TRANSLATIONAL PHYSIOLOGY

PPAR-γ agonist rosiglitazone ameliorates ventricular dysfunction in experimental chronic mitral regurgitation

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PPAR-γ agonist rosiglitazone ameliorates ventricular dysfunction in experimental chronic mitral regurgitation. Am J Physiol Heart Circ Physiol 288: H77–H82, 2005. First published September 2, 2004; doi:10.1152/ajpheart.01246.2003.—Previously we reported that the beneficial effects of β-adrenergic blockade in chronic mitral regurgitation (MR) were in part due to induction of bradycardia, which obviously affects myocardial energy requirements. From this observation we hypothesized that part of the pathophysiology of MR may involve faulty energy substrate utilization, which in turn might lead to potentially harmful lipid accumulation as observed in other models of heart failure. To explore this hypothesis, we measured triglyceride accumulation in the myocardia of dogs with chronic MR and then attempted to enhance myocardial metabolism by chronic administration of the peroxisome proliferator-activated receptor (PPAR)-γ agonist rosiglitazone. Cardiac tissues were obtained from three groups of dogs that included control animals, dogs with MR for 3 mo without treatment, and dogs with MR for 6 mo that were treated with rosiglitazone (8 mg/day) for the last 3 mo of observation. Hemodynamics and contractile function (end-systolic stress-strain relationship, as measured by K index) were assessed at baseline, 3 mo of MR, and 6 mo of MR (3 mo of the treatment). Lipid accumulation in MR (as indicated by oil red O staining score and TLC analysis) was marked and showed an inverse correlation with the left ventricular (LV) contractility. LV contractility was significantly restored after PPAR therapy (K index: therapy, 3.01 ± 0.11*; 3 mo MR, 2.12 ± 0.34; baseline, 4.01 ± 0.29; ANOVA, P = 0.038; *P < 0.05 vs. 3 mo of MR). At the same time, therapy resulted in a marked reduction of intramyocyte lipid. We conclude that 1) chronic MR leads to intramyocyte myocardial lipid accumulation and contractile dysfunction, and 2) administration of the PPAR-γ agonist rosiglitazone ameliorates MR-induced LV dysfunction accompanied by a decline in lipid content.

valvular heart disease; function; lipid accumulation

β-ADRENERGIC RECEPTOR BLOCKERS provide striking long-term improvement in left ventricular (LV) function and mortality from human chronic heart failure (8, 10). The benefits of β-adrenergic receptor blockers are probably in part a class effect (1) that includes reducing mortality in patients who are already receiving angiotensin-converting enzyme inhibitors (16). Although the exact mechanisms by which β-blockers improve survival in patients with heart failure are not yet established, we found that β-blockers ameliorate LV contractile dysfunction in experimental chronic mitral regurgitation (MR) by restoring cellular contractile elements (27). However, this improvement did not occur when bradycardia was prevented by atrial pacing (17). These findings suggested that the LV dysfunction that develops in experimental chronic MR is at least in part heart rate dependent; this notion is supported by clinical heart failure trials in which patients with the fastest pretreatment heart rates had the most improvement when β-blockers were administered (14). How bradycardia is salutary in the failing heart remains unclear, but heart rate dependency could imply a problem with energy utilization whereby slowed heart rate beneficially alters cardiac metabolism, which is known to be abnormal in both human and experimental MR (6, 21, 32) and in heart failure in general.

During heart failure, the myocardium switches from its normally preferred fuel of free fatty acids (FFAs) to glucose. Accordingly there is downregulation of the genes that control FFA oxidation (7, 9, 20, 24, 30), whereas gene expression regulating glucose oxidation enzymes is upregulated (22). This switch may be related to the elevated catecholamine levels that pervade heart failure in general and human and experimental MR specifically (15, 27). Elevated catecholamine levels during heart failure activate lipoprotein lipase, promote lipolysis, and increase circulating FFA levels. When the FFA supply exceeds the heart’s capacity to oxidize fatty acids, a mismatch develops between myocardial fatty acid uptake and oxidation that may lead to myocardial lipid accumulation. Such accumulation results in a constellation of metabolic derangements culminating in programmed cell death in rodent models (3, 33) and human diseases, including idiopathic dilated cardiomyopathy (26), diabetic cardiomyopathy (33), and inherited defects in the mitochondrial fatty acid oxidation pathway (11). These findings led us to postulate that lipid accumulation might occur in MR and that stimulation of peroxisome proliferator-activated receptors (PPARs), which help regulate lipid metabolism, might be therapeutic. We performed the following studies to test those hypotheses.

