Kobe University Repository : Kernel

タイトル Title	Pioglitazone preserves vein graft integrity in a rat aortic interposition model.
著者 Author(s)	Zin Chen / Hasegawa, Tomomi / Tanaka, Akiko / Okita, Yutaka / Okada, Kenji
掲載誌・巻号・ページ Citation	Journal of thoracic and cardiovascular surgery,140(2):408-416.e1
刊行日 Issue date	2010-08
資源タイプ Resource Type	Journal Article / 学術雑誌論文
版区分 Resource Version	author
権利 Rights	
DOI	10.1016/j.jtcvs.2009.11.067
URL	http://www.lib.kobe-u.ac.jp/handle_kernel/90001468

Create Date: 2017-12-20

ARTICLE IN PRESS

Pioglitazone preserves vein graft integrity in a rat aortic interposition model

Zhi Chen, MD, Tomomi Hasegawa, MD, PhD, Akiko Tanaka, MD, Yutaka Okita, MD, PhD, and Kenji Okada, MD, PhD

Objective: Improvement of vein graft patency may be highly beneficial in coronary artery bypass grafting, but graft degeneration is considered to be one of the main pathophysiologic causes for vein graft failure. Because peroxisome proliferator-activated receptor-γ activator pioglitazone was recently reported to possess pleiotropic protective effects on various organs and tissues, we conducted experiments to test the hypothesis that pioglitazone could prevent graft degeneration, leading to the preservation of vein graft integrity.

Methods: In a rat aortic interposition model with autologous femoral vein, pioglitazone (3 mg/kg/d) or vehicle (normal saline) was given to rats by gastric gavage once per day beginning 3 days before surgery and ending 8 weeks after surgery. Vein graft degeneration and remodeling were assessed at 24 hours, 7 days, 8 weeks, and 6 months after surgery.

Results: At 24 hours, pioglitazone significantly reduced endothelial desquamation, reactive oxygen species generation, myeloperoxidase activity, and lipid peroxidation in vein grafts. At 7 days, mRNA expression and gelatinolytic activity of matrix metalloproteinase-2 and 9 in vein grafts were significantly suppressed by pioglitazone treatment. Immunofluorescent staining showed that pioglitazone enhanced peroxisome proliferator-activated receptor-γ expression in vein grafts at 8 weeks, especially in their intimal side. At 6 months, pioglitazone treatment prevented graft dilation (52.3% $\pm$ 3.1% vs 90.7% $\pm$ 9.9%, $P = .0041$) and neointimal hyperplasia (14.6% $\pm$ 1.3% vs 29.9% $\pm$ 2.9%, $P = .0008$), and increased graft flow velocity ratio (0.86 $\pm$ 0.03 vs 0.59 $\pm$ 0.04, $P < .0001$), compared with vehicle treatment.

Conclusion: Pioglitazone prevents graft degeneration under arterial pressure stress and preserves the vein graft integrity in a rat aortic interposition model. (J Thorac Cardiovasc Surg 2010;■:1-9)

ET/BS

Supplemental material is available online.

Although the clinical use of arterial conduits, such as the internal thoracic artery or radial artery, is a current trend in coronary artery bypass grafting (CABG) because of its superior long-term patency, the saphenous vein is still widely used as the bypass conduit. After grafting, the vein grafts are immediately exposed to arterial pressure and pulsatile blood flow, thereby increasing wall tension and shear forces. These hemodynamic changes and physiologic stresses lead to graft overdistention, endothelial damage, and dysfunction, and involve proliferation and migration of smooth muscle cells (SMCs) into the vein graft. Eventually, the vein grafts might undergo vascular remodeling, including neointimal hyperplasia, which is considered to be one of the major causes of vein graft failure.[1,2]

Thiazolidinediones, which include the presently available drug pioglitazone, are well established insulin-sensitizing agents that act as agonists of the ligand-activated transcriptional factor peroxisome proliferator-activated receptor (PPAR)-γ.[3] It was recently reported that pioglitazone possesses pleiotropic cardiovascular protective actions in experimental settings, including anti-inflammatory and antiproliferative properties.[4,5] Migration and proliferation of vascular SMCs are inhibited by pioglitazone,[6] and pioglitazone reduces hypertensive vascular injury and hypertrophy.[7,8] However, there is no report to elucidate an efficacy of pioglitazone on vein grafts in the arterial environment.

This study evaluates whether pioglitazone has the potential to preserve vein graft integrity in the arterial environment associated with preventing graft distension and intimal hyperplasia.

MATERIALS AND METHODS

Animals

Sixty male Sprague-Dawley rats weighing 450 to 550 g (10 weeks of age, CLEA Japan Inc, Tokyo, Japan) were used in the present study. The handling of laboratory animals and their use in experiments conformed to the *Guidelines for Animal Experiment at Kobe University Graduate School*

From the Department of Surgery, Division of Cardiovascular Surgery, Kobe University Graduate School of Medicine, Kobe, Japan.

Disclosures: None.
Supported by the Japan Heart Foundation Research Grant.
Received for publication Sept 2, 2009; revisions received Nov 10, 2009; accepted for publication Nov 27, 2009.

Address for reprints: Kenji Okada, MD, PhD, Department of Surgery, Division of Cardiovascular Surgery, Kobe University Graduate School of Medicine, 7-5-2, Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan (E-mail: yutamo@aol.com).

0022-5223/$36.00

Copyright © 2010 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2009.11.067

ARTICLE IN PRESS

Abbreviations and Acronyms

CABG = coronary artery bypass grafting
MMP = matrix metalloproteinase
PPAR = peroxisome proliferator-activated receptor
SMA = smooth muscle actin
SMC = smooth muscle cell
TIMP = tissue inhibitor of matrix metalloproteinase

of Medicine (permission number: P080120) and the *Guide for the Care and Use of Laboratory Animals* (www.nap.edu/catalog/5140.html).

Drug Treatment

Pioglitazone, provided by Takeda Chemical Industries (Tokyo, Japan), was diluted in normal saline. Pioglitazone (3 mg/kg/d)[9,10] or vehicle (normal saline) was given to rats by gastric gavage once per day beginning 3 days before surgery and ending 8 weeks after surgery.

