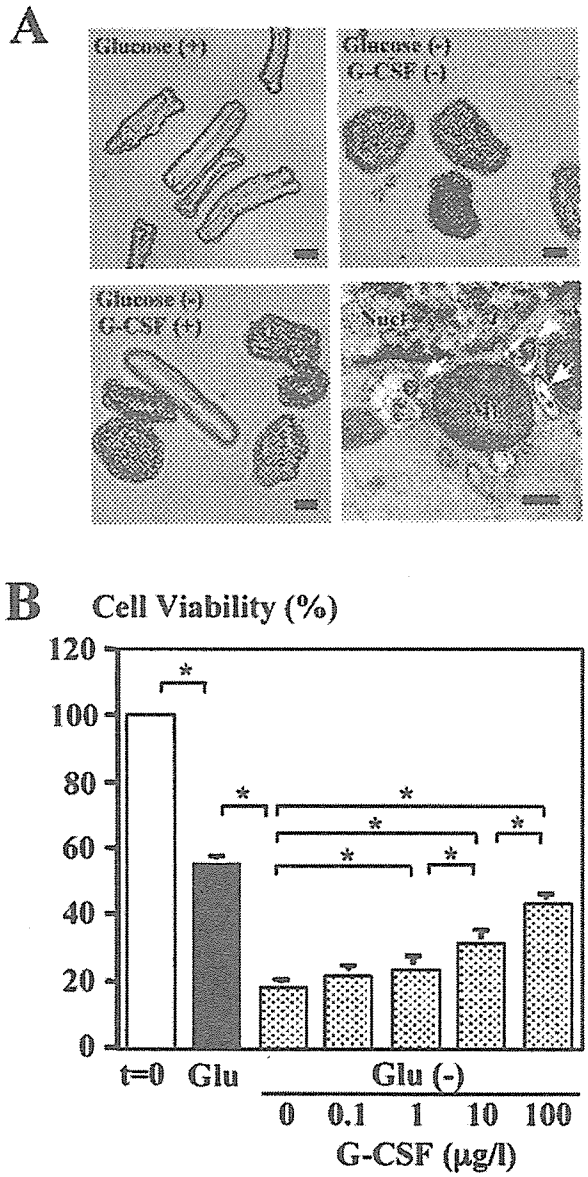

Figure 11. *In vitro* cell death assay. **A:** Light microphotographs of adult cardiomyocytes incubated for 96 hours in glucose-containing media (**top left**), glucose-depleted media without G-CSF (**top right**), and glucose-depleted media with 10 μg/L G-CSF (**bottom left**) and electron microphotograph showing autophagic vacuoles in a glucose-depleted cardiomyocyte (**bottom right**). Nucl, nucleus; Mt, mitochondria. **White arrows** indicate autophagic vacuoles. **B:** Survival rate of cultured cardiomyocytes after 96 hours of incubation. Glu, glucose. $^*P < 0.05$. Scale bars: 10 μm (**top**); 0.5 μm (**bottom**).

approach certainly underestimated the regenerative effect of G-CSF because only ~3 × 10^5 bone marrow mononuclear cells were labeled with DiI and the others were not. Therefore, the negative finding does not deny bone marrow-derived myocardial regeneration. However, we would be able to say that the incidence may not be enough to explain the beneficial effects of G-CSF. In contrast, using an *in vitro* model, we have demonstrated that G-CSF directly protected cultured cardiomyocytes from glucose depletion-induced death. Thus, the beneficial effects of G-CSF may be associated with the cardiomyocyte protection against autophagic degeneration and death of cardiomyocytes rather than cardiac cell regeneration.

The G-CSF treatment induced a further increase in the size (transverse diameter) of cardiomyocytes, compared with the untreated UM-X7.1 where cardiomyocytes were already hypertrophied. Hypertrophic growth of the myocardium is thought to preserve pump function, although prolongation of the hypertrophic state is a leading predictor of arrhythmias, sudden death, and heart failure.[37,38] However, not all forms of cardiac hypertrophy are necessarily pathological, as athletic conditioning can stimulate heart growth without deleterious consequence, ie, a physiological type of cardiac hypertrophy.[39] The observed G-CSF-induced hypertrophy cannot be simply explained as a compensatory response because the hypertrophic reaction was more pronounced in G-CSF-treated hearts, which showed less severe heart failure than was seen in the untreated hearts. In the present study, moreover, we detected increased activation of both Akt and Stat3, ie, increased levels of phospho-Akt and phospho-Stat3, in the G-CSF-treated hearts. G-CSF is known to activate not only Akt, a serine threonine kinase and powerful survival signal in many systems,[32] but also Stat3 in cardiomyocytes.[22] In that regard, transgenic mice with cardiac-specific overexpression of active Akt not only exhibit hypertrophy but also enhanced LV function,[40–42] as do transgenic mice with cardiac-specific overexpression of Stat3.[43] Immunohistochemistry revealed that p-Stat3 was localized on cardiomyocytes in the present model. We therefore suggest that these downstream signals of G-CSF, Jak/Stat in particular, are associated with the cardiomyocyte hypertrophy observed in the G-CSF-treated hearts.

G-CSF resulted in a reduced heart weight despite causing cardiomyocyte hypertrophy. This apparent inconsistency may be partially reconciled by the reduction in fibrotic volume by the treatment. We also observed up-regulation of MMP catalytic activity, and down-regulation of TNF-α in the G-CSF-treated hearts. G-CSF was previously reported to induce both MMP-2 (gelatinase A/type IV collagenase) and MMP-9 (gelatinase B).[44] The increases in MMP activity and down-regulation of TNF-α would be expected to contribute to the reduction in collagen content seen in G-CSF-treated hearts. It is well known that fibrosis, in particular interstitial fibrosis, often becomes excessive in DCM, accelerating cardiac remodeling and dysfunction, as is the case in the present model. Under those circumstances, an increase in MMP family proteins might exert a protective effect by catalyzing the degradation of the excessive collagen. Consistent with that idea, several earlier studies have shown that inhibition of MMP causes CHF,[45] that targeted deletion of MMP-9 attenuates LV remodeling and collagen accumulation caused by overexpression of MMP-2 and MMP-13,[46] and that an increase in MMP-1-induced by hepatocyte growth factor has a beneficial effect on postinfarction heart failure via its anti-fibrotic action.[47]

Coronary vascular abnormalities have been proposed as a primary defect of mice without δ-sarcoglycan.[48]

Although these vascular abnormalities could be secondary to the cardiomyocyte degeneration,[49] verapamil effectively treated this animal model.[50] Moreover, neovascularization seems to be an important part of G-CSF action in the case of a postmyocardial infarction model.[20] In the present model, we noted reduced vascular density, compared with the control heart. However, we did not note its increase in the G-CSF-treated UM-X7.1 hearts even though more activated Akt, an angiogenetic factor, was confirmed in the present study.

G-CSF has already been confirmed to be safe and is widely used in patients with granulocytopenia, as well as in healthy individuals donating bone marrow for transplantation. The daily dose of G-CSF used in the present study (10 μg/kg/day) was well within the clinical dosage range used in humans.[51] Thus, G-CSF administration may represent a new therapeutic strategy for prevention of development of heart failure, although it will certainly be necessary to confirm the safety of its long-term administration and to carefully construct the appropriate administrative protocols.

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Significance of 8-Hydroxy-2′-Deoxyguanosine Levels in Patients With Idiopathic Dilated Cardiomyopathy

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ABSTRACT

Background: Although oxidative stress mediated by reactive oxygen species plays an important role in the pathogenesis of heart failure (HF), good clinical markers for reactive oxygen species in patients with HF have not been established. 8-hydroxy-2′-deoxyguanosine (8-OHdG) is formed from deoxyguanosine in DNA by hydroxyl free radicals and might serve as a sensitive biomarker of intracellular oxidative stress in vivo. Thioredoxin (TRX) is known to be induced in cells as a radical scavenger against oxidative stress. The aim of this study is to evaluate the clinical significance of the serum 8-OHdG and TRX of patients with chronic HF with idiopathic dilated cardiomyopathy (DCM).

Methods and Results: We estimated serum 8-OHdG and TRX levels using enzyme-linked immunosorbent assay in 32 patients with DCM and investigated the impact of these markers to the clinical characteristics of these patients. Serum levels of 8-OHdG, but not TRX were significantly correlated with New York Heart Association functional class, left atrial diameters, left ventricular end-diastolic diameters, left ventricular end-systolic diameters, and plasma levels of brain natriuretic peptide.

Conclusion: These data suggest oxidative DNA damage is increased in patients with DCM according to the severity of HF. Serum levels of 8-OHdG may represent clinically useful markers of left ventricular remodeling.

Key Words: 8-OHdG, Heart failure, Idiopathic dilated cardiomyopathy.

