Pioglitazone, a Peroxisome Proliferator–Activated Receptor-γ Agonist, Attenuates Left Ventricular Remodeling and Failure After Experimental Myocardial Infarction

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Background—Peroxisome proliferator–activated receptor-γ activators have recently been implicated as regulators of cellular proliferation and inflammatory response such as cytokine expression. Because proinflammatory cytokines play a critical role in left ventricular (LV) remodeling after myocardial infarction (MI), we examined the effects of pioglitazone treatment in an experimental model of chronic heart failure.

Methods and Results—Mice with extensive anterior MI were treated with placebo or pioglitazone (3 mg · kg⁻¹ · d⁻¹) as a dietary supplement for 4 weeks starting 6 hours after surgery. Infarct size and glucose levels were similar among all groups. LV cavity dilatation and dysfunction by echocardiography were significantly attenuated in MI mice given pioglitazone. LV end-diastolic pressure was increased in MI mice and was significantly reduced by pioglitazone treatment. Pioglitazone partially normalized LV dP/dt_max and dP/dt_min, indices of LV contractile function, which were significantly reduced in MI mice. Improvement of LV function by pioglitazone was accompanied by a decrease in myocyte hypertrophy and interstitial fibrosis and a reduced expression of tumor necrosis factor-α, transforming growth factor-β, and monocyte chemoattractant protein-1 genes in the noninfarcted LV from MI mice. LV inducible nitric oxide synthase and gelatinase B protein levels were increased in MI and were not altered by pioglitazone treatment.

Conclusions—Pioglitazone improved LV remodeling and function in mice with post-MI heart failure. This effect was associated with an attenuated LV expression of inflammatory cytokines and chemokines. Peroxisome proliferator–activated receptor-γ ligands have promise as preventive and therapeutic agents against heart failure. (Circulation. 2002; 106:3126-3132.)

Key Words: myocardial infarction • heart failure • myocardium • remodeling • receptors

Myocardial infarction (MI) frequently produces left ventricular (LV) dilatation associated with myocyte hypertrophy and interstitial fibrosis of the noninfarcted myocardium. These changes in LV geometry, referred to as remodeling, contribute to the development of depressed cardiac function.¹ Currently, ACE inhibitors have been shown to attenuate LV remodeling and decrease the mortality and morbidity of patients with MI and heart failure.² However, they may not sufficiently antagonize disease progression and thereby reduce the risk of major cardiac events in the patients.² Accordingly, it is of critical importance to develop therapeutic strategies that will effectively inhibit the development of LV remodeling and failure after MI.

Peroxisome proliferator–activated receptors (PPARs) are transcription factors belonging to the nuclear receptor superfamily that bind to specific response elements, called PPAR-responsive elements, in target gene promoters.³ Evidence is emerging that the PPAR regulatory pathway plays a critical role in the regulation of a variety of biological processes within the cardiovascular system.⁴ Synthetic antidiabetic thiazolidinediones such as pioglitazone and rosiglitazone are identified as ligands for PPARs. PPAR activators have been shown to suppress the expression of proinflammatory cytokine genes such as tumor necrosis factor-α (TNF-α) and interleukin (IL)-1β and inducible nitric oxide synthase (iNOS) and matrix metalloproteinase (MMP)-9.⁵ Recent studies have shown that PPAR activators such as rosiglitazone inhibit TNF-α gene expression in cultured cardiac myocytes.⁶ Furthermore, proinflammatory cytokines, including TNF-α and IL-1β, have been demonstrated to be attributable to the development of heart failure.⁷ In addition, the increase in iNOS and MMP-9 is also implicated in the pathophysiology of heart failure.⁸,⁹ On the basis of these results, we hypothesized that PPAR activators might improve LV remodeling and failure by modulating the myocardial expression of inflammatory genes.
Therefore, the purpose of this study was to determine whether chronic administration of pioglitazone attenuates the progressive LV chamber dilatation and dysfunction in a murine model of post-MI heart failure. We also determined whether pioglitazone alters the gene expression of cytokines within the LV.

Methods

Experimental Design
The study was approved by our Institutional Animal Research Committee and conformed to the animal care guidelines of the American Physiological Society. MI was created in male CD-1 mice, 5 to 8 weeks old and 25 to 35 g body weight, by ligation of the left coronary artery. Our previous studies have shown that most mice have infarction of >40% of the LV and impaired systolic function. 