Rat Aortic Bypass Model

Rats were placed in a closed chamber containing diethyl ether (Wako, Osaka, Japan). After loss of consciousness, the rats were weighed and anesthetized with pentobarbiturate (5 mg/kg, intraperitoneally). The anesthetized rats were placed under a Leica M651 operating microscope (Leica Microsystems, Heerbrugg, Switzerland) with 6× to 10× magnification. The right femoral vein was harvested and rinsed in heparinized saline. The infrarenal abdominal aorta was transected and interposed with the autologous femoral vein graft (1.0 cm in length) by end-to-end anastomosis with a continuous running suture of 8-0 polypropylene (PROLENE; Johnson & Johnson Gateway, LLC, Piscataway, NJ). The right femoral vein was not reconstructed. After the anastomosis, graft patency was evaluated by direct visualization before layered surgical closure. The surgeon (Z.C.) was blinded to treatment group in the present study. A 1-week postoperative prophylactic antibiotic regimen was followed for all rats. Their body weight was measured once per week. For monitoring pioglitazone-related adverse effects, the rats were observed every day postoperatively.

Study Design

The present study design is shown in Figure E1. The end points of the present study were 24 hours, 7 days, 8 weeks, and 6 months after surgery in each group. The experiments of 7-day, 8-week, and 6-month end points were performed on 6 rats per each group. The 24-hour experiment was performed in 2 subsets of each group, which provided samples for Evans blue assay (n = 6 in each group) and other assays (n = 6 in each group). The 7-day samples were used for matrix metalloproteinase (MMP) analysis, and the 8-week and 6-month samples were used for physiologic, morphometric, and histologic analyses.

Macroscopic Assessments

Animals were anesthetized, and the abdominal aorta was exposed and photographed with a Leica IC D digital camera (Leica Microsystems). Graft diameter was measured under physiologic conditions with an optical micrometer by the person (A.T.) who was blinded to the different treatment groups. The graft diameter was defined as the maximum dimension of the transverse minor axis of the vein graft, and the graft dilation was calculated by the following formula according to our previous study:[11] graft dilation ratio (%) = (diameter at harvesting − diameter at implantation)/diameter at implantation × 100.

The velocity of blood flow through the vein graft was measured using a Transonic flowmeter (3 mm in size of the probe; Transonic System Inc, New York, NY) and recorded with PowerLab/MacLab800 (AD Instruments Japan Inc, Nagoya, Japan). It was measured at the proximal aorta, vein graft, and distal aorta, and the mean value was taken as the flow velocity. The graft flow velocity ratio was calculated according to the following formula: graft flow velocity ratio = graft flow velocity/proximal aorta flow velocity.

Histology

Harvested tissues were rinsed in saline and fixed in 10% formalin. The formalin-fixed samples were dehydrated in a graded ethanol bath, cleaned in xylene, and embedded in paraffin. Sections (5 μm thick) were stained by hematoxylin–eosin and Elastica von Gieson. After those images were captured using a microscopic system (BZ-8100, KEYENCE Co, Osaka, Japan), histologic analyses of the sections were performed using ImageJ version 1.41 software (National Institutes of Health, Bethesda, Md). The neointimal thickness at the anastomosis site was calculated by converting the number of pixels to micrometers using a stage micrometer on the captured images, and the neointimal hyperplasia at the aneurysmal site was calculated by using the following formula[11]: neointimal hyperplasia (%) = neointimal area/area bounded by the media × 100.

Immunohistochemical Staining

Immunohistochemical staining was performed on the paraffin-embedded section by using ENVISION kit/HRP (DAKO, Kyoto, Japan) with primary antibody, anti-human α-smooth muscle actin (αSMA)/horseradish peroxidase (DAKO). Diaminobenzidine substrate (DAKO) was used as a chromogen, and cell nuclei were counterstained with hematoxylin. The αSMA-positive area in a cross-section was semiquantified using the ImageJ software and calculated by dividing the αSMA-positive area by the cross-sectional vein graft area.

Immunofluorescent Staining

Immunofluorescent staining was performed on the paraffin-embedded section with primary antibody, mouse monoclonal anti-PPAR-γ antibody (Santa Cruz Biotechnology, Santa Cruz, Calif). Fluorescein-linked sheep anti-mouse antibody was used as secondary antibody (Amersham Biosciences, Buckinghamshire, UK). The cell nuclei were counterstained with 4′,6-diamidino-2-phenylindole (Chemicon International, Temecula, Calif).

Evans Blue Staining

To evaluate the endothelial desquamation in the whole area of the vein graft at 24 hours after surgery, Evans blue staining was performed as described previously with some modifications.[11,12] Briefly, Evans blue (10 mg/kg; Sigma-Aldrich, St Louis, Mo) was injected intravenously 2 hours before vein graft harvesting. After perfusion-fixation with 100% methanol, vein grafts including both proximal and distal anastomotic sites were harvested and opened longitudinally for macroscopic examination. After the vein graft was separated from aorta, its uptake of Evans blue was quantified by formamide extraction (55°C for 2 hours) measuring absorbance at 595 nm. The concentration was determined by the calibration curve, and the data were standardized to the protein concentration of each sample as determined by a micro BCA protein assay kit (Pierce, Rockford, Ill).

Myeloperoxidase Assay

MPO activity in vein grafts at 24 hours after surgery was assessed as previously described.[12] MPO values were standardized to the protein concentration of each sample. Data are expressed as the change in absorbance at 450 nm/min/mg of total protein.

Lipid Peroxidation Assay

Lipid peroxidation in vein grafts at 24 hours after surgery was assessed using spectrophotometric assay for malondialdehyde (BIOXYTECH MDA-586; OXIS International Inc, Foster City, Calif), according to the manufacturer's instructions. Data are standardized by protein concentration.