Despite advances in various treatment strategies, the number of patients with chronic heart failure (CHF) is increasing and CHF is still a disease with a poor prognosis.[1] Therefore, an improved understanding of the fundamental mechanisms responsible for the development of heart failure is important for design of novel therapy strategies. CHF is a complex neuro-hormonal and inflammatory syndrome.[2] Inflammatory cells contain nicotinamide adenine dinucleotide phosphate (reduced form) (NADPH) oxidases that produce large amounts of reactive oxygen species (ROS) on stimulation with microbial antigens and cytokines to combat infections. Excess ROS contribute to a condition of oxidative stress in CHF,[3,4] in part caused by dampened antioxidant response systems essential to maintain homeostasis. ROS has been shown to elicit apoptosis of cardiomyocytes,[5] thus playing an important role in the progression and symptoms of CHF. Recently, experimental studies have revealed that ROS are produced in the failing myocardium and that ROS induce the functional and structural damage of cardiac myocytes and may play a role in the pathogenesis of heart failure. Moreover, there is considerable evidence derived from various animal models of heart failure and cardiomyopathy that oxidative DNA damage affects cardiac dysfunction by various mechanisms. Oxidative stress can generate DNA single-strand breaks, and activate poly(ADP-ribose) polymerase, which in turn alters cardiac energetic and eventually leads to cellular dysfunction and necrosis.[6,7] Furthermore, clinical studies have revealed that thiobarbituric acid reactive substances and 8-iso-prostaglandin $F_{2\alpha}$, which are the major biochemical products of ROS generation, are elevated in plasma and pericardial fluid of patients with heart failure.[3] Generally, thiobarbituric acid reactive substances measurement is a good index of lipid peroxidation. However, it may not be completely adequate to study the changes occurring in

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biological membranes because, in addition to membrane lipids, membrane proteins are also affected by oxidative stress.[8]

Thioredoxin (TRX) is a small multifunctional protein and key regulator of cellular redox balance operating in synergy with TRX reductase and NADPH.[9] TRX has gene regulatory activity of several transcription factors and controls in a redox-sensitive "on-off" mechanism decisions for apoptotic or hypertrophic pathways.[10,11] TRX is stress-inducible, which protects cells from various types of stresses, and eliminates hydrogen peroxide and act as a radical scavenger.[12,13] Previous studies have shown that serum levels of TRX were elevated in patients with chronic heart failure.[14,15] However, most of subjects of these studies include CHF patients from ischemic etiology, and increased TRX production might be the net results of activated vascular inflammation (atherosclerotic process) and myocardial or vascular activation in heart failure. In this study, we employed patients with CHF with nonischemic etiology; idiopathic dilated cardiomyopathy (DCM), to elucidate the influence of heart failure process itself on the cardiovascular system.

8-hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage after specific enzymatic cleavage after 8-hydroxylation of the guanine base. Because intracellular ROS can cause strand breaks in DNA and base modifications, including the oxidation of guanine residues to 8-OHdG, 8-OHdG might serve as a sensitive biomarker of intracellular oxidative stress in vivo.[16] 8-OHdG increases in plasma with aging,[17] during the development of tumors caused by ROS[18], and smokers.[19] The increased excretion of urinary 8-OHdG is seen in patients with non−insulin-dependent diabetes mellitus.[20] However, there have been no reports to investigate the possible relationship between serum 8-OHdG level and heart failure.

In this study, to determine the possible contribution of oxidative stress to the pathogenesis of heart failure, we measured the serum levels of TRX and 8-OHdG, which are the representative makers of ROS, in patients with DCM. We also examined the relationships to cardiac function as assessed by echocardiography and plasma brain natriuretic peptide (BNP) and to various clinical parameters.

Methods

Subjects

We have estimated serum levels of TRX and 8-OHdG in 32 patients (24 men and 8 women; mean age, 46.6 ± 18.2 years) with DCM admitted to Tokyo Women's Medical University Hospital (Japan) (Table 1) and 14 healthy volunteers (8 men and 6 women; mean age, 34.6 ± 6.9 years), as control subjects. DCM was diagnosed according to the criteria of the 1995 World Health Organization/International Society and Federation of Cardiology Task Force.[21] None of the patients showed any evidence of coronary artery disease, systemic hypertension, valvular heart disease, or pericardial heart disease. All were in stable heart failure status with a left ventricular ejection fraction (LVEF) <50%. All subjects gave informed consent to participation in this study.

Table 1. Basal Characteristics of Patients With Idiopathic Dilated Cardiomyopathy

Parameter	Mean ± SD (range)
Age (years)	46.6 ± 18.2
Gender: male (n)	24 (75%)
Hemodynamics	
LVEDD (mm)	66.0 ± 8.5
LVESD (mm)	56.7 ± 9.3
Shortening of fraction (%)	14.2 ± 6.1
LVEF (%)	26.7 ± 10.2
LAD (mm)	39.9 ± 9.4
NYHA functional class	
I (n)	7
II (n)	19
II (n)	6
Medications	
Diuretics	27/32 (84%)
ACE inhibitors/ARB	30/32 (94%)
β-receptor blocking drugs	25/32 (78%)
Digoxin	12/32 (38%)

Data are given as mean ± standard deviation.
LVEDD, left ventricular end-diastolic diameter; LVESD, left ventricular end-systolic diameter; LVEF, left ventricular ejection fraction; LAD, left atrial diameter; NYHA, New York Heart Association; ACE, angiotensin-converting enzyme; ARB, angiotensin II receptor blocker.

8-OHdG and TRX Estimation

Blood samples were taken from the patients after an overnight fast and, within 1 hour, sera were separated from blood cells by centrifugation. These samples were stored frozen at −80°C until analysis. Serum 8-OHdG levels were measured by enzyme-linked immunosorbent assay method (8-OHdG check high sensitivity; Japan Institute for the Control of Aging, Fukuroi, Japan). The kit can measure extremely low levels of 8-OHdG ranging from 0.1 to 10 ng/mL, and the specificity of the monoclonal antibody has been established.[22,23]

The serum TRX levels were measured by a sandwich enzyme-linked immunosorbent assay kit using anti-TRX monoclonal antibody according to the procedure described previously.[24]

To evaluate the severity of heart failure, plasma levels of BNP were measured using a commercially available kit (Shionoria BNP kit, Shionogi Pharmaceutical). Echocardiographic evaluation was performed by independent echocardiologists in all patients.

Statistical Analysis

All data are expressed as mean ± SD. Statistical analysis was performed using one-way analysis of variance and Student's *t*-test. Pearson's rank correlation test was used to assess the relationship between variables. Values of $P < .05$ were considered significant.

Results

Table 1 summarizes the baseline characteristics of subjects in this study. Number of patients in New York Heart Association (NYHA) functional class of I, II, and III was 7, 19, and 6, respectively. Mean LVEF of these patients was 26.7 ± 10.2%. Table 2 indicates the comparison of hemodynamic parameters according to the NYHA functional class. Patients in class III showed lower systolic blood pressure and LVEF and higher plasma BNP levels compared

Table 2. Hemodynamic Characteristics of Patients According to the NYHA Functional Class

NYHA Functional Class	I (n = 7)	II (n = 19)	III (n = 6)
HR (beats/min)	66.6 ± 7.0	68.1 ± 9.2	72.7 ± 6.9
SBP (mm Hg)	112.3 ± 9.8	116.1 ± 13.4	96.3 ± 10.9*,†
DBP (mm Hg)	71.7 ± 7.8	69.9 ± 8.2	63.3 ± 9.3
Plasma BNP (pg/mL)	43.8 ± 51.8	214.7 ± 335.6	718.8 ± 200.0†,‡
LVEF (%)	30.8 ± 10.5	28.7 ± 8.7	15.9 ± 7.8*,†

Data are given as mean ± standard deviation.

NYHA, New York Heart Association; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; BNP, brain natriuretic peptide; LVEF, left ventricular ejection fraction.

*$P < .05$ vs. NYHA I.

†$P < .01$ vs. NYHA II.

‡$P < .0001$ vs. NYHA I.

with those in class I and II. Eighty-four percent of patients were on diuretics, 94% on angiotensin-converting enzyme inhibitor or angiotensin II receptor blockers, 78% on β-blockers, and 38% on digoxin.

As shown in Fig. 1A, serum 8-OHdG levels increased in proportion to the severity of the disease, with a mean value of 0.24 ± 0.08 ng/mL in patients of NYHA functional class I and 0.32 ± 0.11 ng/mL in NYHA class II patients vs. NYHA class III (0.75 ± 0.57 ng/mL, $P = .04$, $P = .003$, respectively). However, there was no significant difference between control group (0.23 ± 0.07 ng/mL) and NYHA class I. In contrast, there was no relationship between serum level of TRX and the severity of heart failure (Fig. 1B).

Serum levels of 8-OHdG were significantly correlated with left atrial diameters ($r = 0.54$, $P = .002$), left ventricular end-diastolic diameters ($r = 0.65$, $P < .0001$), and left ventricular end-systolic diameters ($r = 0.66$, $P < .0001$)

