Troglitazone upregulates PTEN expression and induces the apoptosis of pulmonary artery smooth muscle cells under hypoxic conditions

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Abstract. The increased proliferation and decreased apoptosis of pulmonary artery smooth muscle cells (PASMCs) are the main causes of hypoxic pulmonary hypertension. In this study, we investigated the role of troglitazone [peroxisome proliferator-activated receptor γ (PPARγ) agonist] in the regulation of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) expression and the apoptosis of PASMCs under hypoxic conditions. Normal human PASMCs were cultured in growth medium (GM) and treated with troglitazone (0.5-80 µM) under hypoxic conditions (5% CO₂,94% N₂,1% O₂) for 72 h. The gene expression of PTEN, AKT-1 and AKT-2 was determined by quantitative reverse transcription PCR (qRT-PCR). The protein expression level of PTEN, AKT and phosphorylated AKT (p-AKT) was determined by western blot analysis. The apoptosis of PASMCs was determined by measuring the activities of caspase-3, -8 and -9 and by TUNEL assay. The proliferation rate of the PASMCs was altered in a concentration-dependent manner by troglitazone. A significantly reduced proliferation rate was observed at troglitazone concentrations starting from 20 µM under hypoxic conditions (72±5.8%). Although the gene expression levels of PTEN were increased, the gene expression levels of AKT-1 and AKT-2 remained unaltered. Consistent with this, PTEN protein expression was also altered in a concentration-dependent manner by troglitazone. Although AKT expression was unaltered in all the cell samples, reduced p-AKT expression was observed in the troglitazone-treated PASMCs. Troglitazone increased the activities of caspase-3,-8 and -9 in the PASMCs. bpV(HOpic) (PTEN inhibitor) and GW9662 (PPARγ inhibitor) inhibited PTEN protein expression and recovered the proliferation rate of the PASMCs. TUNEL assay demonstrated that troglitazone significantly increased the apoptosis of PASMCs under hypoxic conditions. In conclusion, troglitazone increases PTEN expression under hypoxic conditions in a concentration-dependent manner. Troglitazone increases the apoptosis of PASMCs under hypoxic conditions. The increase in PTEN expression is mediated through the PPARγ signaling pathway.

Introduction

Hypoxic pulmonary hypertension (HPH) is an important pathophysiological process and plays a key role in the development of a variety of pulmonary diseases, including chronic obstructive pulmonary disease (COPD), pulmonary fibrosis and pulmonary heart disease. Multiple pathogenic pathways have been implicated in the development of HPH, including those at the molecular and genetic levels of smooth muscle and endothelial cells and adventitia. The HPH 'phenotype' is characterized by a decreased ratio of apoptosis/proliferation in pulmonary artery smooth muscle cells (PASMCs) and a thickened, disordered adventitia (1). The increased proliferation and reduced apoptosis of PASMCs is a component of pulmonary hypertension, increased pulmonary vascular resistance and pulmonary arterial pressure, which is associated with COPD and right ventricular failure (2). Since the proliferation of SMCs is an essential feature of vascular proliferative disorders, considerable efforts have been made to develop a therapeutic strategy that effectively suppresses SMC proliferation.

Peroxisome proliferator-activated receptor γ (PPARγ) agonists act as negative regulators of SMC growth (3). They are potent inhibitors of vascular SMC proliferation in vitro and in vivo (3). PPARγ agonists can stimulate the production and secretion of apolipoprotein E (ApoE), which has anti-proliferative effects on human PASMCs (4). Nisbet et al (5) found that rosiglitazone (a PPARγ agonist) attenuated hypoxia-induced pulmonary vascular remodelling and hypertension by suppressing oxidative and proliferative signals. These studies provide the mechanisms underlying the therapeutic effects of PPARγ activation in pulmonary hypertension.

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Studies have also suggested that PPARγ agonists upregulate phosphatase and tensin homologue deleted on chromosome 10 (PTEN) expression in allergen-induced asthmatic lungs (6). Zhang et al (7) demonstrated that rosiglitazone inhibits the migration of human hepatocellular BEL-7404 cells by upregulating PTEN. The tumor suppressor gene, PTEN, encodes a dual-specificity phosphatase that recognizes protein and phosphatidylinositol substrates and modulates cellular functions, such as migration and proliferation. However, the role of PPARγ in regulating PTEN expression in PASMCs under hypoxic conditions has not yet been elucidated. Therefore, the present study aimed to examine the molecular mechanisms through which PPARγ activation modulates PTEN expression in PASMCs under hypoxic conditions, since hypoxia is a proliferative stimulus of PASMCs (5).

In the present study, we investigated the effects of troglitazone (a PPARγ agonist) on PTEN gene expression and the apoptosis of PASMCs under hypoxic conditions. We found that troglitazone increased PTEN expression in PASMCs in a concentration-dependent manner and increased the apoptosis of PASMCs under hypoxic conditions.