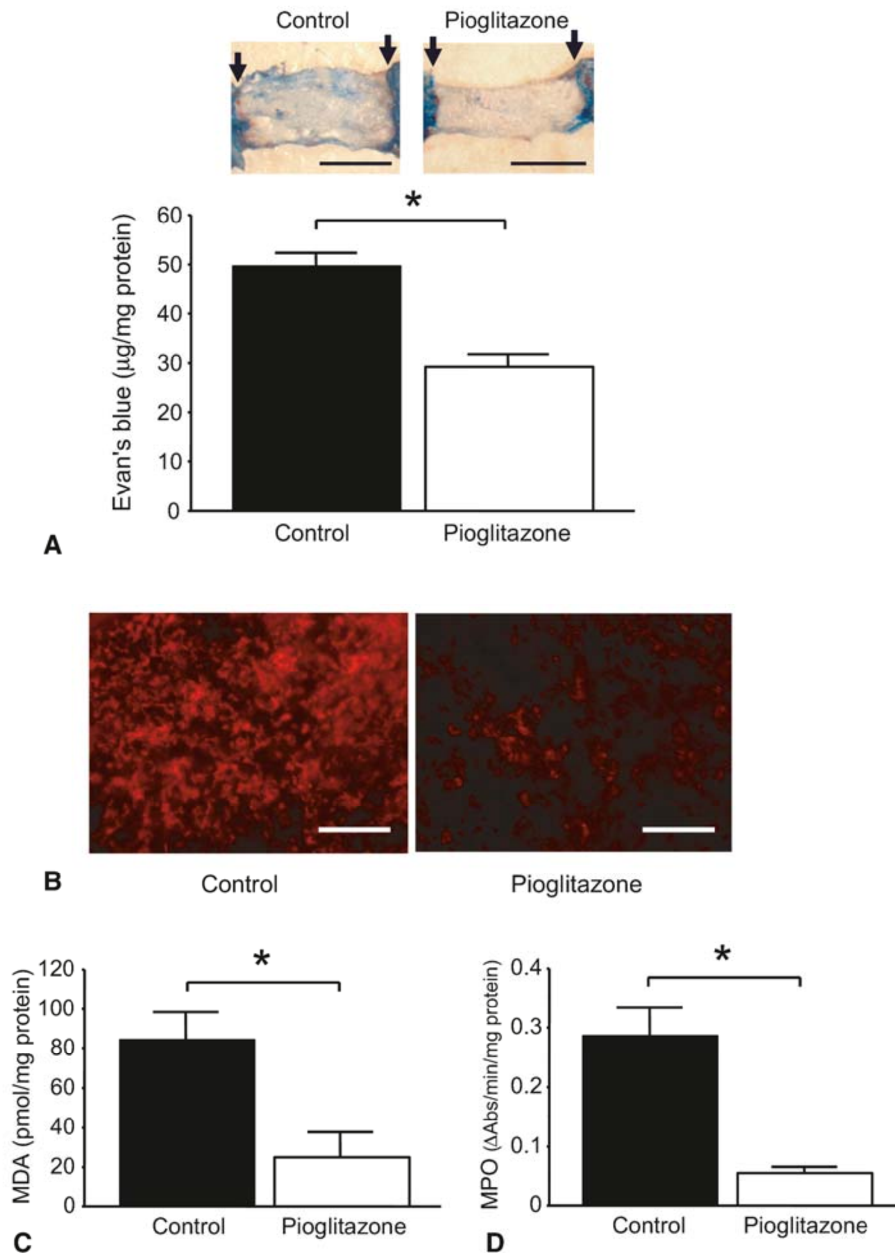

FIGURE 1. Effects of pioglitazone on endothelial desquamation and oxidative stress in vein grafts at 24 hours. A, In vivo Evans blue staining of vein grafts. Anastomotic sites (*arrows*). Bar = 5 mm. B, In situ detection of superoxide generation (*red fluorescence*). Bar = 100 μm. C, Malondialdehyde levels as an indicator of lipid peroxidation. D, MPO activity. ΔAbs, change in absorbance. *$P < .05$. All data are expressed as mean ± standard error of the mean (SEM) for n = 6 per group. *MDA,* Malondialdehyde.

ET/BS

Quantitative Real-Time Polymerase Chain Reaction Analysis

Total RNA was isolated from graft samples using an RNeasy fibrous tissue mini-kit (Qiagen, Venlo, The Netherlands) according to the manufacturer's instructions. The RNA was transcribed and amplified to cDNA using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, Calif). Quantitative real-time polymerase chain reaction analysis for mRNA of MMP-2, MMP-9, tissue inhibitor of MMP (TIMP)-1, and TIMP-2 was performed using the ABI Prism 7500 sequence detector system (Applied Biosystems) with TaqMan universal polymerase chain reaction master mix and TaqMan real-time polymerase chain reaction primers (Applied Biosystems). The expression level of each mRNA was divided by the mRNA level of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase.

Gelatin Zymography

Proteins of graft samples were extracted using a buffer containing 50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1% Triton X-100, 0.2% sodium dodecyl sulfate, and 1 mmol/L EDTA, supplemented with protease inhibitors (20 μg/mL aprotinin, 10 μg/mL leupeptin, and