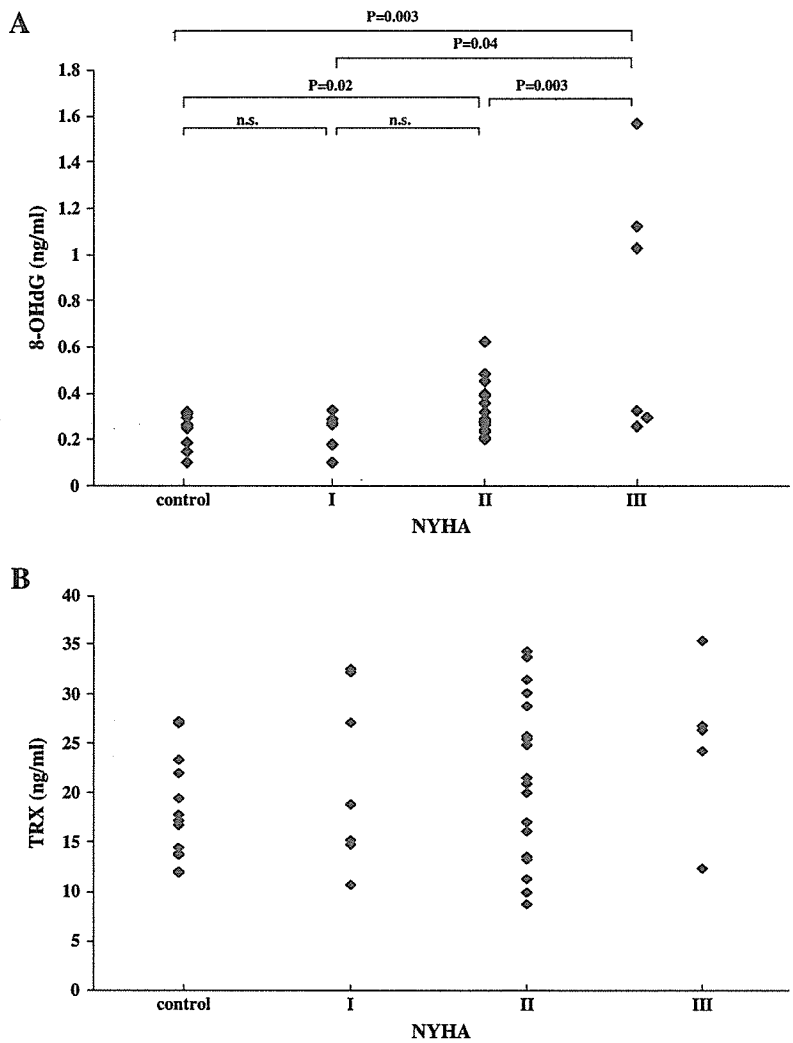

Fig. 1. Serum 8-OHdG (A) and thioredoxin (B) levels in proportion to severity of the New York Heart Association (NYHA) functional class. 8-OHdG levels increased in proportion to severity of the NYHA functional class; however, there was no relationship between serum level of thioredoxin and the severity of heart failure.

(Fig. 2A–C). There was no correlation between serum levels of 8-OHdG and LVEF ($P = .11$). Moreover, serum levels of 8-OHdG were significantly correlated with plasma levels of BNP ($r = 0.44$, $P = .01$) (Fig 3). No association was found between serum TRX levels and the echocardiographic data (left atrial diameter, left ventricular end-diastolic diameters, left ventricular end-systolic diameters, LVEF), BNP levels, and serum 8-OHdG levels.

Discussion

In this study, we have demonstrated that symptomatic severity of heart failure (eg, NYHA functional class) is associated with elevations in the 8-OHdG. Serum 8-OHdG has also correlated with left atrial (LA) size, left ventricular (LV) size, and plasma BNP; however, there was no significant correlation between serum TRX levels and those parameters.

8-OHdG is formed from deoxyguanosine in DNA by hydroxyl-free radicals. Oxidative DNA damage and 8-OHdG have been extensively studied in relation to the development of cancer. Previous studies have shown that increased oxidative stress, accessed by blood glutathione, erythrocyte superoxide dismutase, and membrane SH content, is associated with dilated cardiomyopathy and heart failure.[8] In agreement with those studies, we observed that 8-OHdG is elevated according to the NYHA functional class and plasma BNP level, markers of severity of heart failure. Moreover, we could demonstrate that progression of LV remodeling as expressed by LV and LA enlargement is associated with the elevation of serum 8-OHdG.

In DCM, ventricular systolic dysfunction and chamber dilation are accompanied by architectural remodeling, wall thinning, and cardiac myocyte slippage. BNP is considered to be one of the practical markers of cardiac function and cardiovascular prognosis. Strong production of BNP has been demonstrated in hypertrophied and failing ventricular myocardium. Furthermore, induction of BNP in ventricular myocytes appears to occur more rapidly during mechanical and neurohumoral stimulation, which indicates close correlation between BNP and LV overload and remodeling. Nagaya et al showed that the high levels of plasma BNP in acute phase and the sustained elevation of plasma BNP in chronic phase may be associated with progressive ventricular remodeling occurring after acute myocardial infarction.[25] Serum levels of 8-OHdG were significantly correlated with plasma level of BNP in the present study. Our data suggest an important role of oxidative stress on ventricular remodeling in the progression of heart failure.

Serum levels of 8-OHdG did not correlate with serum levels of TRX. Compared with 8-OHdG, serum levels of TRX were not associated with the severity of CHF in this study. Jekell et al[15] showed that mean plasma level of TRX was significantly higher in patients with CHF than in the healthy subjects. They also showed that TRX levels increased in proportion to severity of disease. The inconsistencies in the role of TRX patients with heart failure may be attributed to the differences of patients' characteristics and the severity of heart failure between 2 studies.

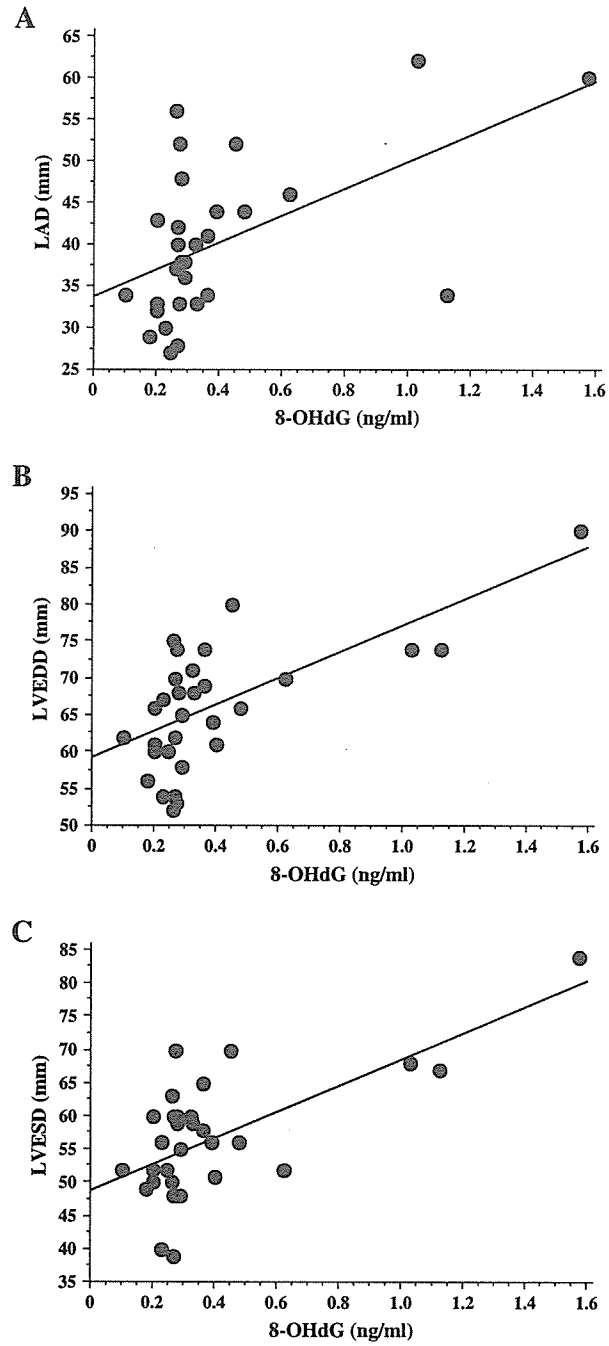

Fig. 2. Correlation of 8-OHdG and left atrial diameter, left ventricular end-diastolic diameter, and left ventricular end-systolic diameter. Serum levels of 8-OHdG were significantly correlated with left atrial diameters (A: $r = 0.54$, $P = .002$), left ventricular end-diastolic diameters (B: $r = 0.65$, $P < .0001$), and left ventricular end-systolic diameters (C: $r = 0.66$, $P < .0001$). LAD: left atrial diameter, LVEDD: left ventricular end-diastolic diameter. LVESD: left ventricular end-systolic diameter.

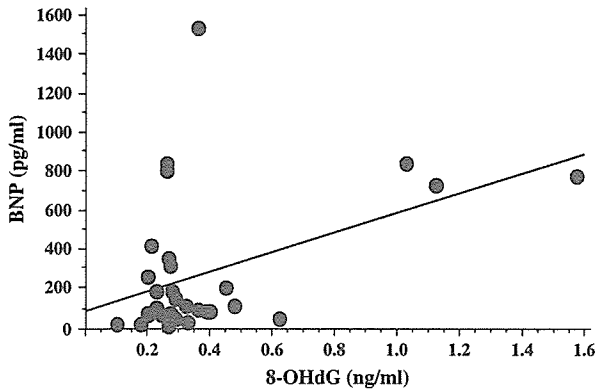

Fig. 3. Correlation of serum 8-OHdG and plasma BNP levels. Serum levels of 8-OHdG were significantly correlated with plasma levels of BNP ($r = 0.44$, $P = .01$). BNP: brain natriuretic peptide.

Angiotensin II has been shown to be a potent stimulation of NAD(P)H oxidase activity and O_2^- production in vascular smooth muscle, fibroblasts, endothelial cells, and cardiomyocytes. Of the drugs in common clinical use, there is evidence that angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers have beneficial effects on oxidative stress.[26,27] β-adrenergic antagonist carvedilol is also known to have a potent antioxidant activity.[28] Therefore, these medications might affect the levels of 8-OHdG in our patients. However, because most of our patients were receiving these medications, the effects of these drugs will not explain the difference of 8-OHdG levels according to the severity of heart failure and LV remodeling in this study.

Although we observed the relationship between 8-OHdG and the severity of heart failure in patients with DCM, the number of patients in this study was comparatively small. Further investigations in larger population-based studies are warranted to corroborate to current findings, particularly the potential of 8-OHdG to detect the severity of heart failure and cardiac function.

In conclusion, significant relationship is evident between serum level of 8-OHdG and biochemical and echocardiographic parameters of LV dysfunction. Serum levels of 8-OHdG may represent clinically useful markers of LV remodeling.