Materials and methods

Cell culture of PASMCs. Human PASMCs were purchased from ScienCell Research Laboratories (Carlsbad, CA, USA). The PASMCs were cultured and expanded in SMC growth medium (GM): smooth muscle cell basal medium (BM; ScienCell Research Laboratories) supplemented with SMC growth supplement (ScienCell Research Laboratories), 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (P/S). The cells were cultured at 37˚C in a 5% CO2 incubator, and cell culture medium was changed every 2 days. Cells were regularly passaged every 4-5 days.

Hypoxia treatment. The PASMCs were cultured under hypoxic conditions in order to determine the anti-proliferative effects of troglitazone on PASMCs. A hypoxic environment for PASMC culture was created in an incubator: 5% CO2, 1% O2, 94% N2, and 1% O2. The cells were cultured at 37˚C in a 5% CO2 incubator, and the cell culture medium was changed every 2 days. Cells were regularly passaged every 4-5 days.

PASMC culture with PTEN inhibitor or PPARγ antagonist. The PASMCs were seeded at 1x10^4 cells/well in a 12-well plate and cultured in GM supplemented with 2.5 µM dipotassium bisperoxo(5-hydroxyppyridine-2-carboxyl)oxovanadate (V) [bpV(HOpic)], a PTEN inhibitor, or 1 µM 2-chloro-5-nitro-N-phenylbenzamide (GW9662), a PPARγ antagonist (both were purchased from EMD4Biosciences, Darmstadt, Germany). bpV(HOpic) and GW9662 were added to the cell culture medium 1 h prior to the addition of troglitazone. The PASMCs were cultured in an incubator under hypoxic conditions for 72 h. Cell proliferation was determined using the CyQUANT® Cell Proliferation assay kit (Invitrogen, Carlsbad, CA, USA). Proteins were extracted from the treated cells using PhosphoSafe™ Extraction Reagent (EMD Millipore, Billerica, MA, USA). Following quantification, the proteins were used for western blot analysis.

Quantitative reverse transcription PCR (qRT-PCR) analysis. PASMCs were analyzed by qRT-PCR to determine gene expression at 0.5, 1, 2, 4, 8 and 24 h after treatment with troglitazone. Total RNA was isolated as previously described (8). DNase I was used to remove DNA contamination. cDNA was synthesized using the Maxima® First Strand cDNA Synthesis kit (Fermentas, Hanover, MD, USA).

To quantify gene expression levels, Maxima® SYBR-Green qPCR Master Mix (2X) (Fermentas) was used. The qPCR thermal cycling protocol was programmed for 40 cycles: 1 cycle of initial denaturation for 10 min, then denaturation at 95˚C for 15 sec, annealing for 30 sec and extension at 72˚C for 30 sec. The primers used are listed in Table I.

Cell proliferation. PASMC proliferation was determined using the CyQUANT® Cell Proliferation assay kit (Invitrogen). Briefly, 1x10^4 PASMCs/well were seeded into 24-well plates and cultured with GM for 24 h. The cell culture medium was changed to GM supplemented with various concentrations of troglitazone (stock solution 20 mM in 100% DMSO) for 72 h in an incubator under hypoxic conditions at 37˚C. Subsequently, the cell culture supernatant was removed. The cells were washed with PBS and frozen at -80˚C for 1 h. Each well was then incubated with 200 µl CyQUANT® cell-lysis buffer containing DNase-free RNase (1.35 U/ml) for 1 h at room temperature to eliminate the RNA component of the fluorescence signal. Subsequently, 200 µl cell-lysis buffer containing 2X solution of CyQUANT® GR dye were added to each well for 10 min. The fluorescence intensity was measured using a fluorescent microplate reader (Tecan Infinite M200 microplate reader; LabX, Canada) with an excitation wavelength of 560 nm and a emission wavelength of 590 nm.

Lactate dehydrogenase (LDH) release for cytotoxic determination. The cytotoxicity of troglitazone towards PASMCs was determined using CytoTox-ONE™ Homogeneous Membrane Integrity assay (Promega, Madison, WI, USA). Briefly, 5x10^4 PASMCs/well were cultured in a 12-well plate with GM supplemented with troglitazone for 72 h under normoxic conditions at 37˚C. Subsequently, cell culture supernatant was collected and mixed with CytoTox-ONE™ Reagent for 20 min. After the addition of stop solution, the fluorescence signal was measured at an excitation wavelength of 560 nm and an emission wavelength of 590 nm.