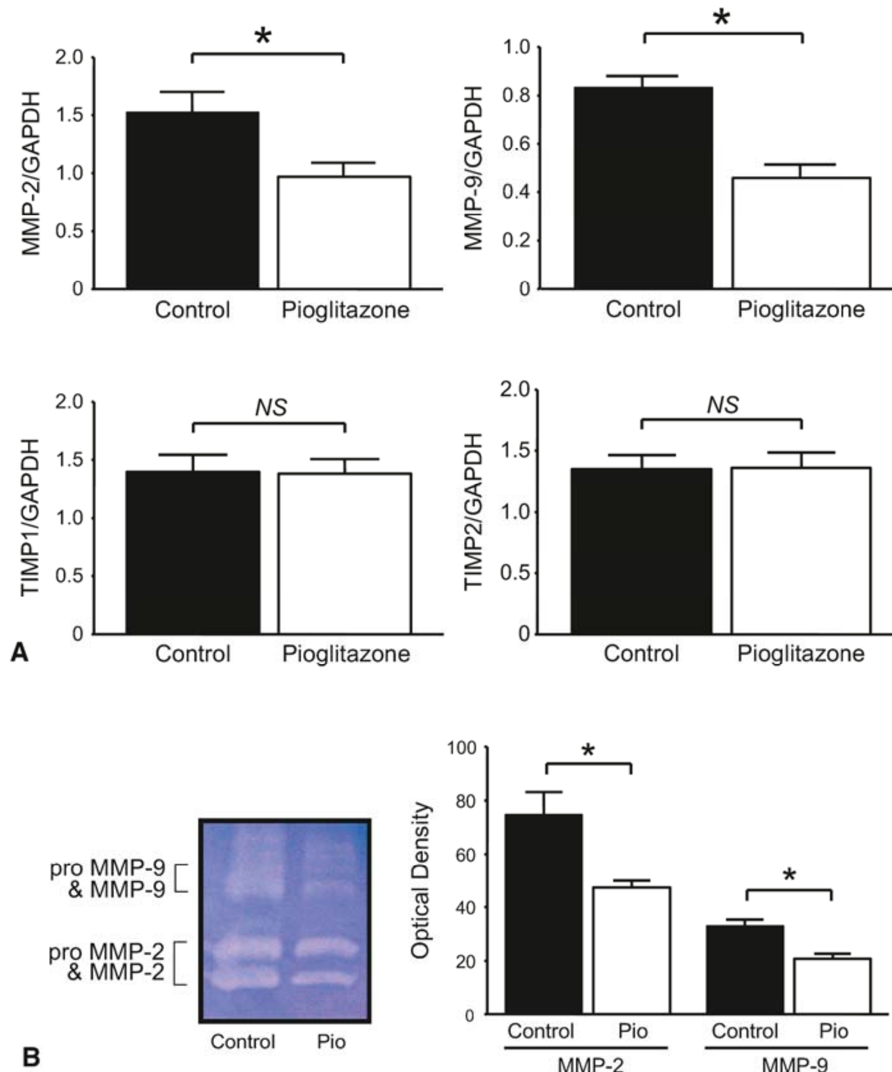

FIGURE 2. Protease activity and mRNA expression in vein grafts at 7 days. A, mRNA expression of MMP-2, MMP-9, TIMP-1, and TIMP-2. B, Gelatinolytic activities of MMP-2 and MMP-9 by gelatin zymography, and their densitometric analysis. *$P < .05$. All data are expressed as mean ± SEM for n = 6 per group. *Pio,* Pioglitazone; *NS,* not significant; *MMP,* matrix metalloproteinase; *GAPDH,* glyceraldehyde-3-phosphate dehydrogenase.

1 mmol/L phenylmethylsulfonyl fluoride). To determine gelatinolytic activities of MMP-2 and MMP-9 in vein grafts, the gelatin-zymography kit (Primary Cell Co, Hokkaido, Japan) was used according to the manufacturer's instructions. After the protein concentration was standardized, 20 μg of protein was applied in each lane to the electrophoresis. Densitometric analysis of lytic bands for MMP-2 and MMP-9 was performed by ImageJ version 1.41.

Statistics

Database management and statistical analysis were performed with Statview Software Version 5.0 (SAS Institute Inc, Cary, NC). All values are expressed as mean ± standard error of the mean. Statistical analyses of data were performed by unpaired Student *t* test or 1-way analysis of variance by the Bonferroni post hoc test, as appropriate.

RESULTS

The surgical ischemic time during an end-to-end interposition of the vein graft was approximately 20 minutes in both the pioglitazone and control groups. There was no significant difference in the ischemic time between the 2 groups. There was neither technical failure nor death intraoperatively, and all animals have uneventfully survived until each end point. No thrombus formation in vein grafts was observed postoperatively. During the postoperative observation, there were no significant differences of body weight change or histologic findings of heart, lung, liver, and kidney between the pioglitazone and control groups postoperatively.

Preservation of Endothelial Function

To evaluate endothelial desquamation of vein grafts caused by exposure to the arterial environment, the grafts were stained with Evans blue dye, which is believed to penetrate intimal areas that were not covered by endothelium.[11]

ARTICLE IN PRESS

At 24 hours after surgery, vein grafts in the control treatment were stained more deeply with Evans blue than those in pioglitazone treatment. Quantitatively, the uptake of Evans blue in vein grafts was significantly less in the pioglitazone group compared with the control group (29.1 ± 2.5 μg/mg protein vs 45.6 ± 2.8 μg/mg protein, $P = .0003$; Figure 1, *A*). Because the arterial pressure stretch of vein grafts would induce a generation of reactive oxygen species and oxidative injury,[13,14] we next evaluated oxidative stress in vein grafts at 24 hours. The intensity of red oxidative fluorescence in the pioglitazone group was apparently lower than in the control group (Figure 1, *B*). The graft oxidative damage, quantified by malondialdehyde levels in vein grafts, was significantly reduced by pioglitazone treatment compared with the control treatment (25 ± 13 pmol/mg protein vs 85 ± 14 pmol/mg protein, $P = .0104$; Figure 1, *C*). MPO activity in the pioglitazone group was also significantly less than in the control group (0.06 ± 0.01 ΔAb/min/mg vs 0.29 ± 0.05 ΔAb/min/mg protein, $P = .0009$; Figure 1, *D*).

Suppression of Matrix Metalloproteinase

The arterial pressure stretch of vein grafts also enhances the activity of MMPs, which are especially important regulators of vein graft architecture because of their specificity for elastin.[15] At 7 days after surgery in the present study, the MMP-2 and MMP-9 mRNA expressions were significantly down-regulated by pioglitazone treatment compared with control treatment (MMP-2, 0.97 ± 0.12 vs 1.53 ± 0.18, $P = .0254$; MMP-9, 0.46 ± 0.06 vs 0.83 ± 0.05, $P = .0004$; Figure 2, *A*). Gelatinolytic activities of MMP-2 and MMP-9 proteins were also significantly lower in the pioglitazone group than in the control group (MMP-2, 47.4 ± 2.6 vs 74.6 ± 8.4, $P = .0113$; MMP-9, 20.9 ± 1.7 vs 33.0 ± 2.5, $P = .0023$; Figure 2, *B*). Because the MMP activities are tightly regulated by TIMPs,[15] we also evaluated mRNA expression of TIMP-1 and TIMP-2 in vein grafts. However, there were no significant differences in these mRNA expressions between the pioglitazone and control groups (Figure 2, *A*).