Pioglitazone (3 mg · kg⁻¹ · d⁻¹) was administered as a dietary supplement for 4 weeks. To adjust the daily dose of the drug, the body weight was measured twice a week. Our preliminary studies demonstrated that plasma pioglitazone concentration in the animals treated at this dose was 250±58 ng/mL (mean±SEM, n=8), within the range achieved in humans after oral administration of its clinical dosage (30 mg/d). 

Echocardiographic and Hemodynamic Measurements
After 4 weeks, echocardiographic studies were performed under light anesthesia with trichlorethanol/amylyne hydrate (Avertin; 2.5% wt/vol, 8 µL/g IP) and spontaneous respiration. A 2D parasternal long-axis view of the LV was obtained at the level of the papillary muscles. In general, the best views were obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was then gently moved cephalad or caudal and angulated until desirable images were obtained. After it had been ensured that the imaging was on axis (based on roundness of the LV cavity), 2D targeted M-mode tracings were recorded at a paper speed of 50 mm/s. Under the same anesthesia with Avertin, a 1.4F micromanometer-tipped catheter (Millar Instruments) was inserted into the right carotid artery and then advanced into the LV to measure LV pressures. Two investigators (S.H. and N.S.), who were not informed of the experimental groups, performed in vivo LV function studies.

Interpretive Variability and Reproducibility of M-Mode Echocardiographic Measurements
Interpretive variability of M-mode measurements was determined according to the methods described previously. To determine intraobserver variability, one observer (S.H.) remeasured LV dimensions on a second occasion in 15 sham and 15 MI animals. To determine interobserver variability, LV dimension data were determined by 2 individuals (S.H. and N.S.) using the same 3 beats in 15 sham and 15 MI animals. To determine reproducibility, studies were repeated on separate days in 10 sham and 10 MI animals. All data were analyzed in a blinded fashion.

Tissue Preparation and Morphometric Analysis
After in vivo hemodynamic studies, the heart was excised and dissected into the right and left ventricles, including the septum. The LV was cut into 3 transverse sections: apex, middle ring, and base. From the middle ring, 5-µm sections were cut and stained with Masson’s trichrome. The boundary lengths of the infarcted and noninfarcted endocardial and epicardial surfaces were traced with a planimeter digital image analyzer. Infarct size (fraction of the infarcted LV) was calculated as the average of all slices and expressed as the percentage of circumference length. Myocyte cross-sectional area and collagen volume fraction were determined by quantitative morphometry of tissue sections from the mid LV.

Gene Expression
The myocardial tissues with MI were carefully dissected into 2 parts, consisting of the infarcted LV with the peri-infarct rim (a 0.5- to 1-mm rim of normal-appearing tissue) and the remaining noninfarcted (remote) LV. In all subsequent assays, the comparison was made between noninfarcted LV from MI and control LV from sham. Total RNA was isolated from noninfarcted LV myocardium, and an RNase protection assay (PharMingen) with 15 µg of total RNA was performed to determine the expression level of genes, including RANTES (regulated on activation, normal T cell expressed and secreted), IL-1β, IL-6, TNF-α, transforming growth factor β (TGF-β), and monocyte chemoattractant protein-1 (MCP-1) as described previously. The value of each hybridized probe was normalized to that of GAPDH in each template set as an internal control.

Western Blot Analysis
Crude protein extracts (20 µg) were subjected to 7.5% SDS-PAGE and transferred to nitrocellulose membranes. iNOS protein levels were detected by use of a specific antibody against mouse iNOS and were visualized by enhanced chemiluminescence. MMP-9 protein levels were also quantified by use of a specific antibody against recombinant mouse MMP-9.

Gelatin Zymography
Myocardial gelatinase (MMP-2 and MMP-9) activities were measured by gelatin zymography as described previously. Within a given experiment, the densitometric values were normalized by use of standards run concurrently within the same gel, and the value for each MI or MI plus pioglitazone (MI+Pio) was calculated as a ratio of that from sham samples.

Plasma Biochemical Measurement
Before euthanasia, venous blood samples (1 mL) were collected for determination of plasma glucose and AST.

Statistical Analysis
Data are expressed as mean±SEM. Comparison of survival was performed by Kaplan-Meier analysis. For multiple-group comparisons, ANOVA followed by Bonferroni’s t test was performed. A value of P<0.05 was considered statistically significant.