Acknowledgment

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Pioglitazone Prevents Acute and Chronic Cardiac Allograft Rejection

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Pioglitazone Prevents Acute and Chronic Cardiac Allograft Rejection

Hisanori Kosuge, MD; Go Haraguchi, MD; Noritaka Koga, MD; Yasuhiro Maejima, MD; Jun-ichi Suzuki, MD; Mitsuaki Isobe, MD

Background—Peroxisome proliferator–activated receptor-γ plays an important role in regulating inflammation. Although cardiac transplantation is an established therapy for patients with end-stage heart disease, allograft rejection is a major concern for long-term survival. We investigated the role of pioglitazone in acute and chronic rejection in a murine cardiac transplantation model.

Methods and Results—We performed heterotopic murine cardiac transplantation in total allomismatch or major histocompatibility complex class II–mismatched combinations. Recipient mice were given standard chow or chow containing pioglitazone (3 mg · kg^{-1} · d^{-1}) beginning 1 day before cardiac transplantation. In acute rejection, animals given pioglitazone showed significantly longer cardiac allograft survival than control mice (mean survival time, 34.6±7.8 versus 8.4±0.4 days; $P<0.003$). Treatment with pioglitazone significantly suppressed graft expression of interferon-γ and monocyte chemoattractant protein-1. In chronic rejection, neointimal hyperplasia was significantly lower in allografts from mice treated with pioglitazone (luminal occlusion, 25.1±8.8%) than in those from control mice (65.8±7.3%, $P<0.001$). Pioglitazone-treated allografts showed significantly reduced expression of interferon-γ, interleukin-10, and monocyte chemoattractant protein-1. We performed mixed lymphocyte reactions and in vitro proliferation assays of smooth muscle cells. Addition of pioglitazone to mixed lymphocyte reactions inhibited proliferation of T cells. Smooth muscle cells showed significant proliferation when cocultured with activated splenocytes. This proliferation was significantly inhibited by the addition of pioglitazone (1 μmol/L).

Conclusions—Pioglitazone prolongs allograft survival and attenuates neointimal hyperplasia through the suppression of proliferation of smooth muscle cells. Pioglitazone may be a novel means to prevent acute and chronic allograft rejection. (***Circulation.* 2006;113:2613-2622.**)

Key Words: inflammation ■ pharmacology ■ rejection ■ smooth muscle ■ transplantation

Despite advances in immunosuppressive agents, acute rejection and chronic rejection remain the major causes of graft failure after cardiac transplantation.[1] Graft rejection involves immune responses and inflammation. In acute rejection, expression of adhesion molecules by graft endothelial cells is increased, and inflammatory cells, including T cells and macrophages, infiltrate into the allografts and produce various cytokines and chemokines.[2] In chronic rejection, graft vasculopathy is characterized by intimal thickening resulting from infiltration of inflammatory cells, proliferation of smooth muscle cells (SMCs), and accumulation of extracellular matrix.[3] Therefore, it is important not only to suppress acute rejection with conventional immunosuppressive agents but also to prevent the development of graft vasculopathy to improve prognosis after transplantation.

Clinical Perspective p 2622

Peroxisome proliferator–activated receptors (PPARs) constitute a superfamily of ligand-dependent transcription factors.[4] Three PPAR isotypes, α, β (or δ), and γ, have been identified. PPARγ is expressed mainly in adipose tissue and is an important determinant of adipocyte differentiation and insulin sensitivity. PPARγ agonists such as pioglitazone, troglitazone, and rosiglitazone are used as insulin-sensitizing compounds. It has been reported that PPARγ is expressed in macrophages, T cells, endothelial cells, and SMCs.[4–8] PPARγ agonists inhibit T-cell proliferative responses[6] and SMC proliferation and migration.[8,9] PPARγ agonists are associated with the expression of adhesion molecules, cytokines, and chemokines.[10–13] Furthermore, treatment with PPARγ agonists has been shown to inhibit atherosclerosis, cardiac hypertrophy, experimental autoimmune myocarditis, development of left ventricular remodeling, failure after myocardial infarction, and intimal hyperplasia after vascular injury.[13–17] However, it is not known whether PPARγ agonists prevent acute and chronic rejection after cardiac transplantation.

To explore the role of PPARγ agonists in acute and chronic rejection after organ transplantation, we performed cardiac transplantation in mice and found that pioglitazone is asso-

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ciated with immune response and SMC proliferation. Furthermore, administration of pioglitazone can prolong cardiac allograft survival and suppress the development of graft vasculopathy.

Methods

Reagents

Pioglitazone was provided by Takeda Chemical Industries (Tokyo, Japan). Anti-mouse interferon (IFN)-γ, CD4, CD8, and CD11b monoclonal antibodies were purchased from Pharmingen (San Diego, Calif). Anti-goat monocyte chemoattractant protein-1 (MCP-1) and PPARγ antibodies were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif). Anti-mouse actin monoclonal antibody was purchased from CHEMICON International (Temecula, Calif).

Animals

Male BALB/c, C3H/He, and C57BL/6 (B/6, H-2^b) mice 6 to 8 weeks of age were obtained from Japan Clea (Tokyo, Japan). B6.C-H-2^{bm12}KhEg (Bm12, H-2^{bm12}) mice were obtained from the Jackson Laboratory (Bar Harbor, Me). Animals were maintained in our animal facility and weighed 20 to 25g. The study protocol conformed to the *Guide for the Care and Use of Laboratory Animals* of Tokyo Medical and Dental University.

Cardiac Transplantation

Donor hearts were heterotopically transplanted into recipient mice as described previously.[18] The aorta and pulmonary artery of donor hearts were anastomosed to the recipient abdominal aorta and inferior vena cava, respectively. Survival of cardiac allografts was evaluated by daily palpation, and cessation of beating was interpreted as rejection. Recipient mice were given standard chow or chow containing pioglitazone (3 mg · kg^{-1} · d^{-1}) beginning 1 day before cardiac transplantation.[13] We measured body weight of the mice twice a week, and adjusted the daily dose of pioglitazone accordingly. Serum total cholesterol, triglycerides, and glucose were measured by enzymatic assays. After 5 days in total allomismatch combinations and 8 weeks in major histocompatibility complex (MHC) class II–mismatched combinations, pioglitazone did not affect serum concentrations of total cholesterol, triglycerides, and glucose (data not shown).

In total allomismatch combinations, allografts were harvested at 5 days after transplantation or at the time of graft failure. In MHC class II–mismatched combinations, allografts were harvested at 2 and 8 weeks after transplantation. After harvest, allografts were sectioned transversely into 3 parts. The basal section was fixed in 10% formalin and embedded in paraffin for morphological examination. The midsection was embedded immediately in OCT compound (Tissue-Tek, Sakura FineTech Inc) and flash-frozen in liquid nitrogen for immunohistochemistry. The apical section was used to extract proteins for Western blot analysis.

Immunohistochemistry

Frozen sections (5 μm) were fixed in acetone for 10 minutes at 4°C.[19] After sections were washed in phosphate-buffered saline, they were incubated with primary antibodies overnight at 4°C. Sections were then incubated with biotinylated secondary antibodies at room temperature for 30 minutes. Antigen-antibody conjugates were detected with avidin-biotin-horseradish peroxidase complex (Nichirei, Tokyo, Japan) according to the manufacturer's instructions. We used 3-amino-9-ethylcarbazole as chromogen and counterstained sections with hematoxylin.

Histological Evaluation

Grafts and arteries were analyzed by Mallory staining, hematoxylin and eosin, and elastica van Gieson staining. The areas within the internal elastic lamina (IEL), the external elastic lamina, and the lumen were carefully traced, and planimetric areas were calculated with an image analysis system (Scion Image Beta 4.02). The cross-sectional area of luminal stenosis was calculated as follows: luminal occlusion=[(IEL area−luminal area)/IEL area]×100 (%). The intima-to-media (I/M) ratio was calculated as follows: I/M=(IEL area−lumen area)/(external elastic lamina area−IEL area).

Parenchymal rejection (PR) was assessed in allografts at 5 days after transplantation and at the time of failure in total allomismatch combinations.[20,21] PR severity was graded with a scale modified from the International Society for Heart and Lung Transplantation (0=no rejection, 1=focal mononuclear cell infiltrates without necrosis, 2=focal mononuclear cell infiltrates with necrosis, 3=multifocal infiltrates with necrosis, 4=widespread infiltrates with hemorrhage and/or vasculitis).[20,21] We measured the fibrotic areas with an image analysis system (Scion Image Beta 4.02). The fibrotic area ratio (fibrotic areas/entire area as a percentage) was calculated in allografts at 8 weeks after transplantation.

Mixed Lymphocyte Reaction

Splenocyte suspensions were obtained by disrupting spleens between sterile glass slides. Red blood cells were lysed with ammonium chloride. Mixed lymphocyte reaction (MLR) was performed with responder splenocytes from C3H/He mice (n=3) at 5 days after transplantation and mitomycin-C–inactivated stimulator splenocytes from naïve BALB/c mice. A total of 3×10^5 responder cells and an equal number of stimulator cells were cocultured in 96-well plates at 37°C under 5% CO_2 for 4 days. Pioglitazone was added to each well at various concentrations on day 0. T-cell proliferation was assessed with Cell Counting Kit-8 (Dojindo, Kumomoto, Japan) according to the manufacturer's instructions. Cell proliferation was expressed as the optical density of the responder cells.