Maintenance of Graft Blood Flow Velocity

There was no significant difference in mean graft blood flow velocity immediately after surgery between the groups. Although the graft flow velocity ratio gradually decreased after surgery, it was significantly higher in the pioglitazone group than in the control group at both 8 weeks and 6 months after surgery (8 weeks, 0.87 ± 0.02 vs 0.66 ± 0.05; 6 months, 0.86 ± 0.03 vs 0.59 ± 0.04, $P < .0001$ by analysis of variance; Figure 3).

Prevention of Graft Dilation

Graft dilation was observed under physiologic conditions at 8 weeks and 6 months. At both 8 weeks and 6 months, the graft dilation ratios were significantly lower in the pioglitazone group than in the control group (8 weeks, 36.3% ± 2.2% vs 61.4% ± 3.9%, $P = .0002$; 6 months, 52.3% ± 3.1% vs 90.7% ± 9.9%, $P = .0041$; Figure 4, *A*)

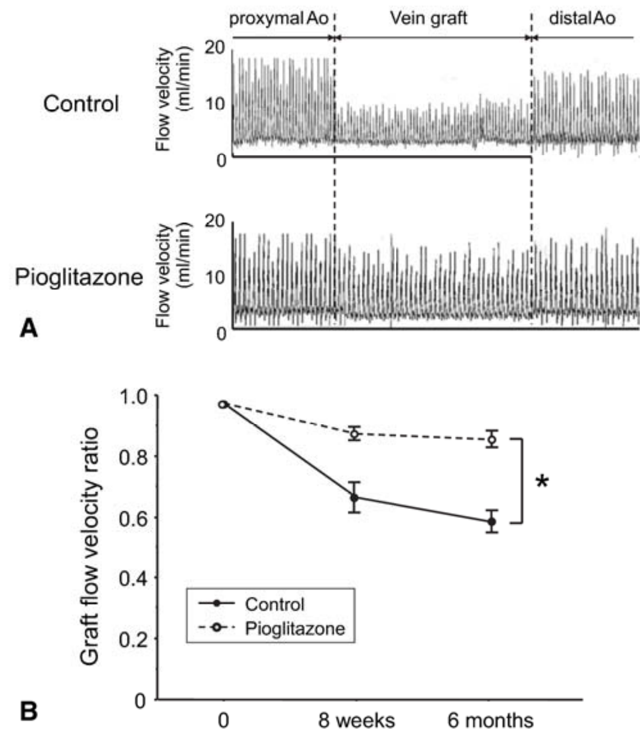

FIGURE 3. Graft flow velocity in vein grafts. A, Blood flow waveforms in proximal aorta, vein graft, and distal aorta at 6 months. B, Graft flow velocity ratio at 8 weeks and 6 months. *$P < .05$ by analysis of variance. All data are expressed as mean ± SEM for n = 6 per group. *Ao*, Aorta.

Enhancement of Peroxisome Proliferator-Activated Receptor-γ Expression

Immunofluorescent staining has shown that pioglitazone treatment up-regulates PPAR-γ expression in vein grafts at 8 weeks after surgery compared with the control (Figure 4, *B*). There was especially a stronger enhancement of PPAR-γ expression in the intimal side of grafts than in the adventitial side by pioglitazone.

Reduction of Neointimal Hyperplasia

At 6 months, neointimal hyperplasia and SMC proliferation in vein grafts were assessed by Elastica von Gieson staining and αSMA immunostaining, respectively (Figure 5, *A, B*). Pioglitazone treatment reduced both neointimal hyperplasia and αSMA-positive area significantly compared with the control group (neointimal hyperplasia: 14.6% ± 1.3% vs 29.9% ± 2.9%, $P = .0008$; αSMA-positive area: 27.7% ± 2.0% vs 47.6% ± 3.6%, $P = .0007$). At the anastomosis site of vein grafts, neointimal thickness was also significantly thinner in the pioglitazone group than in the control group (67.6 ± 2.1 μm vs 134.6 ± 19.6 μm, $P = .0069$; Figure 5, *C*).

ET/BS

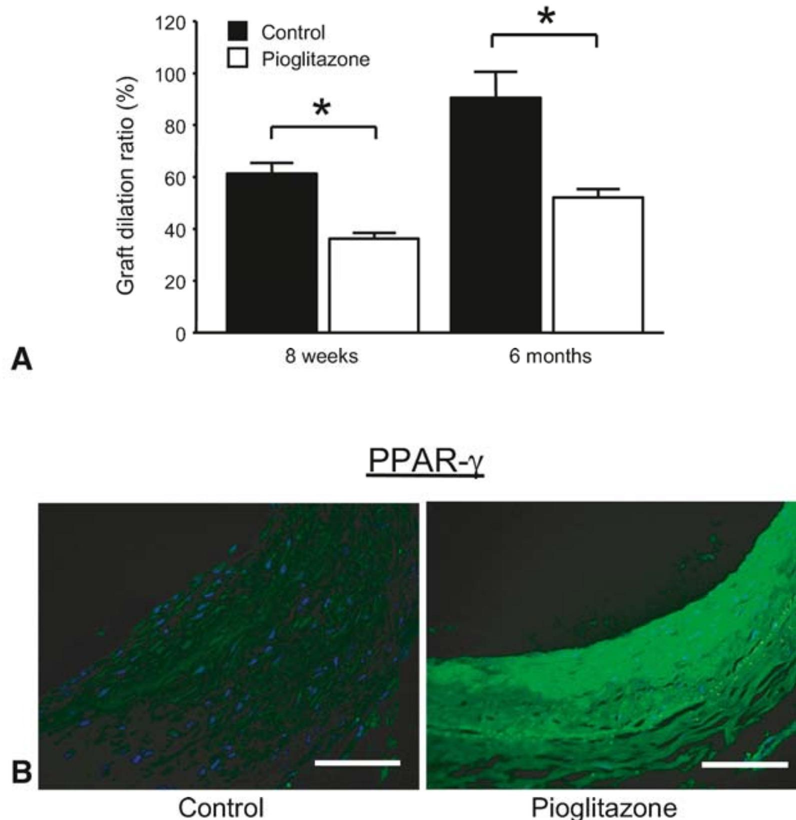

FIGURE 4. A, Graft dilation ratio at 8 weeks and 6 months. *$P < .05$. All data are expressed as mean ± SEM for n = 6 per group. B, Immunofluorescent staining of PPAR-γ in vein grafts at 8 weeks. Intracellular expression of PPAR-γ is identified as the *green* emission. Cell nuclei are identified by the *blue* emission. Bar = 200 μm. *PPAR,* Peroxisome proliferator-activated receptor.