Results

Mortality and Serum Chemistry
The survival rate of MI mice at 6 hours after ligation was 92% (48/52). They were divided into MI (n=27) or MI+Pio (n=21). The survival rate up to 4 weeks was comparable between MI and MI+Pio (67% versus 76%, P=0.59). Death was suspected to be attributable to heart failure and/or arrhythmia. One MI+Pio (5%) and 3 MI (11%) mice died of LV rupture (P=NS).

After 4 weeks, plasma glucose level was not lowered by pioglitazone. Serum AST used to assess potential hepatic toxicity was not increased by pioglitazone (55±6 versus 48±4 IU/L, P=NS).

Interpretive Variability and Reproducibility
Intraobserver and interobserver variability and reproducibility data for LV dimensions, fractional shortening, and wall thickness, assessed in sham and MI animals, are summarized in Table 1. The intraobserver and interobserver variabilities for LV dimensions and fractional shortening were excellent. These measurements made in the same animals on separate days were highly reproducible.
Echocardiography and Hemodynamics

Echocardiography demonstrated that pioglitazone significantly attenuated LV dilatation and dysfunction caused by MI after 28 days (Figure 1 and Table 2). In comparison with sham, MI animals showed a significant increase in the thickness of the noninfarcted region, which was attenuated by pioglitazone.

Heart rate was slightly higher in MI than in sham ($P=0.06$), and pioglitazone tended to inhibit this increase, which, however, did not reach statistical significance ($P=0.08$). Mean aortic blood pressure was lower in the MI groups, but there was no significant difference between MI and MI+Pio. LV end-diastolic pressure was increased in MI; this increase was attenuated in MI+Pio. Pioglitazone partially normalized LV $dP/dt_{max}$ and $dP/dt_{min}$, which were significantly reduced in MI.

Organ Weights and Histomorphometry

Body weight values at baseline and 28 days after operation were similar among the 3 groups (Table 3). The ratio of right ventricular weight to body weight was increased in the MI group, and pioglitazone attenuated this increase. Coinciding with an increased LV end-diastolic pressure, the ratio of lung weight to body weight was increased in the MI group; this increase was also attenuated by pioglitazone. The prevalence of pleural effusion was significantly lower in MI+Pio than MI.

Infarct size determined 28 days after operation was comparable (59±2% versus 56±2%; $P=0.31$) between MI (n=9) and MI+Pio (n=8). It was also comparable (63±2% versus 59±3%; $P=0.20$) 3 days after coronary ligation, at which time LV cavity size was similar between MI (n=8) and MI+Pio (n=8). Myocyte cross-sectional area was increased in MI; this increase was significantly attenuated by pioglitaza-
TABLE 2. Echocardiographic and Hemodynamic Data

<table>
<thead>
<tr>
<th></th>
<th>Sham (n = 16)</th>
<th>MI (n = 16)</th>
<th>MI + Pio (n = 16)</th>
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<tbody>
<tr>
<td>Echocardiographic data</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>LV LVEDD, mm</td>
<td>3.93 ± 0.07</td>
<td>5.99 ± 0.05*</td>
<td>5.37 ± 0.11**††</td>
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<tr>
<td>LV ESVD, mm</td>
<td>2.49 ± 0.06</td>
<td>5.31 ± 0.05*</td>
<td>4.59 ± 0.12**††</td>
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<tr>
<td>Fractional shortening, %</td>
<td>36.9 ± 0.9</td>
<td>11.3 ± 0.3**</td>
<td>14.8 ± 0.7**††</td>
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<tr>
<td>Wall thickness, mm</td>
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<td></td>
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<tr>
<td>Infarct</td>
<td>...</td>
<td>0.43 ± 0.01</td>
<td>0.46 ± 0.01</td>
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<tr>
<td>Noninfarct</td>
<td>0.77 ± 0.02</td>
<td>1.01 ± 0.03**</td>
<td>0.94 ± 0.01**††</td>
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<td>Hemodynamic data</td>
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<tr>
<td>Heart rate, bpm</td>
<td>458 ± 10</td>
<td>490 ± 17</td>
<td>460 ± 8</td>
</tr>
<tr>
<td>Mean AoP, mm Hg</td>
<td>77 ± 2</td>
<td>69 ± 3*</td>
<td>69 ± 3*</td>
</tr>
<tr>
<td>LV LVEDP, mm Hg</td>
<td>4.1 ± 2.7</td>
<td>20.7 ± 1.4**</td>
<td>11.0 ± 1.6**††</td>
</tr>
<tr>
<td>LVdP/dtmax, mm Hg/s</td>
<td>6465 ± 278</td>
<td>4023 ± 278</td>
<td>5124 ± 339**††</td>
</tr>
<tr>
<td>LVdP/dtmin, mm Hg/s</td>
<td>4972 ± 212</td>
<td>3091 ± 180**</td>
<td>3673 ± 210**††</td>
</tr>
</tbody>
</table>