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Methods

Study design. As shown in Fig. 1, six previously unreported adult male mongrel dogs weighing 28.7 ± 3.8 kg were studied longitudinally from baseline, when they were normal, through 6 mo of chronic MR. At 3 mo of MR, all animals were given 8 mg of oral rosiglitazone daily. The animals were then observed for another 3 mo. Because all dogs were studied longitudinally from when they were normal, they served as their own controls. At the 6-mo study, the animals were humanely euthanized while under deep anesthesia, and cardiac tissues were stored at 70°C for histological and biochemical studies. Because lipid staining was performed only at death, four normal dogs and four dogs with identically severe MR to the experimental group served as additional controls.

The experimental protocol was approved by the Animal Subjects Committee of Baylor College of Medicine and the Houston Veterans Affairs Medical Center. All animals in this study received humane care in compliance with the animal use principles of the American Physiological Society and the Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, Revised 1985).

Catheterization. All studies were performed while dogs were under light anesthesia produced by the combination of fentanyl-droperidol and inhaled nitrous oxide (3:1 nitrous oxide-to-oxygen ratio). This light anesthesia produced by the combination of fentanyl-droperidol and inhaled nitrous oxide (3:1 nitrous oxide-to-oxygen ratio).

Creation of MR. MR was created using a technique we have previously described (2, 12, 17, 18, 27, 28). Briefly, after baseline measurements were made in the initial study, a urologic calculus-retrieving forceps was advanced to mitral valve apparatus via a 7-Fr sheath that was introduced into the left ventricle retrograde through the right carotid artery. The forceps was used to rupture chordae tendineae and thereby create MR. When PCWP increased to 20 mmHg and forward stroke volume was reduced to 50% of its baseline value, a ventriculogram was taken to confirm angiographically that severe MR had been created and to calculate the regurgitant fraction (RF) to quantify the amount of MR.

This model of MR is characterized as follows: 1) RF is unchanged over the course of the MR; 2) LV end-diastolic volume increases chronically and increasingly after MR creation; 3) LV mass-to-body weight ratio increases by ≥45% at 3 mo and plateaus after 6 mo; and 4) end-systolic stiffness (K index) is severely depressed at 3 mo, and this depression persists without treatment.

Assessment of in vivo LV contractile function. In vivo contractile function was assessed in this experiment using the end-systolic stiffness constant (K index), which is derived from end-systolic stress-strain relationship analysis (19). Because strain is a dimensionless property, this index is independent of LV chamber size. It has correlated well with changes in contractile function of isolated cardiac myocytes in our previous studies in which sarcomere contractility served as an independent standard of contractile function (17, 18, 27, 28).

At all times, studies were made during acute β-blockade induced by the infusion of esmolol given intravenously with a loading dose of 0.5 mg·kg⁻¹·min⁻¹, followed by a constant infusion of 0.3 mg·kg⁻¹·min⁻¹. Acute β-blockade was used to prevent adrenergic reflexes from confounding measurements of intrinsic contractility and to provide consistency of β-adrenergic effects at three experimental observations (18).