DISCUSSION

It is well recognized that inevitable exposure of vein grafts to the arterial system with high pressure and pulsatility contributes to their circumferential stretch. It leads to structural and functional changes in the graft wall, which is known as vascular remodeling. We have first described that pioglitazone can regulate the distended vascular remodeling of vein graft, likely as the result of PPAR-γ up-regulation. The main findings of this study are that pioglitazone treatment can (1) prevent acute graft damage caused by both mechanical and oxidative stresses, (2) regulate the extracellular matrix integrity by suppressing MMP-2 and MMP-9 activities, (3) inhibit the graft dilation and maintenance of the graft flow velocity, and (4) reduce neointimal hyperplasia with less SMC proliferation in vein grafts.

It is not surprising that pioglitazone enhances PPAR-γ expression in vein grafts. Previous experimental studies have demonstrated that PPAR-γ activation with thiazolidinediones has protective effects against hypertensive vascular injury and hypertrophy.[7,8] Notably, treatment with PPAR-γ agonists, such as pioglitazone and rosiglitazone, reduces aortic aneurysmal formation in mice.[16,17] Therefore, there would be obvious potential benefits of vein graft protection induced by PPAR-γ activation with pioglitazone. In the present study, the enhancement of PPAR-γ expression was clearly observed in the intimal side of vein grafts, possibly because of a direct pharmacologic effect of pioglitazone carried through the bloodstream. Although pioglitazone has some clinical drawbacks, such as causing fluid retention and congestive heart failure, we believe that pioglitazone has numerous advantages as long as the dose is optimal. In accordance with the previous report,[9,10] we administered the same dosage (3 mg/kg/d) of pioglitazone in this study without those adverse effects.

Circumferential pressure stretch of the vein graft is believed to contribute to the degradation of its endothelium. The average circumferential pressure stress in the vein graft can be increased by 140 times or greater compared with that in a native vein.[2] Liu and colleagues[18] reported that vein grafts exposed to arterial pressure lost up to 60% of the endothelial cells and SMCs within 12 hours after surgery. Our previous study also demonstrated that the endothelium of vein grafts was desquamated and that the media disrupted at 24 hours after surgery.[11] In the present study, the endothelial desquamation of vein graft, which was evaluated with Evan's blue staining, was significantly reduced by

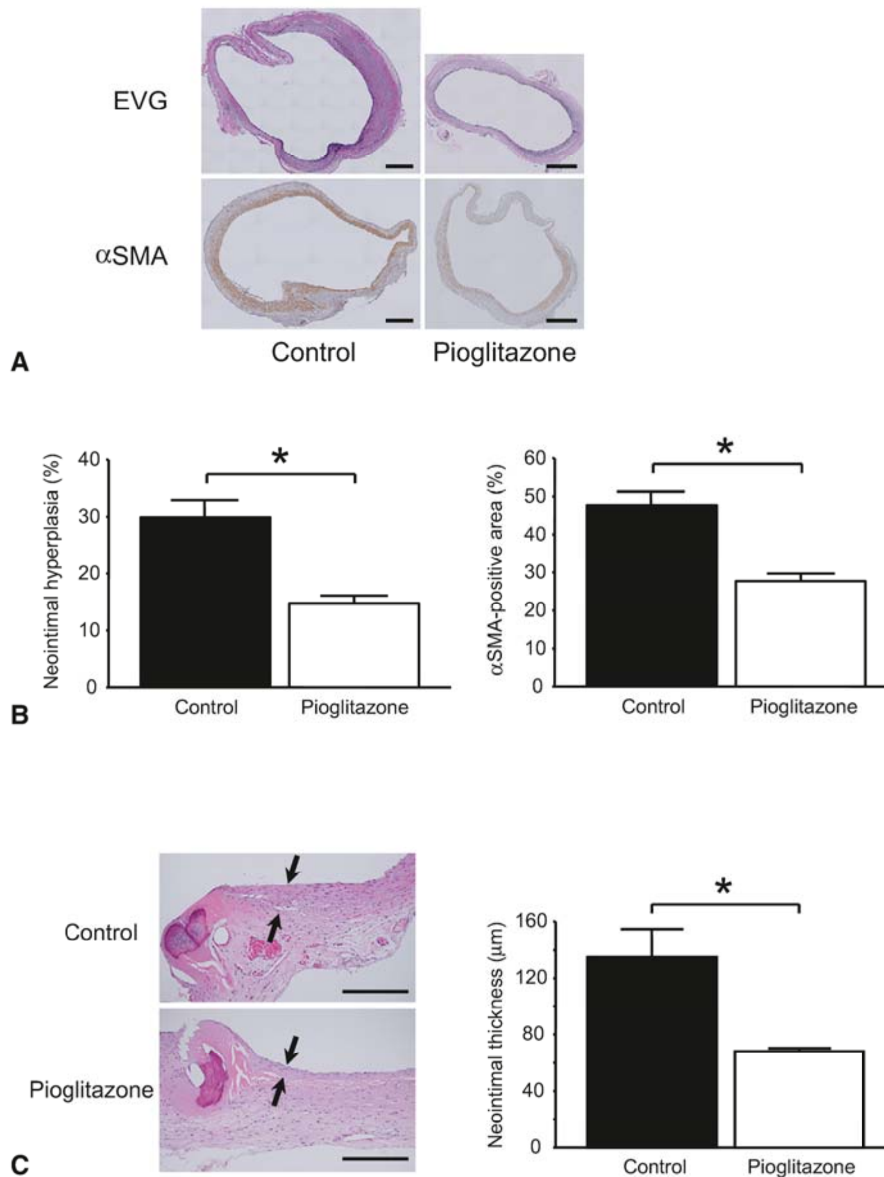

FIGURE 5. Histologic findings in vein grafts at 6 months. A, Elastin von Gieson staining and αSMA immunostaining. Bar = 500 μm. B, Area of neointimal hyperplasia and αSMA-positive area. C, Neointimal thickness at anastomosis site (hematoxylin–eosin staining). Bar = 200 μm. *$P < .05$. All data are expressed as mean ± SEM for n = 6 per group. *EVG,* Elastin von Gieson; *SMA,* smooth muscle actin.

