Transplantation

Pioglitazone Prevents Acute and Chronic Cardiac Allograft Rejection

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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.

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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 responses⁶ 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-
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 PPARY 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-2b) mice 6 to 8 weeks of age were obtained from Japan Clea (Tokyo, Japan). B6.C-H-2*bm12/XHeg (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 with 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. 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. 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.

Immunohistochemistry
Frozen sections (5 μm) were fixed in acetone for 10 minutes at 4°C. 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 (%).

Parenchymal rejection (PR) was assessed in allografts at 5 days after transplantation and at the time of failure in total allomismatch combinations. PR severity was graded with a scale modified from the International Society for Heart and Lung Transplantation and stained with 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–luminal 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. 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=multiple focal infiltrates with necrosis, 4=widespread infiltrates with hemorrhage and/or vasculitis). 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=5) at 5 days after transplantation and mitomycin-C–inactivated stimulator splenocytes from naïve BALB/c mice. A total of 3×10⁵ responder cells and an equal number of stimulator cells were cocultured in 96-well plates at 37°C under 5% CO₂ 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, Kumamoto, 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. 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% CO₂. 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, 5×10⁵) 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) or MCP-1 antibody (2 μg/mL). 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 mmol/L NaF, 1 mmol/L Na₃P₂O₅, 2 mmol/L Na₂VO₃, 100 μg/mL phenylmethylsulfonyl fluoride, and cocktail tablets (Roche, Basel, Switzerland). 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 (Amer sham 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. In vitro transcription was performed with the template set, T7 polymerase, and [α-32P]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).

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 allotransplantation 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.
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 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 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
survival after transplantation has improved substantially. 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. PPARγ agonists also inhibit synthesis of inflammatory cytokines, including IFN-γ, IL-1β, and TNF-α, in human peripheral blood mononuclear cells. Shioi et al 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. 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; 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 allogeneic combination, treatment of mice with pioglitazone significantly prolonged cardiac allograft survival compared with controls. Because PPARγ agonists are associated with T-cell responses, 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. We previously reported that coculture of SMCs and T cells induces SMC proliferation. 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

Discussion

Organ allograft rejection limits long-term survival after transplantation, and immunosuppressive agents have been used clinically to prevent allograft rejection. Although 1-year

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).

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.
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.
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 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, blockade of IL-10 activity did

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<0.0001 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.

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^{-5}. †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.
not attenuate neointimal thickening after transplantation.30
In the present study, treatment with pioglitazone signifi-
cantly 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 al-
lografts 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 piogli-
tazone may provide a novel strategy for managing acute and
chronic rejection in clinical cardiac transplantation.

Acknowledgments
We thank Noriko Tamura and Masahito Ogawa for excellent
technical assistance.

Sources of Funding
This study was supported by grants-in-aid of the Japanese
Ministry of Education, Culture, Sports, Science and Technology
and the Organization for Pharmaceutical Safety and Research.

Disclosures
None.

