Inhibitory Activity of Clinical Thiazolidinedione Peroxisome Proliferator Activating Receptor-γ Ligands Toward Internal Mammary Artery, Radial Artery, and Saphenous Vein Smooth Muscle Cell Proliferation

Stephanie T. de Dios, BAppSC(Hons); Dennis Bruemmer, MD; Rodney J. Dilley, PhD; Melanie E. Ivey, BAppSci; Garry L.R. Jennings, MBBS, PhD; Ronald E. Law, PhD; Peter J. Little, PhD

Background—The proliferation of vascular smooth muscle cells (VSMCs) is a known response to arterial injury that is an important part of the process of restenosis and atherosclerosis. People with diabetes have an increased risk of cardiovascular disease resulting from accelerated coronary atherosclerosis. The newest drugs for Type 2 diabetes are thiazolidinediones, which are insulin-sensitizing peroxisome proliferator activating receptor-γ (PPARγ) ligands. We investigated the antiproliferative effects of troglitazone, rosiglitazone, and pioglitazone on VSMCs derived from the three vascular beds used for coronary artery by-pass grafting: the internal mammary and radial artery and saphenous veins.

Methods and Results—The three vessels yielded proliferating cells of slightly differing morphology. Inhibition of cell proliferation was assessed by cell counting and cell cycle studies by Western blotting for phosphorylated retinoblastoma protein. All three thiazolidinediones showed inhibitory potency toward cell proliferation with a potency troglitazone > rosiglitazone = pioglitazone, and this potency profile was maintained toward the growth factor and insulin-stimulated phosphorylation of the retinoblastoma protein, which controls cell cycle progression.

Conclusion—The inhibitory potency of clinical thiazolidinediones toward different vascular sources is dependent on the individual thiazolidinedione and very little on the vascular source. (Circulation. 2003;107:2548-2550.)

Key Words: muscle, smooth ■ diabetes mellitus ■ restenosis ■ grafting

Proliferation of vascular smooth muscle cells (VSMCs) is an important response to arterial injury and contributes to the formation of atherosclerotic lesions and coronary heart disease (CHD), and the process is markedly accelerated in the setting of diabetes. The main methods of treatment for CHD are angioplasty and coronary artery bypass grafting (CABG). The three most common vessels used in CABG are internal mammary artery (IMA), radial artery (RA), and saphenous veins (SV).

Individuals with diabetes experience hyperproliferation of VSMCs limiting angioplasty, and some consider that the preferred line of intervention in this group is CABG. Thiazolidinediones (TZDs), ligands for peroxisome proliferator-activated receptor-γ (PPARγ), are the latest group of therapeutic agents for the treatment of Type 2 diabetes. TZDs inhibit the proliferation of VSMCs but whether the inhibitory activity is dependent on the particular PPARγ agent or the vascular bed from which the cells are derived is unknown.

We investigated the effects of troglitazone (TRO), rosiglitazone (ROS), and pioglitazone (PIO) on the proliferation of VSMCs derived from IMA, RA, and SV. The order of potency was TRO > ROS = PIO, which was the same in each vascular smooth muscle cell preparation, and thus it is the particular TZD, not the vascular bed, that determines the inhibitory response.

Methods

Materials

Troglitazone was obtained as a gift from Parke Davis Pharmaceutical Research, Ann Arbor, Mich, rosiglitazone from SmithKline Beecham International, Australia, and pioglitazone was donated by Eli Lilly Pharmaceuticals.

VSMC Preparations

Primary human VSMCs were isolated by the explant technique from IMA, RA, and SV segments excess to heart by-pass operations and cultured in nominally 12.5 mmol/L glucose Dulbecco’s modified Eagle medium (DMEM) (Gibco-BRL) containing 10% fetal bovine serum (FBS) (CSL Limited, Parkville, Australia). The acquisition of the vessel segments was approved by the Alfred Hospital Ethics Committee.
Cell Proliferation Studies
VSMCs at passage 4 to 6 were grown in 5 mmol/L glucose DMEM media containing 10% FBS at 37°C in an atmosphere of 5% CO₂. VSMCs were seeded at low density (3×10⁴ cells per 30-mm diameter plate) in media with 10% FBS (2 mL) grown for 48 hours then serum deprived for 48 hours and at day 4, media containing 5% FBS was used with various concentrations of TZDs or DMSO (0.01%). Incubation proceeded for 3 days followed by cell counting (Coulter counter, Coulter Corporation).

Western Immunoblotting for Rb Phosphorylation
Human aortic VSMCs (HASMCs) were obtained from BioWhittaker Inc (Walkersville, Md) and cultured in recommended media. Cells were harvested at the indicated time after the addition of growth factors (PDGF-BB (Sigma) and insulin (Eli Lilly) at the final concentration of 20 ng/mL and 1 µmol/L, respectively, for 24 hours and drugs. Rb phosphorylation was determined exactly as previously described.

Statistical and Mathematical Analyses
All data were expressed as the mean±SEM. Computer-assisted statistical analysis (Sigma Stat 2.0 statistical program) was used for one-way ANOVA and a Tukey test. In some experiments, a Student’s t test was used to determine differences between groups.

The hydrophobicity values for the thiazolidinediones were calculated using the ACD/log D version 5.0 module (Advanced Chemistry Development).