pioglitazone treatment. The arterial pressure stretch with endothelial damage in vein grafts induced a generation of reactive oxygen species and an activation of early inflammation.[19] Reactive oxygen species were produced by all vascular cells, including endothelial cells, SMCs, and infiltrated leukocytes. Increased superoxide generation was observed in the vein grafts by dihydroethidium staining in the present study. Pioglitazone treatment effectively attenuated the superoxide generation. Moreover, both MPO activity as a producer of leukocytic activity and lipid peroxidation as an indicator of oxidative lipid damage in vein grafts were also significantly suppressed by pioglitazone. Because oxidative stress including lipid peroxidation has been implicated in the initiation and progression of vein graft failure,[13] these results suggest the subsequent vein graft failure.

The activities of extracellular matrix-degrading MMPs, which are important regulators of vein graft architecture,[15] have a strong mechanistic link to radical oxygen species generation in vascular aneurysm formation.[20] In addition, the upregulation of MMPs, especially MMP-2 and MMP-9, have been shown to induce SMC proliferation and migration leading to neointimal formation in vein grafts.[15] The present study demonstrated that pioglitazone treatment downregulated MMP-2 and MMP-9 expressions and activities at

7 days after surgery. Although the MMP activities are regulated by TIMPs,[15] pioglitazone did not affect significant changes of TIMP-1 and TIMP-2 mRNA expression in vein grafts. These results suggest that pioglitazone can regulate the extracellular matrix integrity by suppressing MMP-2 and MMP-9, eventually resulting in the regulation of vein graft remodeling, such as dilation and neointimal formation.

Poiseuille's law defines that increasing diameter in vessels induces a decrease of flow velocity with low shear stress. The present study showed distinct circumferential dilation with low flow velocity after vein grafting. Pioglitazone treatment significantly suppressed the graft dilation and maintained the graft flow velocity possibly with good shear stress. In addition, both high circumferential pressure stress and low shear stress are the main triggers for the development of neointimal hyperplasia in vein grafts, associated with subsequent SMC proliferation.[2,21] The present study showed that pioglitazone treatment significantly suppressed SMC proliferation and neointimal hyperplasia at the aneurysmal site and the anastomosis site of vein grafts. These results suggest that pioglitazone could preserve vein graft integrity and regulate its remodeling in the chronic phase. Although the mechanism of preservation of the vein graft integrity was not elucidated in the present study, Duan and colleagues[22] currently reviewed the effects of PPAR-γ in the vasculature and showed that PPAR-γ activation inhibits SMC proliferation and migration, as well as endothelial inflammation. They introduced several mechanisms of the inhibitory effect on SMC proliferation in their review, and one of the interesting mechanisms is that the suppression of ROS production via PPAR-γ activation contributes to the inhibition of SMC proliferation and migration via an inhibition on telomerase activity or MMP-9 activity. Further studies are needed to elucidate the mechanism of preservation of the vein graft integrity by pioglitazone.

Study Limitations

This study has some limitations that deserve attention. There is some discrepancy between the rat aortic interposition model and clinical CABG, with regard to species, heterotopic grafting, grafting technique, and physiologic hemodynamics. The present study has not established the dose-response relation of pioglitazone to a preservation of the vein graft integrity. Although other doses or dosing regimens of pioglitazone were not applied in the present study, previous studies have demonstrated that plasma pioglitazone concentration in the animals treated with 3 mg/kg/d is within the range achieved in humans after oral administration of its clinical dosage (30 mg/d).[9,23] Current studies have shown that pioglitazone prevented reactive hypoglycemia in patients with an impaired glucose tolerance[24] and improved coronary endothelial function without any change in blood glucose, insulin, or hemoglobin-A1c levels in nondiabetic patients with coronary artery disease.[25] Although adverse effects of pioglitazone were not observed in the present study, the use of pioglitazone in the patients who underwent CABG, particularly those with congestive heart failure and cardiomyopathies, might be the high incidence of fluid retention associated with its use. Further studies of pioglitazone for the vein graft integrity preservation, including clinical trials, are needed.

CONCLUSIONS

Pioglitazone treatment prevents graft dilation and neointimal hyperplasia, which leads to preservation of the vein graft integrity in a rat aortic interposition model. Pioglitazone has a potential use for post-CABG regimens by providing an improvement of the long-term patency of the vein grafts. Further investigation will be required to determine if this will hold true in a clinical setting.