Calculations. LV volumes and LV mass were determined by the area-length method. This method was previously validated as providing an accurate measurement of LV volume and mass in dogs with MR in studies in our laboratory (2, 12). The RF was calculated as RF (in %) = [(SVa - SVf)/SVa] × 100, where SVa is angiographically measured stroke volume (end-diastolic volume — end-systolic volume), and SVf is forward stroke volume (actual cardiac output measured by thermodilution and heart rate). The K index was determined by fitting end-systolic stress and end-systolic wall-thickness

Table 1. Comparison of rosiglitazone treatment with and without mitral regurgitation in dogs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Heart rate, beats/min</th>
<th>Ejection fraction, %</th>
<th>Regurgitant fraction, %</th>
<th>End-diastolic volume-to-body weight ratio, ml/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline, No MR</td>
<td>84 ± 4</td>
<td>56.4 ± 3.9</td>
<td>0</td>
<td>2.60 ± 0.27</td>
</tr>
<tr>
<td>3 Mo of MR</td>
<td>96 ± 3</td>
<td>55.6 ± 3.6</td>
<td>70.9 ± 7.1</td>
<td>5.73 ± 1.03*</td>
</tr>
<tr>
<td>Rosiglitazone + 6 Mo of MR</td>
<td>84 ± 4</td>
<td>54.5 ± 4.0</td>
<td>66.2 ± 6.1</td>
<td>5.00 ± 0.95</td>
</tr>
<tr>
<td>P Value, ANOVA</td>
<td>0.055</td>
<td>0.945</td>
<td>0.755</td>
<td>0.494</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 6 dogs. MR, mitral regurgitation. *P < 0.05 vs. baseline (Newman-Keuls test).
data to the curvilinear equation as \( \sigma = C e^{\kappa \ln(1/H)} \), where \( \sigma \) is end-systolic wall stress, \( C \) is a constant, \( \kappa \) is the end-systolic stiffness constant, and \( \ln(1/H) \) is the natural logarithm of the reciprocal of wall thickness. A detailed discussion of the mathematical derivation of this index was previously described (19).

Histological examination for lipid accumulation. Snap-frozen LV tissues were sectioned and stained with oil red O. Lipid content was estimated from oil red O staining using a semiquantitative score (from 1 to 4) graded by two blinded observers (interobserver correlation, 0.91). For comparison, four control dogs and four dogs that had MR for 3 mo without therapy were killed for this study in addition to the rosiglitazone-treated MR dogs.

Thin-layer chromatography. TLC was performed as previously described (4). Briefly, lipids were extracted from the myocardial samples after homogenization in Folch reagent (2:1 chloroform-methanol ratio). The homogenate was then centrifuged, and the tissue pellet was separated from the liquid phase. The liquid phase was again centrifuged into lipid and aqueous phases. The lipid phase was withdrawn and prepared for TLC. TLC results were quantified using Kodak 1D software, which expresses the net intensity of the TLC brightness bands as the sum of the pixel intensity of the band with the background pixel intensity subtracted out. Intensity is expressed as arbitrary units.

Statistics. All data are expressed as means ± SE. Comparisons made regarding given parameters during this study represented multiple repeated comparisons. Therefore, we used two-way ANOVA to test statistical differences, followed by a Newman-Keuls test if ANOVA testing demonstrated that significant differences were present. Comparison of oil red O staining among the three groups was made by one-way ANOVA, followed by a Newman-Keuls test if ANOVA testing demonstrated that significant differences were present. A \( P < 0.05 \) was considered statistically significant.

RESULTS

Hemodynamics and LV contractile function. Heart rate (Table 1) tended to increase after MR creation and decrease to baseline during rosiglitazone therapy. Ejection fraction did not change throughout this study. RF also did not vary significantly during the study and was consistently >60%. As shown in Table 1, LV volume normalized for body weight significantly increased 3 mo after creation of MR and did not change significantly thereafter. As shown in Fig. 2, the ratio of angiographically calculated LV mass to body weight significantly increased at 3 mo of MR and remained elevated throughout the study. Figure 3 demonstrates PCWP, which significantly increased at 3 mo of MR and decreased significantly with rosiglitazone therapy. Figure 4 demonstrates end-systolic elastance normalized for LV mass. Peak elastance decreased after 3 mo of MR and then increased to values that were not different from baseline values after rosiglitazone therapy. Figure 5 shows the change in the K index, which is a load-independent contractility index. The K index was depressed...
after 3 mo of MR and increased significantly with rosiglitazone therapy to values that were significantly greater than for untreated MR and not different from baseline.