Data are mean ± SEM. n indicates number of animals studied; AoP, aortic pressure; EDP, end-diastolic pressure. Other abbreviations as in Table 1. *P < 0.05, **P < 0.01 vs Sham; †P < 0.05, ††P < 0.01 vs MI.

zone. These results are concordant with LV wall thickness data obtained from echocardiography (Table 2). Collagen volume fraction was also increased in MI; this increase was inhibited by pioglitazone.

Myocardial Expression of Cytokines, iNOS, and MMP-9

Gene expression of proinflammatory cytokines, including TNF-α, IL-1β and IL-6, and TGF-β1, was significantly increased in MI at day 28. An increase in TNF-α and TGF-β1 gene expression was significantly attenuated by pioglitazone, whereas other cytokine genes were not affected (Figure 2A and B). Chemokine mRNA levels, including RANTES and MCP-1, were also increased in MI, and increased MCP-1 expression was inhibited by pioglitazone.

The LV iNOS protein level was significantly increased in MI at day 28, and this increase was similarly observed in pioglitazone-treated animals (Figure 2C and D).

Gelatinolytic activity of MMP-9 was increased in MI 3 days after ligation, which, however, was not altered by pioglitazone (Figure 3A). Similarly, MMP-9 protein level was increased in MI, and this increase was not altered by pioglitazone (Figure 3B). In addition, pioglitazone did not affect MMP-2 zymographic activity in MI.

Discussion

The major novel finding of this study was that chronic pioglitazone treatment improved LV systolic function in mice with heart failure after experimental MI. It attenuated remodeling and reduced chamber dilatation and hypertrophy and fibrosis of the noninfarcted myocardium. These beneficial effects were independent of glucose lowering and were associated with an attenuated LV expression of TNF-α, TGF-β, and MCP-1 genes.

Previous studies have shown that myocardial ischemia-reperfusion injury can be prevented by PPAR activators. The present study extends the previous observations by demonstrating that they can inhibit not only ischemia-reperfusion injury but also heart failure. Our results are also consistent with a recent study showing that PPAR activators inhibited myocyte hypertrophy induced by mechanical stretch and by angiotensin II or phenylephrine.

The beneficial effects of pioglitazone were not a result of its MI size–sparing effect, because the administration was started 6 hours after coronary ligation. In fact, the infarct size was comparable between MI and MI + Pio mice (Table 3). Furthermore, its effects might not be attributable to the effects on hemodynamics, because blood pressure and heart rate were not altered (Table 2).

Recent experimental and clinical studies have suggested that TNF-α plays an important role in the development of LV remodeling and failure. The present study demonstrated that TNF-α gene expression was increased in MI animals, and this increase was significantly attenuated by pioglitazone (Figure 2). In addition, in vitro studies have shown that the PPAR-γ activators troglitazone and rosiglitazone inhibit the production of TNF-α from cardiac myocytes exposed to lipopolysaccharide. Therefore, one proposed mechanism of pioglitazone for reverse remodeling is related to the attenuation of TNF-α gene expression after MI. Pioglitazone significantly reduced collagen volume fraction without affecting myocardial MMPs. However, it significantly attenuated TGF-β gene expression (Figure 2), which might explain the decrease of collagen volume fraction in MI + Pio.