Results
IMA, SV, and RA Smooth Muscle Cell Cultures
Cells established from IMA and SV proliferated well in culture, and RA had a different morphology and grew more slowly; these results are from 7 or more independent IMA and SV cultures, and 6 of 7 RA cultures showed a spindle phenotype. The increase in cell number between day 4 and day 7 of cells grown in control medium with 5% FCS averaged 85.39±4.29×10⁴, 1.94±0.08×10⁴, and 50.91±1.19×10⁴ for IMA, RA, and SV, respectively.

Clinical Thiazolidinedione (PPARγ) Ligands and Inhibition of VSMC Proliferation
TRO significantly inhibited proliferation at 3 µmol/L in both RA and SV smooth muscle cells, and significantly inhibited all cell types at 10 µmol/L (Figures 1A through 1C). Similarly, ROSI and PIO also inhibited all cell types, but at 10-fold higher concentrations. Statistically significant inhibition was observed for both ROSI and PIO at 30 µmol/L, with over 50% inhibition observed at 100 µmol/L for both agents (Figure 1). Therefore, the order of potency was TRO>ROSI=PIO, and this is the same in each VSMC type. Neither TRO, ROSI, or PIO had any visible effects on cell morphology (data not shown).

TZDs Inhibit Mitogen-Induced Rb Phosphorylation
One possible mechanism of inhibition of proliferation by TZDs would be due to cell cycle arrest associated with phosphorylation of the retinoblastoma (Rb) gene product. HASMCs were treated with PDGF-BB+insulin, and after 24 hours, Rb phosphorylation was clearly increased (Figure 2, lanes 1 and 2). PIO and ROSI caused concentration-dependent inhibition at 10 and 30 µmol/L (Figure 2) and TRO (10 µmol/L) completely inhibited Rb phosphorylation (Figure 2).

Discussion
Thiazolidinediones are the latest class of drugs for the treatment of the hyperglycemia but the first that appear to have beneficial antiatherosclerotic actions directly on vascular cells. We examined the effects of three clinically relevant TZDs of widely varying PPARγ binding affinity for their ability to inhibit the proliferation of VSMCs obtained and cultured from three vessels used for CABG. All three agents inhibited cell proliferation by a cell cycle–dependent mechanism, and the inhibitory potency, which is seemingly unrelated to PPARγ binding affinity, was dependent on the individual TZD and not the specific vascular origin.

Although the insulin-sensitizing activity of PPARγ ligands correlates closely with their binding affinity for the nuclear receptor, it is unknown whether this relationship also holds for their vascular effects. An important finding in the present study is that the antiproliferative activities of different
PPARγ ligands do not parallel their affinity for the ligand-binding domain of the nuclear receptor. Although ROSI and PIO displayed similar potencies in inhibiting VSMC proliferation, ROSI has a 2-log greater affinity for PPARγ than PIO.12 The most potent vascular antiproliferative agent detected in our studies was TRO, which has a 2-fold lower affinity for PPARγ than PIO.12 Regulation of the expression and/or activity of a subset of genes that control the cell cycle, like Rb, by a specific PPARγ ligand may be of much greater importance than its binding affinity or transactivation potency in explaining or predicting antiproliferative properties.

TZDs have been reported to inhibit the growth of PPARγ−/− embryonic stem cells, suggesting that their antiproliferative effects can occur independent of nuclear receptor activation.13 Our group has recently used adenoviral-mediated overexpression of constitutively active and dominant-negative forms of PPARγ, as well as delivery of a small molecule PPARγ antagonist, to demonstrate that ligand-activation of PPARγ can inhibit VSMC proliferation by blocking cell cycle progression.14 Depending on the concentration used and physiological context, it is likely that TZDs can inhibit cell proliferation through both PPARγ-dependent and PPARγ-independent mechanisms.

A further consideration is the role of hydrophobicity in determining inhibitory potency. Hydrophobicity values as reflected in the calculated partition coefficients (Log P) for TRO, ROSI, and PIO are 4.95, 2.56, and 3.16, respectively; distribution coefficients (Log D) reflecting distribution at physiological pH are 3.90, 1.47, and 2.11, respectively. Thus, all the compounds are relatively hydrophobic but TRO is almost two orders of magnitude more hydrophobic than ROSI or PIO, and indeed, the vascular inhibitory potency closely follows this hydrophobicity data. Hydrophobicity could affect inhibitory potency through modulating access to the intracellular receptors or in the interaction between the ligand binding domain and downstream effects on transcription. The in vitro effects described in this article occur at concentrations above plasma concentrations after oral dosing; however, it has recently been shown that these high dose effects may also be PPARγ-dependent.9

Inhibition of VSMC proliferation in vitro by PPARγ ligands is an important mechanism for their observed in vivo effect to inhibit neointima formation in rat models of restenosis.15 More importantly, early clinical studies demonstrate that TRO administration reduced intimal hyperplasia after coronary stent implantation.16 Data presented in this study carry important implications for future PPARγ-based pharmacology. Identifying novel PPARγ ligands based on their direct vascular activity, rather than their portfolio of metabolic effects on glucose and lipids, may yield compounds with even greater efficacy against proliferative vascular diseases.

**Acknowledgments**

Support was provided to P.J.L. by The Alfred Hospital Foundation, an Eli Lilly Endocrinology Research Grant, and Diabetes Australia Research Trust Grant. We thank Associate Prof Franklin Rosenfeldt and Dr Olivier van den Brink and the staff of the C.J. Officer Brown Cardiac Theaters at the Alfred Hospital for assistance with the acquisition of the vessels used to develop the cell cultures and Brian Jones for assistance with the preparation of the photomicrograph.

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Circulation. 2003;107:2548-2550; originally published online May 12, 2003; doi: 10.1161/01.CIR.0000074040.31731.96

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