolated cardiomyocyte fractional shortening. Furthermore, cardiomyocyte fractional shortening was markedly enhanced in response to Iso. This may be due to increased myofibrillar content, as previously reported by Carabello and coworkers (18). In addition, β1-RB improved Ca2+ transients, a finding that is consistent with studies in chronic dilated cardiomyopathy in humans (6) and in tropomodulin-overexpressing transgenic mice (12), where β1-RB restored Ca2+ handling and improved Ca2+ transients in cardiomyocytes from failing hearts. Interestingly, Iso-induced Ca2+ transients did not differ from normal in untreated and β1-RB-treated MR dogs. This suggests that post-β-receptor/Ca2+ signaling pathway(s) remain intact in MR. Despite these improvements in cardiomyocyte function, we did not find a concomitant decrease in cardiomyocyte remodeling. Cardiomyocytes isolated from MR dogs treated with β1-RB were longer than untreated MR and normal cells. It is likely that this may have contributed to the changes in LV remodeling.

After 4 mo of MR, there were significant increases in LVEDV/mass and 3-D LVED R/T and decreases in LVED and LVES circumferential curvature, which were not attenuated by β1-RB. Of interest is the finding that β1-RB caused a significant increase in LV length from the base to apex over MR alone. This increase could be due to a lower conscious heart rate, resulting in a longer diastolic filling time. However, we were unable to detect a significant decrease in heart rate at the time of death under general anesthesia. Nevertheless, peak positive and peak negative dP/dt were decreased in MR dogs treated with β1-RB, consistent with an effect of β1-RB. The increase in LVEDV in MR dogs treated with β1-RB further supports the finding of an increase in cardiomyocyte length. In effect, further elongation of the LV cardiomyocyte without an increase in CSA suggests that β1-RB causes more eccentric LV remodeling despite improvements in function. In terms of overall LV function, β1-RB normalized LVEDP pressure and cardiac output and did not appear to cause a worsening of the MR condition, as evidenced by improved survival, in albeit a small cohort of dogs. We observed the same survival trend in MR dogs treated for 4 wk with β1-RB (17).

Insight into the discordance between cardiomyocyte function and cardiomyocyte and LV chamber remodeling in the MR dog with β1-RB may be explained in part by our finding that the drug failed to prevent the loss of endocardial collagen. We (17) have previously reported that interstitial collagen decreases by 50% as early as 2 wk after MR induction. At 4 wk, β1-RB improved epicardial collagen but not endocardial collagen. The same finding was seen after 4 mo of MR in β1-RB-treated dogs. Taken together, it would appear that endocardial collagen loss is a persistent feature during the course of the volume overload of MR. The difference in ECM homeostasis between LV wall compartments with β1-RB may be reflective of the different types of hemodynamic stresses that are placed on the endocardium versus epicardium. Nevertheless, an improvement in epicardial matrix components does not prevent further eccentric remodeling of the LV, suggesting that the ECM of the entire wall is important in maintaining LV geometry.

In summary, β1-RB improved LV cardiomyocyte function and β-adrenergic receptor responsiveness despite further cell elongation. The failure to attenuate LV remodeling associated with MR could be due to a failure to improve ultrastructural changes in ECM organization. We speculate that a second therapy targeted at preventing matrix degradation will provide additional benefit to β1-RB in MR. Future studies will focus on elucidating the mechanisms responsible for maintaining the ECM and other scaffolding proteins that provide matrix support that may improve LV remodeling associated with the pure volume overload of isolated MR.

REFERENCES