Coculture of SMCs and Splenocytes

Primary SMCs were obtained from the thoracic aortas of Bm12 mice by the explant technique described previously.[22] Cells were grown in Dulbecco's modified Eagle's medium (Sigma Chemical Co, St Louis, Mo) containing 50 μg/mL streptomycin, 50 IU/mL penicillin, and 10% fetal bovine serum at 37°C and 5% CO_2. Cultured SMCs were identified by the typical hill-and-valley morphology and by immunostaining with monoclonal antibody to α-smooth muscle actin. All experiments were performed with cells between passages 3 and 8.

SMCs were trypsinized and seeded into 96-well plates. At confluence, SMCs were arrested in medium with 0.4% fetal bovine serum for 5 days. Mitomycin-C–inactivated splenocytes from B/6 mice (n=3) after transplantation (total, 5×10^5) were washed with phosphate-buffered saline and added with pioglitazone to each well. We investigated SMC proliferation in response to anti–IFN-γ (1 μg/mL)[23] or MCP-1 antibody (2 μg/mL).[24] After 4 days, SMC proliferation was assessed with Cell Counting Kit-8 (Dojindo) according to the manufacturer's instructions. Cell proliferation is expressed as the optical density.

Western Blot Analysis

Heart sections were homogenized in extraction buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1% Triton X-100, 2 mmol/L EGTA, 10 mmol/L EDTA, 100 nmol/L NaF, 1 nmol/L $Na_4P_2O_7$, 2 mmol/L Na_3VO_4, 100 μg/mL phenylmethylsulfonyl fluoride, and cocktail tablets (Roche, Basel, Switzerland).[23] After centrifugation, the supernatant was stored. The protein concentration of each sample was measured with a Bio-Rad Protein Assay Kit (Bio-Rad, Milan, Italy). Protein concentrations of all samples were equal in subsequent experiments.

Proteins were separated by sodium-dodecyl sulfate–polyacrylamide gel electrophoresis, transferred to nitrocellulose membranes, and incubated with primary antibodies at 4°C overnight. The membranes were then incubated with secondary antibody for 2 hours and developed with enhanced chemiluminescence reagent (Amersham Biosciences). Enhanced chemiluminescence was detected with LAS-1000 (Fujifilm, Tokyo, Japan). The level of MCP-1 protein was normalized to that of actin.

Ribonuclease Protection Assay

mRNA was isolated with TRIzol (Invitrogen, Rockville, Md) according to the manufacturer's protocol.[22] In vitro transcription was performed with the template set, T7 polymerase, and [α-^{32}P]UTP. Total RNA (10 μg) was hybridized with probes at 56°C for 16 hours. All samples were then treated with RNase before treatment with proteinase K. Samples were separated by electrophoresis on denaturing gels containing 5% polyacrylamide. Detection of the mRNA bands was performed with an image analyzer (BAS2000, Fujifilm). Levels of cytokine mRNAs were normalized to that of glyceraldehyde-3-phosphate dehydrogenase mRNA.

Enzyme-Linked Immunoassay Procedure

Production of IFN-γ and MCP-1 was measured in supernatants of MLR or coculture of SMCs and splenocytes. Supernatants were stored at −80°C before enzyme-linked immunoassay (ELISA) analysis. The concentrations of IFN-γ and MCP-1 were determined with an ELISA kit (BioSource International, Camarillo, Calif) according to the manufacturer's instructions.

Statistical Analysis

All data are expressed as mean±SEM. Kaplan-Meier analysis was used to estimate graft survival, and the Mann-Whitney *U* test was used for survival differences between the 2 groups. Differences between groups for PR scores, infiltrating cell number, normalization of RNase protection assays and Western blot data, luminal occlusion, I/M ratio, and fibrotic areas were analyzed by Student *t* test. For infiltrating cell number and ELISA data, logarithmic transformation was performed before statistical analysis. One-way ANOVA was used for comparisons between groups for MLR, cytokine ELISA, and SMC proliferation. Values of $P<0.05$ were considered statistically significant.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agree to the manuscript as written.

Results

Pioglitazone Prolongs Cardiac Allograft Survival

To investigate the effect of pioglitazone on acute rejection, we performed cardiac transplantation using C3H/He recipients and BALB/c donors. In the total allomismatch combination, the survival of cardiac allografts in mice given pioglitazone was significantly prolonged (34.6±7.8 days; n=8) compared with allografts in mice fed standard chow (8.4±0.38 days; n=8; $P<0.005$; Figure 1A). PR scores were significantly lower in allografts treated with pioglitazone (1.6±0.27) than in controls (3.1±0.23) at 5 days after transplantation ($P<0.001$; Figure 1B). However, PR scores were comparable in allografts at the time of failure (Figure 1C).

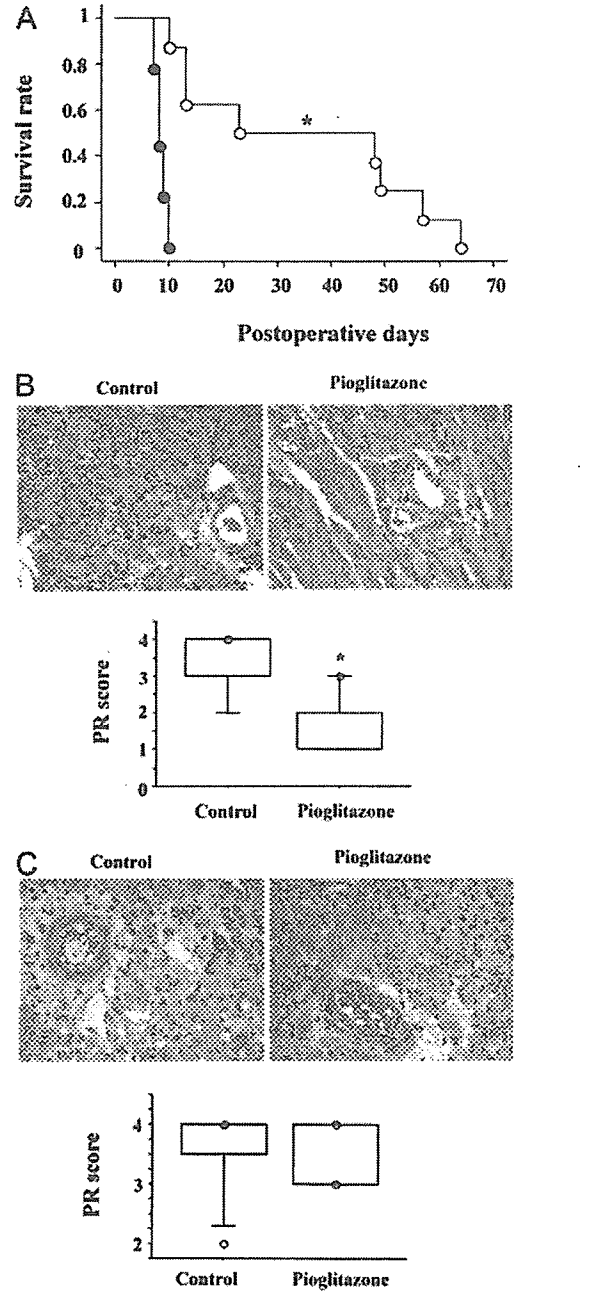

Figure 1. A, Survival of cardiac allografts in mice receiving standard chow (filled ovals) or chow containing pioglitazone (3 mg · kg^{-1} · d^{-1}, open ovals) from 1 day before cardiac transplantation. Treatment with pioglitazone prolonged cardiac allograft survival compared with controls. *$P<0.005$ vs control. B, PR scores were significantly lower in allografts treated with pioglitazone than in controls at 5 days after transplantation. *$P<0.001$ vs control. C, PR scores were not altered in allografts at the time of failure.

Pioglitazone Prevents Expression of IFN-γ and MCP-1 in Allografts From Total Allomismatch Combination

Expression of PPARγ was enhanced in infiltrating cells in cardiac allografts at 5 days after transplantation (Figure 2A). The numbers of infiltrating CD4-, CD8-, and CD11b-positive cells in pioglitazone-treated allografts at 5 days after transplantation were significantly lower than in controls (Figure 2B and 2C). The numbers of infiltrating CD4-, CD8-, and CD11b-positive cells in pioglitazone-treated allografts at the time of failure did not differ between the 2 groups (Figure 2D and 2E). We examined whether pioglitazone was associated with cytokine expression in allografts at 5 days after transplantation. Expression of IFN-γ mRNA and MCP-1 was significantly lower in allografts treated with pioglitazone than in controls (Figure 3A and 3B). Expression of interleukin (IL)-10, IL-15, and IL-6 mRNAs did not differ between the 2 groups (Figure 3A).