Interestingly, pioglitazone significantly reduced gene expression of MCP-1 in post-MI hearts (Figure 2). This is consistent with recent studies showing that PPAR activators decreased MCP-1 in vascular endothelial cells, colonic epithelial cells, and myocardium. Conversely, it disagrees with the previous studies, in which PPAR-γ agonists could not exert any significant alterations. The reasons for these discrepant results might be related to the differences in the...
types of tissue examined. Although the functional role of MCP-1 and its significance in myocardial failure remain unexplained, its inhibition could reduce the infiltration of monocytes and prevent the progression of heart failure.22 These data provide another potential mechanism by which pioglitazone protects against post-MI remodeling. However, the present study does not provide a direct proof of a cause-and-effect relation, and further investigation is clearly needed. In addition, the results of cytokine gene expression should be interpreted with caution, because there was substantial variability in our ribonuclease protection assay.

PPARγ activators have been reported to suppress the expression of iNOS.5 They also inhibit the expression of MMPs,5,23 iNOS and MMPs are shown to be involved in the progression of LV remodeling and failure.8,9 However, the present results could not demonstrate any significant inhibition by pioglitazone against increased iNOS and MMP-9 proteins in post-MI mice (Figures 2 and 3). Therefore, even though the reasons why pioglitazone exerts differential effects on various genes are not clear, the contribution of iNOS and MMP-9 might be minor.

The plasma concentrations of pioglitazone in our animals are within the range achieved after oral administration in clinical dosages (30 mg/d).11 Furthermore, these concentrations are comparable to those that decrease cytokine expression in vitro (1 to 10 μmol/L).6 Therefore, the present results imply that clinically relevant doses of pioglitazone can attenuate LV remodeling after MI. However, we should be cautious in extending our results to the clinical setting. Pioglitazone and other thiazolidinediones are known to cause fluid retention.24 Even though fluid retention is reduced, not exacerbated, in MI animals treated by pioglitazone in the present study, thiazolidinediones are contraindicated in patients with heart failure at the present time and should be used with extreme caution in post-MI patients. In fact, the usefulness of PPAR activators for the morbidity and mortality has not been confirmed in these patients. Therefore, large-scale clinical trials specifically designed to assess this issue are warranted. It should be also determined whether pioglitazone exerts the same beneficial effects on LV remodeling in diabetic animals.

There are several methodological issues to be acknowledged in this study. First, even though in vivo assessment of LV function with echocardiography is feasible and reproducible in the mouse, it might still be difficult to interpret the
indexes in dilated post-MI LV. Our validation study has shown that the variability of our echocardiographic measurements is small and generally resembles those reported previously (Table 1). Therefore, our technique could allow noninvasive assessment of LV structure even in mice with large MI. Importantly, even though the differences between MI and MI+Pio mice were small (Table 2), they were considered to be meaningful. Second, the LV dP/dt max values in the present study (6465 mm Hg/s) were lower than those (12 000 mm Hg/s) reported in previous studies, in which they were measured at a heart rate of 600 bpm in mice anesthetized with α-chloralose and urethane, but were within the ranges (6000 to 7000 mm Hg/s) obtained from normal mice with the use of Avertin. Therefore, these disparate results might be explained by the differences in anesthetic regimens and experimental conditions. We need to acknowledge that the dP/dt max values in anesthetized mice are lower than those obtained from conscious mice (≈16 000 mm Hg/s), indicating that our hemodynamic values might not reflect the physiological conditions. Third, LV weight data were not parallel to myocyte size in the noninfarcted area. At day 28, MI mice displayed marked wall thinning and sometimes aneurysm at the infarcted region. The hypertrophic changes in the noninfarcted LV might be canceled out by the decrease in tissue weight at the infarcted area.

There are several limitations to this study. It is not clear whether in vivo cardioprotection by pioglitazone is shared by other PPARγ agonists and also by PPARα agonists. More PPAR agonists with different structures should be tested. Specifically, rosiglitazone is the most potent thiazolidinedione for the treatment of diabetes mellitus and has been shown to be cardioprotective against ischemia-reperfusion injury. Further studies are necessary to clarify the molecular mechanisms by which PPARγ agonists inhibit cytokine gene expression. We administered pioglitazone into the animals 6 hours after ligation. The amount of chow diet that animals received was smaller during this early phase, indicating that its effects might have been even greater if it had been administered at the time of coronary ligation or before ligation.

In conclusion, pioglitazone, a PPARγ activator, inhibits the development of LV remodeling and failure after MI. These effects were associated with the attenuation of an increase of myocardial cytokine/chemokine expression. These drugs might be beneficial in postinfarction patients irrespective of their plasma glucose levels.

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References

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