References
Hertz MJ. Registry of the International Society for Heart and Lung
Transplantation: Twenty-Second Official Adult Heart Transplant
2. Zhang QW, Kish DD, Fairchild RL. Absence of allograft ICAM-1
attenuates alloantigen-specific T cell priming, but not primed T cell
in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression
proliferation-activated receptor-γ ligands ameliorate experimental auto-
5. Marx N, Kehrle B, Kohlhammer K, Grub M, Koenig W, Hombach V,
Law RE, Meehan WP, Xi XP, Graf K, Wuthrich DA, Coats W, Faxon D,
Harrison DG, Marsden PA. Expression of multiple isoforms of nitric
1997;149:2312–2318.
6. Clark RB, Bishop-Bailey D, Estrada-Hernandez T, Hla T, Puddington L,
Harrison DG, Marsden PA. Expression of multiple isoforms of nitric
1997;149:2312–2318.
Y, Okazaki H, Yahagi N, Izuza Y, Shionoiri F, Ohashi K, Harada K,
Suzuki T, Hagiwara N, Nagai R, Yamada N. Pioglitazone inhibits atherosclerosis
in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression
8. Shiomi T, Tsutsui H, Hayashidani S, Soematsu N, Ikeuchi M, Wen J,
Ishibashi M, Kubota T, Egashira K, Takeshita A. Pioglitazone, a
peroxisome proliferator-activated receptor-gamma agonist, attenuates left
ventricular remodeling and failure after experimental myocardial
9. de Dios ST, Bruemmer D, Dillely RJ, Ivey ME, Jennings GL, Law RE,
Little PJ. Inhibitory activity of clinical thiazolidinedione peroxisome
proliferator activating receptor-γ ligands toward internal mammary
artery, radial artery, and saphenous vein smooth muscle cell proliferation.
inflammation in vitro and in vivo by peroxisome proliferator-activated
11. Wang N, Verna L, Chen NG, Chen J, Li H, Forman BM, Steenmer MB.
Constitutive activation of peroxisome proliferator-activated receptor-γ
suppresses pro-inflammatory adhesion molecules in human vascular en-
12. Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of
proliferation-activated receptor-γ ligands ameliorate experimental auto-
14. Law RE, Meehan WP, Xi XP, Graf K, Wuthrich DA, Coats W, Faxon D,
Harrison DG, Marsden PA. Expression of multiple isoforms of nitric
1997;149:2312–2318.
Y, Okazaki H, Yahagi N, Izuza Y, Shionoiri F, Ohashi K, Harada K,
Suzuki T, Hagiwara N, Nagai R, Yamada N. Pioglitazone inhibits atherosclerosis
in apolipoprotein E-knockout mice: pleiotropic effects on CD36 expression
proliferation-activated receptor-γ ligands ameliorate experimental auto-
17. Law RE, Meehan WP, Xi XP, Graf K, Wuthrich DA, Coats W, Faxon D,
Harrison DG, Marsden PA. Expression of multiple isoforms of nitric
1997;149:2312–2318.
18. Kosuge H, Suzuki J, Gotoda T, Koga N, Inobe M, Uede T. Induction of
imunologic tolerance to cardiac allograft by simulta-
neous blockade of inducible co-stimulator and cytotoxic T-lymphocyte
19. Wilcox JN, Subramanian RR, Sundell CL, Tracey WR, Pollock JS,
Harrison DG, Marsden PA. Expression of multiple isoforms of nitric
oxide synthase in normal and atherosclerotic vessels. Arterioscler Thromb
20. Cooper JD, Billingham M, Egan T, Hertz MJ, Higenbottom T, Lynch J,
Mauer J, Paradies I, Patterson GA, Smith C, Trulock EP, Vreim C,
Yousem S. A working formulation for the standardization of nomen-
clature and for clinical staging of chronic dysfunction in lung allografts:
International Society for Heart and Lung Transplantation. J Heart
ligand deficiency induces long-term allograft survival and donor-specific
tolerance in mouse cardiac transplantation but does not prevent graft
Gotoh R, Inobe M, Isobe M, Uede T. Attenuation of graft arterial disease
of the interaction between PD-1 and PD-L1 accelerates graft arterial
disease in cardiac allografts. Arterioscler Thromb Vasc Biol. 2004;24:
2057–2062.
24. Watanabe T, Pakara R, Katagiri T, Benedict CR. Monocyte chemotactic
protein 1 amplifies serotonin-induced vascular smooth muscle cell pro-
25. Hosenpud JD, Bennett LE, Keck BM, Boucek MM, Novick RJ. The
Registry of the International Society for Heart and Lung Transplantation: Eigh
805–815.
WL. Activation of human T lymphocytes is inhibited by peroxisome
proliferator-activated receptor γ (PPAR-γ) agonists. PPARγ co-association with transcription factor NFAT. J Biol Chem.
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.
Pioglitazone Prevents Acute and Chronic Cardiac Allograft Rejection
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Circulation. 2006;113:2613-2622; originally published online May 30, 2006; doi: 10.1161/CIRCULATIONAHA.105.594101
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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