References

1. Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N Engl J Med*. 1994;330:1431-8.
2. Goldman J, Zhong L, Liu SQ. Degradation of alpha-actin filaments in venous smooth muscle cells in response to mechanical stretch. *Am J Physiol Heart Circ Physiol*. 2003;284:H1839-47.
3. Yki-Jarvinen H. Thiazolidinediones. *N Engl J Med*. 2004;351:1106-18.
4. Abdelrahman M, Sivarajah A, Thiemermann C. Beneficial effects of PPAR-gamma ligands in ischemia-reperfusion injury, inflammation and shock. *Cardiovasc Res*. 2005;65:772-81.
5. Kasai T, Miyauchi K, Yokoyama T, Kajimoto K, Sumiyoshi K, Kubota N, et al. Pioglitazone attenuates neointimal thickening via suppression of the early inflammatory response in a porcine coronary after stenting. *Atherosclerosis*. 2008;197:612-9.
6. Aizawa Y, Kawabe J, Hasebe N, Takehara N, Kikuchi K. Pioglitazone enhances cytokine-induced apoptosis in vascular smooth muscle cells and reduces intimal hyperplasia. *Circulation*. 2001;104:455-60.
7. Nakamura T, Yamamoto E, Kataoka K, Yamashita T, Tokutomi Y, Dong YF, et al. Beneficial effects of pioglitazone on hypertensive cardiovascular injury are enhanced by combination with candesartan. *Hypertension*. 2008;51:296-301.
8. Yoshimoto T, Naruse M, Shizume H, Naruse K, Tanabe A, Tanaka M, et al. Vasculo-protective effects of insulin sensitizing agent pioglitazone in neointimal thickening and hypertensive vascular hypertrophy. *Atherosclerosis*. 1999;145:333-40.
9. Shiomi T, Tsutsui H, Hayashidani S, Suematsu N, Ikeuchi M, Wen J, et al. Pioglitazone, a peroxisome proliferator-activated receptor-gamma agonist, attenuates left ventricular remodeling and failure after experimental myocardial infarction. *Circulation*. 2002;106:3126-32.
10. Tanaka Y, Hasegawa T, Chen Z, Okita Y, Okada K. Renoprotective immunosuppression by pioglitazone with low-dose cyclosporine in rat heart transplantation. *J Thorac Cardiovasc Surg*. 2009;138:744-51.
11. Haraguchi T, Okada K, Tabata Y, Maniwa Y, Hayashi Y, Okita Y. Controlled release of basic fibroblast growth factor from gelatin hydrogel sheet improves structural and physiological properties of vein graft in rat. *Arterioscler Thromb Vasc Biol*. 2007;27:548-55.
12. Hasegawa T, Bouis D, Liao H, Visovatti SH, Pinsky DJ. Ecto-5' nucleotidase (CD73)-mediated adenosine generation and signaling in murine cardiac allograft vasculopathy. *Circ Res*. 2008;103:1410-21.
13. Huynh TT, Davies MG, Trovato MJ, Barber L, Safi HJ, Hagen PO. Reduction of lipid peroxidation with intraoperative superoxide dismutase treatment decreases intimal hyperplasia in experimental vein grafts. *J Surg Res*. 1999;84:223-32.
14. Shi Y, Patel S, Davenpeck KL, Niculescu R, Rodriguez E, Magno MG, et al. Oxidative stress and lipid retention in vascular grafts: comparison between venous and arterial conduits. *Circulation*. 2001;103:2408-13.
15. Chung AW, Rauniyar P, Luo H, Hsiang YN, van Breemen C, Okon EB. Pressure distention compared with pharmacologic relaxation in vein grafting upregulates matrix metalloproteinase-2 and -9. *J Vasc Surg*. 2005;42:747-56.

ARTICLE IN PRESS

16. Diep QN, El Mabrouk M, Cohn JS, Endemann D, Amiri F, Virdis A, et al. Structure, endothelial function, cell growth, and inflammation in blood vessels of angiotensin II-infused rats: role of peroxisome proliferator-activated receptor-gamma. *Circulation*. 2002;105:2296-302.
17. Jones A, Deb R, Torsney E, Howe F, Dunkley M, Gnaneswaran Y, et al. Rosiglitazone reduces the development and rupture of experimental aortic aneurysms. *Circulation*. 2009;119:3125-32.
18. Liu SQ, Moore MM, Yap C. Prevention of mechanical stretch-induced endothelial and smooth muscle cell injury in experimental vein grafts. *J Biomech Eng*. 2000; 122:31-8.
19. Paravicini TM, Touyz RM. Redox signaling in hypertension. *Cardiovasc Res*. 2006;71:247-58.
20. Satoh K, Nigro P, Matoba T, O'Dell MR, Cui Z, Shi X, et al. Cyclophilin A enhances vascular oxidative stress and the development of angiotensin II-induced aortic aneurysms. *Nat Med*. 2009;15:649-56.
21. Human P, Franz T, Scherman J, Moodley L, Zilla P. Dimensional analysis of human saphenous vein grafts: Implications for external mesh support. *J Thorac Cardiovasc Surg*. 2009;137:1101-8.
22. Duan SZ, Usher MG, Mortensen RM. Peroxisome proliferator-activated receptor-gamma-mediated effects in the vasculature. *Circ Res*. 2008;102: 283-94.
23. Yamashita K, Murakami H, Okuda T, Motohashi M. High-performance liquid chromatographic determination of pioglitazone and its metabolites in human serum and urine. *J Chromatogr B Biomed Appl*. 1996;677:141-6.
24. Arii K, Ota K, Suehiro T, Ikeda Y, Nishimura K, Kumon Y, et al. Pioglitazone prevents reactive hypoglycemia in impaired glucose tolerance. *Diabetes Res Clin Pract*. 2005;69:305-8.
25. Wohrle J, Marx N, Koenig W, Hombach V, Kestler HA, Hoher M, et al. Impact of pioglitazone on coronary endothelial function in non-diabetic patients with coronary artery disease. *Clin Res Cardiol*. 2008;97:726-33.

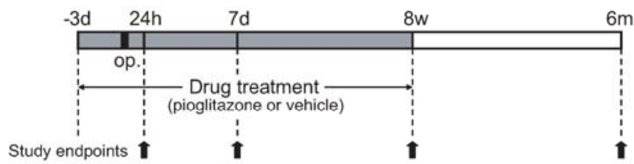

FIGURE E1. Schematic representation of the present study design. Drug treatment (pioglitazone or vehicle) was provided to rats by gastric gavage once per day beginning 3 days before operation and ending 8 weeks after operation. The end points of the present study were 24 hours, 7 days, 8 weeks, and 6 months after operation. *Op,* Operation.

ARTICLE IN PRESS

000 Pioglitazone preserves vein graft integrity in a rat aortic interposition model

Zhi Chen, MD, Tomomi Hasegawa, MD, PhD, Akiko Tanaka, MD, Yutaka Okita, MD, PhD, and Kenji Okada, MD, PhD, Kobe, Japan

We evaluated the efficacy of peroxisome proliferator-activated receptor-γ activator pioglitazone on vein grafts in an arterial environment in a rat aortic interposition model with autologous femoral vein. Pioglitazone treatment prevented vascular degeneration, including graft dilation and neointimal hyperplasia, leading to preserved vein graft integrity.