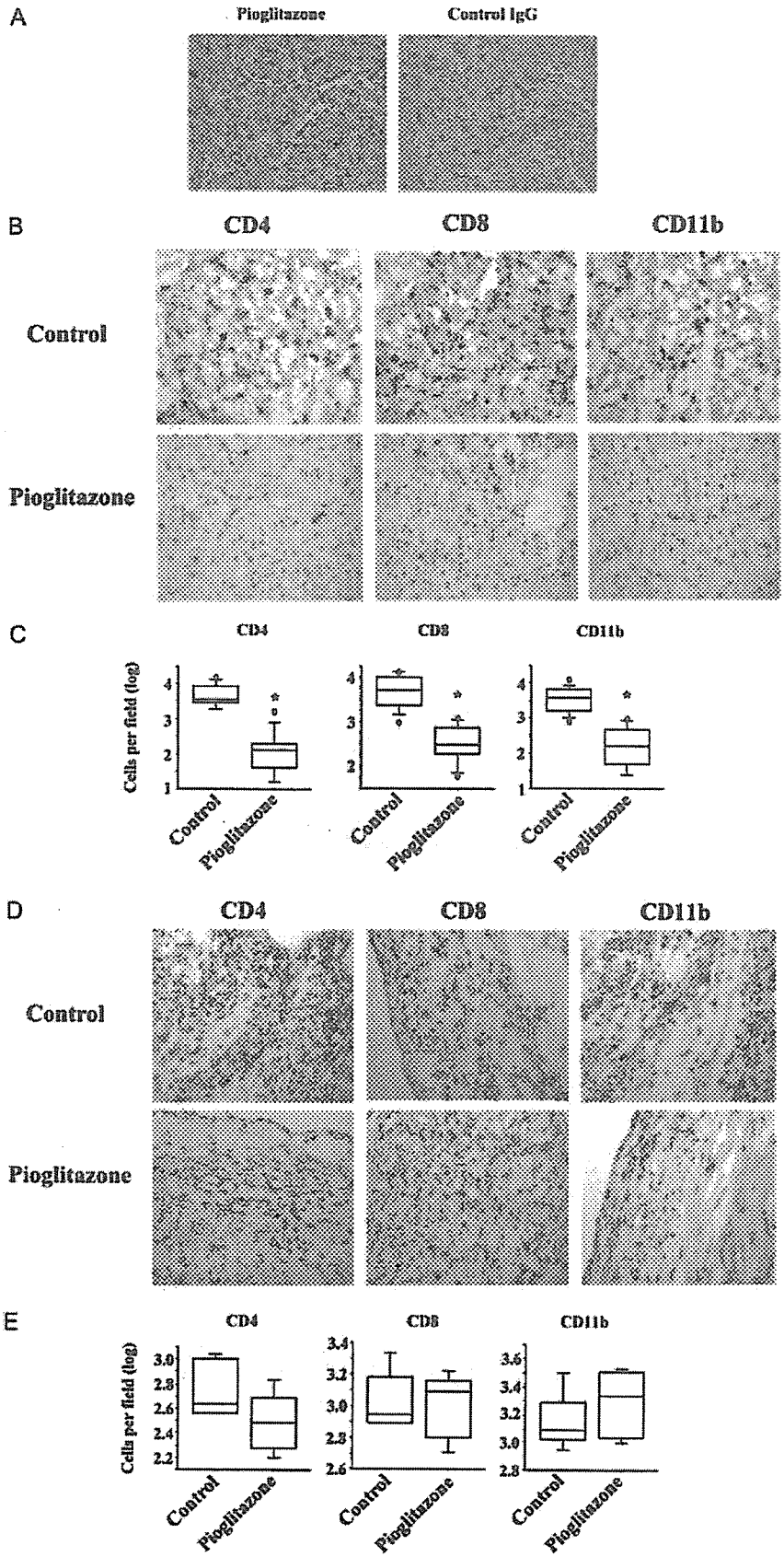

Figure 2. Expression of PPARγ and inhibition of infiltration by CD4-, CD8-, and CD11b-positive cells in donor hearts in the total allomismatch combination. A, Immunohistochemical staining of PPARγ in cardiac allografts at 5 days after transplantation. Representative frozen sections stained with antibody against PPARγ (left) and isotype-matched control IgG (right) are shown. PPARγ expression was identified in cells infiltrating the allografts. Original magnification ×400. B, D, Immunohistochemical staining of CD4-, CD8-, CD11b-positive cells in allografts at 5 days after transplantation (B) and the time of failure (D). Top, Allografts in mice that received standard chow; bottom, allografts in mice that received chow with pioglitazone. Representative frozen sections stained with antibodies against CD4, CD8, and CD11b are shown. Original magnification ×400. C, E, Quantitative analysis of CD4-, CD8-, and CD11b-positive cells. Data are expressed as mean±SEM of 20 fields per graft. *$P<0.0001$ vs control.

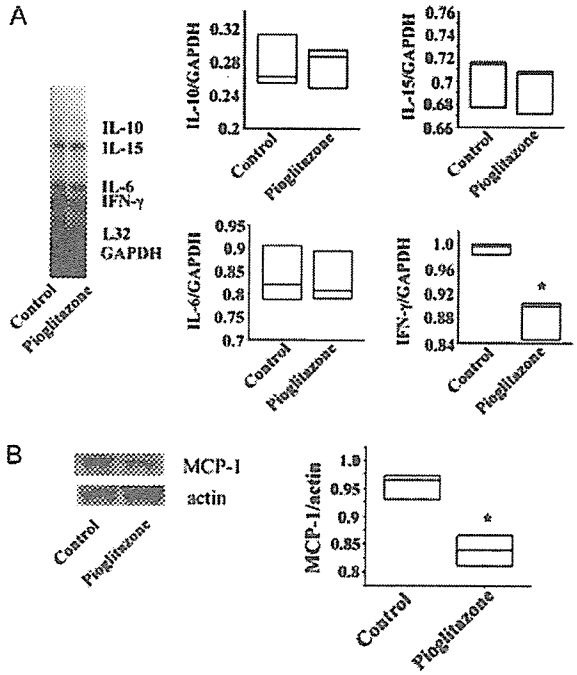

Figure 3. Expression of cytokines and MCP-1 in allografts in the total allomismatch combination. A, Representative data of 3 independent RNase protection assays showing cytokine mRNA expression. Expression of IFN-γ mRNA was significantly reduced in allografts treated with pioglitazone compared with controls. Expression of each cytokine mRNA was normalized to that of GAPDH mRNA. *$P<0.05$ vs control. B, Representative data of 3 independent Western blots showing MCP-1 expression. MCP-1 expression was significantly reduced in allografts treated with pioglitazone compared with controls. MCP-1 levels were normalized to those of actin. *$P<0.05$ vs control.

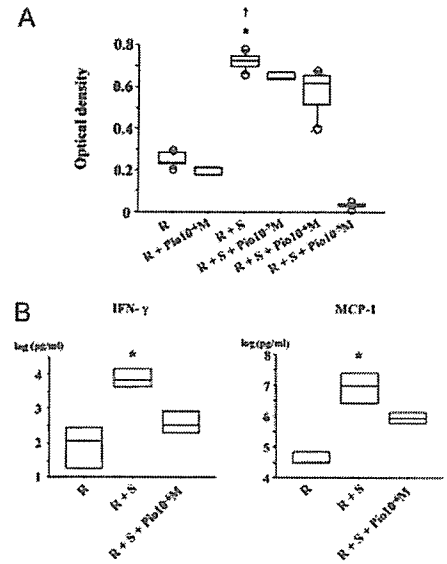

Figure 4. Inhibition of MLR by pioglitazone (Pio). A, MLR responder (R) splenocytes (C3H/He mice) and mitomycin-C–inactivated stimulator (S) splenocytes (BALB/c mice) were incubated. Cell proliferation was significantly suppressed by pioglitazone. *$P<0.01$ vs R+S+Pio 10^{-6}. †$P<0.0001$ vs R, R+Pio 10^{-6}, and R+S+Pio 10^{-5}. B, Production of IFN-γ and MCP-1 in supernatants was suppressed significantly by pioglitazone. Data are expressed as mean±SEM in each group. *$P<0.05$ vs R and R+S+Pio.

Pioglitazone Inhibits MLR

To assess the effect of pioglitazone on allogeneic responses in vitro, we performed MLR with sensitized splenocytes after cardiac transplantation. Pioglitazone at concentrations >1 μmol/L significantly inhibited MLR proliferation (Figure 4A). Production of IFN-γ and MCP-1 in supernatants of MLR was suppressed significantly by pioglitazone (Figure 4B).

Pioglitazone Attenuates Graft Vasculopathy

To investigate the effect of pioglitazone on chronic rejection, we performed cardiac transplantation with B6 recipients and Bm12 donors. In the MHC class II–mismatched combination, neointimal hyperplasia developed characteristically in mice that received standard chow (n=8), whereas neointimal thickening was significantly reduced in mice that received chow containing pioglitazone (n=8; Figure 5A and 5B). The degree of luminal occlusion was 65.8±7.3% for standard chow and 25.1±8.8% for chow containing pioglitazone ($P<0.001$; Figure 5C). The I/M ratio was significantly lower in allografts treated with pioglitazone than in controls (Figure 5D). The fibrotic areas did not differ between the 2 groups (Figure 5E).

Pioglitazone Prevents Expression of IFN-γ, IL-10, and MCP-1

Expression of PPARγ was enhanced in infiltrating cells in cardiac allografts at 2 weeks after transplantation (Figure 6A). Infiltration of inflammatory cells was examined immunohistochemically in allografts at 2 and 8 weeks after transplantation. Pioglitazone-treated allografts at 2 and 8 weeks after transplantation showed significantly lower numbers of CD4-, CD8-, and CD11b-positive cells than controls (Figure 6B and 6E).

Because infiltration of inflammatory cells was decreased by treatment with pioglitazone, we examined whether pioglitazone could modulate expression of cytokines in allografts at 2 and 8 weeks after transplantation. IFN-γ mRNA and MCP-1 protein levels were significantly reduced in pioglitazone-treated allografts at 2 weeks after transplantation (Figure 7A and 7B). Expression of IL-10, IL-15, and IL-6 mRNAs was not altered (Figure 7A). At 8 weeks after transplantation, expression of IFN-γ and IL-10 mRNAs was significantly lower in allografts treated with pioglitazone than in controls. Expression of IL-6 and IL-15 mRNAs did not differ significantly between control and pioglitazone-treated mice (Figure 7C). Furthermore, Western blotting showed that MCP-1 expression was significantly suppressed in allografts treated with pioglitazone compared with controls (Figure 7D).