Intramyocyte triglycerides. Representative images of oil red O staining are shown in Fig. 6. Intense fat staining is obvious at 3 mo of MR. However, fat staining markedly diminished with rosiglitazone treatment. Figure 7 demonstrates semiquantitative oil red O staining score data. With chronic MR, we observed significantly increased lipid staining in the myocardium. However, lipid staining was significantly decreased with rosiglitazone treatment to levels that were not different from controls. Moreover, there was a good inverse correlation between LV contractility expressed by the K index and the oil red O score in the terminal study as shown in Fig. 8. TLC results for triglycerides are demonstrated in Fig. 9.

DISCUSSION

There are two major findings of this study. First, experimental chronic MR led to substantial intramyocyte lipid accumulation and contractile dysfunction. Second, therapy with the PPAR-γ agonist rosiglitazone improved LV contractile function, which correlated well with a decrease in lipid content.

Lipid accumulation and MR. Virchow (29) first reported intramyocyte lipid accumulation in patients with congestive heart failure in 1858, when he referred to it as “lipid atrophy” of the myocardium. Since then, several studies have confirmed that lipid (triglyceride) accumulation develops during cardiomyopathy and heart failure in animal models of diabetic cardiomyopathy (33), transgenic mice with disrupted cardiac long-chain-FFA-CoA synthetase (a key enzyme in β-oxidation of FFA; Ref. 3), and obese rats (31). These reports proposed lipid-induced programmed cell death (lipoapoptosis) as a possible mechanism of lipotoxic cardiac injury. However, these data were accrued from animals with primary metabolic abnormalities. The present study is unique in implicating lipid intoxication in a hemodynamic model of myocardial dysfunction. Taken together, these data suggest that myocyte lipid accumulation plays a role in heart failure of many etiologies. The mechanisms of lipid accumulation and lipotoxicity still need to be elucidated.

Effects of rosiglitazone on LV function and myocardial lipid levels in MR. Rosiglitazone, a PPAR-γ agonist, significantly lowered myocardial lipid levels and simultaneously improved...
LV function caused by volume overload in this study, which raises the possibility of a cause-and-effect relationship between intramyocellular lipid accumulation and LV dysfunction. This notion is further supported by the good correlation between lipid content determined by oil red O staining and LV contractility. The relationship between lipid content and function is also supported by findings in a previous study (33), which shows that another thiazolidinedione, troglitazone, reduced lipid content in addition to decreasing plasma FFA levels and prevented LV dysfunction as seen in an obese diabetic rat model. That report (33) also showed that troglitazone prevented apoptosis and elevation in levels of the potentially toxic intramyocellular lipid accumulation and LV dysfunction. This raises the possibility of a cause-and-effect relationship between LV function caused by volume overload in this study, which suggests that cardiac dysfunction is caused by lipoproteinosis and is prevented by reducing cardiac lipids. These findings, including ours, might provide important insight into the role of disrupted myocardial lipid metabolism in the pathogenesis of LV dysfunction in various types of heart failure.

Limitations. The data obtained in this study are descriptive and are limited to an experimental model of MR; therefore, applicability to humans is uncertain. This study lacks a 6-mo sham group (with no MR creation) in which animals are treated with rosiglitazone for 3 mo. However, rosiglitazone does not have direct inotropic effects in normal subjects. Therefore, the improvement in function observed with thiazolidinedione therapy is unlikely to be due to a direct inotropic effect of rosiglitazone. In conclusion, our data suggest that the ventricular dysfunction of MR might be at least partly due to PPAR-mediated metabolic pathways that result in intramyocellular lipid accumulation. Rosiglitazone therapy results in reduced lipid content together with improved ventricular function. Although our study does not prove a cause-and-effect relationship between these two findings, the possibility obviously exists and requires further study.

REFERENCES