Pioglitazone Suppressed the Proliferation of SMCs Induced by Splenocytes

We previously reported that interaction between SMCs and T cells or splenocytes induces SMC proliferation.[22,23] In the

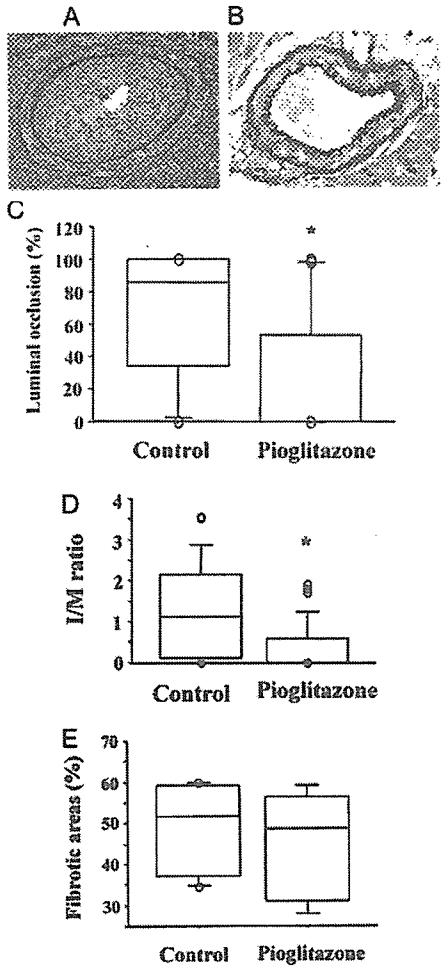

Figure 5. Effect of pioglitazone on graft vasculopathy. Representative elastica van Gieson staining of allografts in mice that received standard chow (A) or chow containing pioglitazone (B). Pioglitazone attenuates graft vasculopathy in allografts at 8 weeks after transplantation in the MHC class II–mismatched combination. Original magnification ×400. C, The degree of graft vasculopathy in each group was quantified. Data are expressed as mean±SEM of 8 mice in each group. *$P<0.001$ vs control. D, Quantitative analysis of I/M ratio in each group is shown. Data are expressed as mean±SEM of 8 mice in each group. *$P<0.0005$ vs control. E, Fibrotic areas did not differ between the control and pioglitazone-treated groups.

present study, SMCs proliferated significantly in response to activated splenocytes. Pioglitazone at concentrations >1 μmol/L significantly reduced SMC proliferation (Figure 8A). The effect of the suppression of SMC proliferation did not differ between pioglitazone and anti–IFN-γ or anti–MCP-1 antibody, although there was a trend toward suppressing SMC proliferation. Production of IFN-γ and MCP-1 in supernatants of coculture of SMCs and splenocytes was suppressed significantly by pioglitazone (Figure 8B).

Discussion

Organ allograft rejection limits long-term survival after transplantation, and immunosuppressive agents have been used clinically to prevent allograft rejection. Although 1-year survival after transplantation has improved substantially,[25] long-term administration of immunosuppressive agents does not prevent chronic rejection, which is characterized by neointimal thickening and fibrosis, and may have adverse side effects, including development of opportunistic infections and neoplasms. Therefore, it is important to improve prognosis by inhibiting acute and chronic allograft rejection with agents other than conventional immunosuppressive drugs.

Inflammation is characterized by the expression of adhesion molecules and infiltration by inflammatory cells such as macrophages and T cells. PPARγ agonists play important roles in regulating inflammation. It has been reported that PPARγ agonists suppress expression of vascular cell adhesion molecule-1 and intercellular adhesion molecule-1 by activated human endothelial cells.[10,11] PPARγ agonists also inhibit synthesis of inflammatory cytokines, including IFN-γ, IL-1β, and TNF-α, in human peripheral blood mononuclear cells.[12] Shiomi et al[13] reported that treatment with pioglitazone reduced the expression of MCP-1 in an experimental model of chronic heart failure. PPARγ agonists also are associated with T-cell activation.[6,26] Expression of PPARγ mRNA occurs in human peripheral blood T cells. PPARγ agonists inhibit IL-2 secretion by T cells and decrease cell proliferation. Several studies have shown the effects of PPARγ agonists on neointimal hyperplasia after vascular injury[17,27]; however, the role of PPARγ agonists in allograft rejection is not known. To the best of our knowledge, we are the first to show that PPARγ agonists play an important role in suppressing allograft rejection after cardiac transplantation.

To explore the effects of PPARγ agonists on allograft rejection, we performed murine cardiac transplantation. In the total allomismatch combination, treatment of mice with pioglitazone significantly prolonged cardiac allograft survival compared with controls. Because PPARγ agonists are associated with T-cell responses,[6] we then examined the allogeneic response of T cells by MLR with splenocytes. Pioglitazone significantly suppressed MLR proliferation at a concentration of 1 μmol/L. This result indicates that pioglitazone is associated with T-cell responses and may be useful as an immunosuppressive agent in organ transplantation.

In the MHC class II–mismatched combination, neointimal thickening in mice treated with pioglitazone was significantly attenuated compared with that in mice fed normal chow. Neointimal formation is associated with proliferation of SMCs. It has been reported that PPARγ is expressed in SMCs, and PPARγ agonists inhibit migration and proliferation of SMCs.[8,9] We previously reported that coculture of SMCs and T cells induces SMC proliferation.[22,23] In the present study, we examined whether pioglitazone suppressed the SMC proliferation induced by the interaction of SMCs with splenocytes. We showed that SMC proliferation was increased by interactions of SMCs with splenocytes and that pioglitazone suppressed this proliferation.

Allograft rejection contributes to the expression of cytokines and chemokines. In the present study, during acute

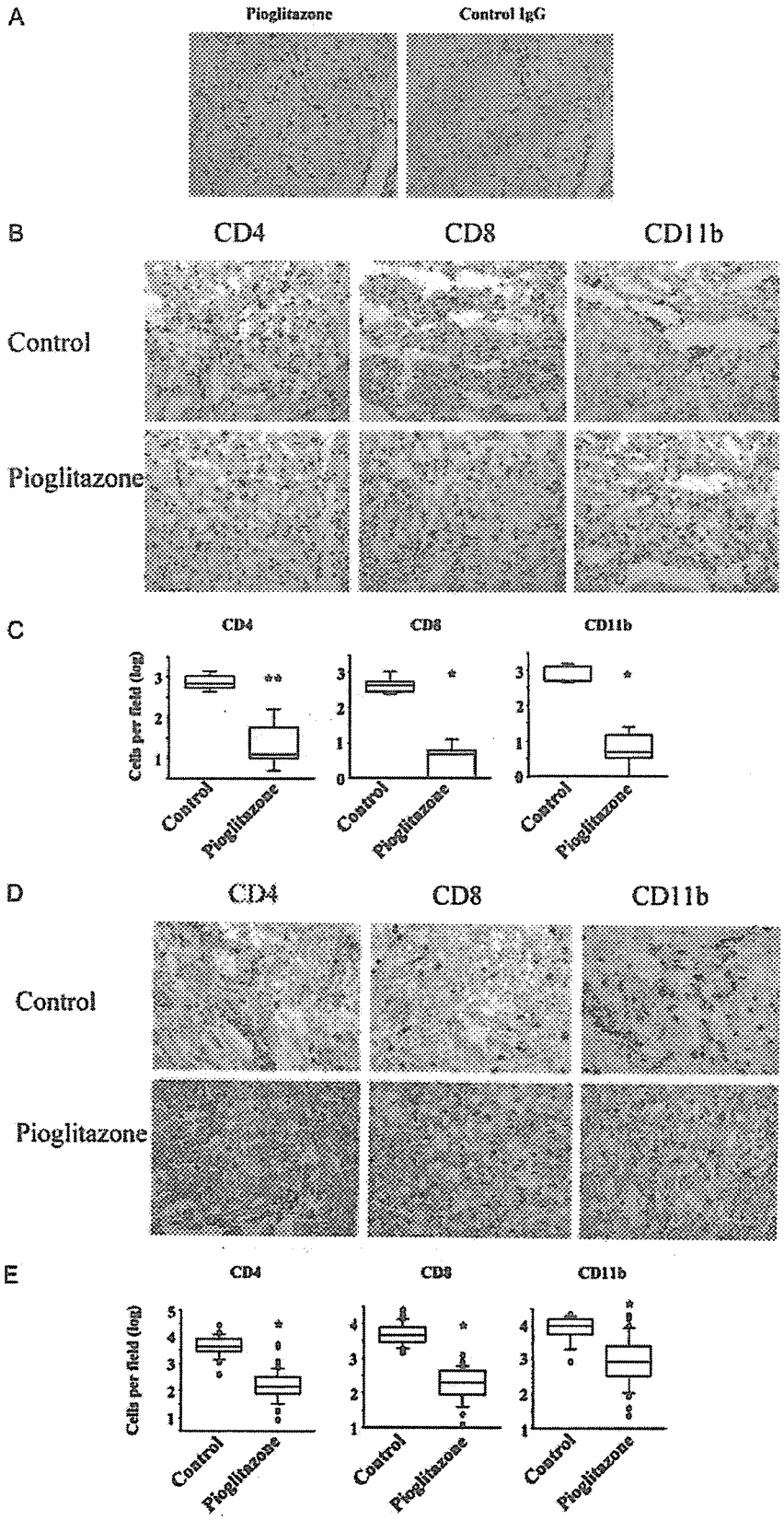

Figure 6. Expression of PPARγ and inhibition of infiltration by CD4-, CD8-, and CD11b-positive cells in donor hearts in the MHC class II–mismatched combination. A, Immunohistochemical staining of PPARγ in cardiac allografts at 2 weeks after transplantation. Representative frozen sections stained with antibody against PPARγ (left) and isotype-matched control IgG (right) are shown. PPARγ expression was identified in cells infiltrating the allografts. Original magnification ×400. B, D, Immunohistochemical staining for CD4, CD8, and CD11b in allografts at 2 and 8 weeks after transplantation. Top, Allografts in mice that received standard chow; bottom, allografts in mice that received chow containing pioglitazone. Representative frozen sections stained with antibodies against CD4, CD8, and CD11b are shown. Original magnification ×400. C, E, Quantitative analysis of CD4-, CD8-, and CD11b-positive cells. Data are expressed as mean±SEM of 20 fields per graft. $^{*}P<0.0001$ vs control. $^{**}P<0.001$ vs control.

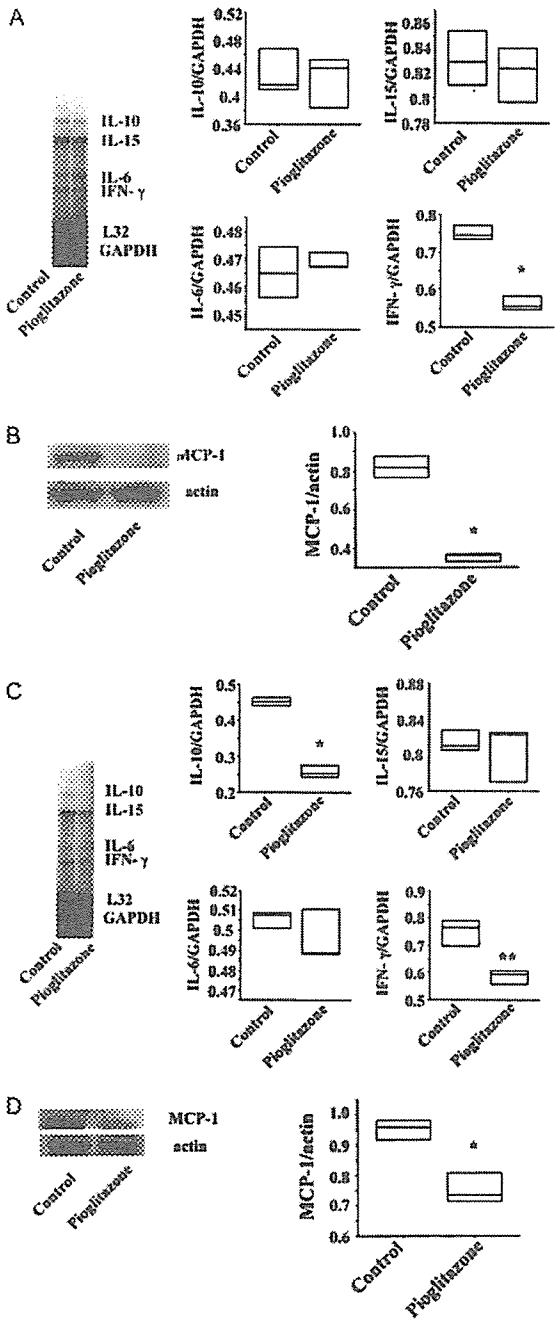

Figure 7. Expression of cytokines and MCP-1 in allografts in the MHC class II–mismatched combination. A, C, Representative data of 3 independent RNase protection assays for expression of cytokine mRNAs. Expression of cytokine mRNAs was normalized to that of GAPDH mRNA. A, Expression of IFN-γ mRNA was significantly lower in allografts treated with pioglitazone than in controls at 2 weeks after transplantation. *$P<0.001$ vs control. C, Expression of IFN-γ and IL-10 mRNAs was significantly lower in allografts treated with pioglitazone than in controls at 8 weeks after transplantation. *$P<0.0005$ vs control. **$P<0.05$ vs control. B, D, Representative data of 3 independent Western blots showing MCP-1 expression. MCP-1 levels were normalized to those of actin. B, MCP-1 levels were significantly lower in allografts treated with pioglitazone than in controls at 2 weeks after transplantation. *$P<0.001$ vs control. D, MCP-1 expression was significantly lower in allografts treated with pioglitazone than in controls at 8 weeks after transplantation. *$P<0.05$ vs control.

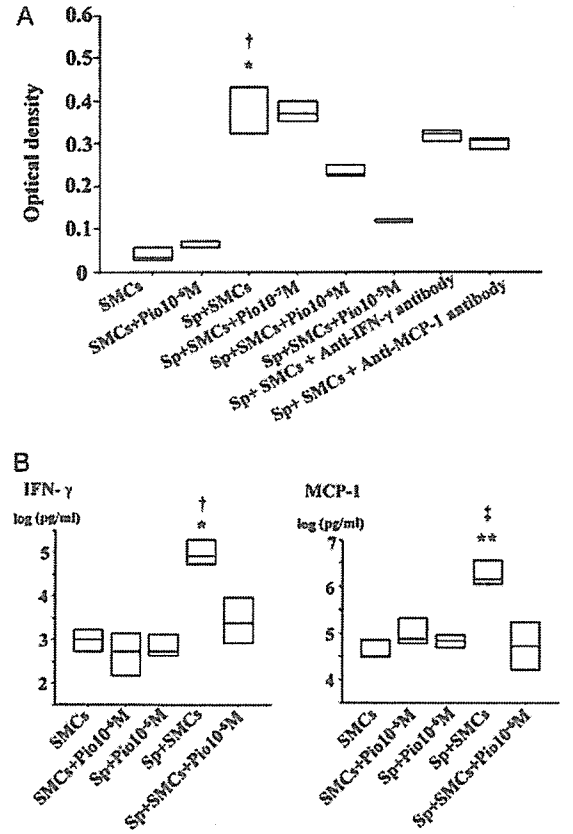

Figure 8. Proliferation of SMCs induced by interactions with splenocytes. A, SMCs and activated splenocytes (Sp) were incubated for 4 days. SMC proliferation was significantly suppressed by pioglitazone (Pio). *$P<0.05$ vs Sp+SMCs+Pio 10^{-6}. †$P<0.0001$ vs SMCs, SMCs+Pio 10^{-6}, and Sp+SMCs+Pio 10^{-5}. B, Production of IFN-γ and MCP-1 in supernatants was suppressed significantly by pioglitazone. Data are expressed as mean±SEM in each group. *$P<0.05$ vs SMCs and Sp+SMCs+Pio. †$P<0.01$ vs SMCs+Pio and Sp+Pio. **$P<0.05$ vs SMCs+Pio and Sp+Pio. ‡$P<0.01$ vs SMCs and Sp+SMCs+Pio.

rejection, expression of IFN-γ and MCP-1 was significantly lower in allografts treated with pioglitazone than in controls. In chronic rejection, treatment with pioglitazone significantly reduced the expression of IFN-γ and MCP-1 in allografts compared with controls. We also found that infiltration of CD4-, CD8-, and CD11b-positive cells was significantly reduced in allografts treated with pioglitazone in acute and chronic rejection; suppression of IFN-γ expression in allografts treated with pioglitazone may be associated with the decrease of graft infiltrating cells, and suppression of MCP-1 expression is associated with infiltration of monocytes. Saubermann et al[28] showed that treatment of PPARγ agonist was associated with reduced expression of Th1 cytokines and increased expression of Th2 cytokines in a murine model of acute colitis. Th2 cytokines play a pivotal role in improving allograft survival and inducing tolerance. However, the effect of IL-10 is controversial. Although IL-10 treatment attenuated the development of autoimmune myocarditis,[29] blockade of IL-10 activity did

not attenuate neointimal thickening after transplantation.[30] In the present study, treatment with pioglitazone significantly suppressed the expression of IL-10 in allografts compared with that in controls in chronic rejection. Suppression of IL-10 expression may be associated with a decrease in the number of graft-infiltrating cells. Further studies are needed to clarify the mechanism involved in the development of neointimal thickening after transplantation.

In conclusion, the present study provides evidence that pioglitazone plays important roles in preventing acute and chronic rejection in a murine model of cardiac transplantation. Several mechanisms are involved in the prevention of allograft rejection by pioglitazone. Pioglitazone suppresses T-cell responses and proliferation of SMCs. Treatment with pioglitazone suppresses the expression of cytokines by allografts in vivo and in vitro. In addition, pioglitazone inhibits recruitment of inflammatory cells in allografts. Suppression of cytokine expression may be associated not only with the regulation of inflammation but also with the decrease in numbers of graft-infiltrating cells. Treatment with pioglitazone may provide a novel strategy for managing acute and chronic rejection in clinical cardiac transplantation.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Cardiac transplantation developed as a therapy for end-stage congestive heart failure. Although the survival rate has improved by administration of immunosuppressive agents, long-term survival is still not satisfactory. Therefore, alternative strategies are needed to regulate acute and chronic allograft rejection. Peroxisome proliferator–activated receptor-γ (PPARγ) plays a crucial role in regulating inflammation. It has been reported that treatment of PPARγ agonists suppresses expression of inflammatory cytokines and the development of atherosclerosis and neointimal hyperplasia after vascular injury. However, the effect of PPARγ agonists on allograft rejection after transplantation has not been fully elucidated. We observed that pioglitazone prolongs allograft survival and attenuates the development of graft vasculopathy in a murine cardiac transplantation model. Furthermore, pioglitazone suppresses T-cell responses and smooth muscle cell proliferation. Our present study provides evidence that treatment of PPARγ agonists prevents acute and chronic allograft rejection after transplantation. However, further studies are necessary to evaluate the therapeutic usefulness of PPARγ agonists in clinical cardiac transplantation.