Western blot analysis of PASMCs. Protein expression in the treated and non-treated PASMCs was determined by western blot analysis as previously described (9). Cells were lysed with PhosphoSafe™ Extraction Reagent (EMD Millipore) and the protein concentration was determined using Bradford reagent (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Proteins were separated and electrophoretically blotted onto a nitrocellulose membrane. After washing with 10 mM Tris/HCl wash buffer (pH 7.6) containing 0.05% Tween-20, the membrane was incubated in blocking buffer (5% non-fat dry milk, 10 mM Tris pH 7.5, 100 mM NaCl, 0.1% Tween-20) for 3 h at room temperature. Subsequently, the blots were incubated with diluted primary antibodies: AKT (1:1,000), phosphorylated (Ser 473) AKT (p-AKT) (1:500), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (1:1,000) and...
Table I. Primers used for RT-PCR.

<table>
<thead>
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<th>Gene</th>
<th>Primer sequence</th>
<th>Annealing temp. (˚C)</th>
<th>Size (bp)</th>
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<tr>
<td>GAPDH</td>
<td>Forward 5'-AGCCACATCGCTCAGACAC-3' Reverse 5'-TAAAGCAGCCCTGTTGAC-3'</td>
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<td>90</td>
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<tr>
<td>PTEN</td>
<td>Forward 5'-TCACCAACTGAAGTGCTAAGA-3' Reverse 5'-CTCCATTCCCTCAACCCGA-3'</td>
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<td>155</td>
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<tr>
<td>AKT-1</td>
<td>Forward 5'-TAACCTTTTCCGCTCAGC-3' Reverse 5'-ATGGTGTAAGGAACCGCCG-3'</td>
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<td>125</td>
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<tr>
<td>AKT-2</td>
<td>Forward 5'-GGTCGCCAACACACCTCAG-3' Reverse 5'-CAGTTCAGCCGCTTGTGG-3'</td>
<td>58</td>
<td>127</td>
</tr>
</tbody>
</table>

RT-PCR, reverse transcription PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PTEN, phosphatase and tensin homologue deleted on chromosome 10.

PTEN (1:200) (all purchased from Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA) overnight at 4˚C. Subsequently, anti-rabbit IgG conjugated with HRP (dilution, 1:3,000-1:8,000) was used to detect the binding of antibodies. The binding of the specific antibody was visualized using the SuperSignal Chemiluminescent Substrate kit and exposed to X-ray film (both from Pierce Biotechnology, Inc., Rockford, IL, USA).

Apopotisis assay. Proteins from the treated and non-treated PASMCs were used to determine the apoptosis of PASMCs by determining the activities of caspase-3,-8 and -9.

Caspase-3 and -8 activities were determined using caspase-3 and -8 assay kits, Fluorimetric (Sigma-Aldrich, Sigma, St. Louis, MO, USA). The fluorescence intensity of caspase-3 was recorded at a wavelength of 360 nm for excitation and a wavelength of 460 nm for emission, and that of caspase-8 was recorded at an excitation of 360 nm and an emission of 440 nm. The activity of caspase was calculated as fluorescence intensity (FI)/min/ml = ΔFt/(t x v), where ΔFt is the difference in fluorescence intensity between time zero and time t minutes; t is the reaction time in min; and v is the volume of the sample in ml.

Similarly, caspase-9 activity was determined using the caspase-9 assay kit, Fluorimetric (QIA72; EMD4Biosciences). The fluorescence intensity was recorded at a wavelength of 400 nm for excitation and a wavelength of 505 nm for emission. The same formula as the one used for the calculation of caspase-3 activity was used to calculate caspase-9 activity.

TUNEL assay. To detect the apoptosis of PASMCs following treatment with troglitazone, the PASMCs were cultured in BM and incubated for 72 h under hypoxic conditions. TUNEL assay was performed using the In situ Cell Death Detection kit (Roche) as per the manufacturer's instructions. In brief, the cells were fixed with 4% paraformaldehyde for 20 min at 25˚C. After washing with PBS for 30 min, the cells were incubated in permeabilization solution (0.1% Triton X-100, 0.1% sodium citrate) for 10 min at 4˚C. After washing, the cells were incubated with rabbit anti-calponin antibody overnight at 4˚C. On the second day, reaction mixture from the kit supplemented with goat anti-rabbit IgG conjugated with FITC was applied to the cells for 1 h at 37˚C in the dark. The samples were counterstained with DAPI to visualize the nuclei after TUNEL staining. The apoptotic index was calculated as TUNEL+ cells/total cells/low magnification field.

Statistical analysis. All statistical analyses were performed using SPSS software (version 10.0). All experiments were performed at least 3 times. The data are presented as the mean ± standard error means (SEM) and analyzed with the method of analysis of variance (ANOVA) using the Bonferroni test. A Student's t-test was performed to determine statistical difference. All tests were performed with a significance level of 5%.

Results

Toxicity of troglitazone towards PASMCs. The toxicity of troglitazone towards the cells was determined in order to exclude the possibility that the decrease in PASMC proliferation was due to the toxicity of troglitazone towards PASMCs. PASMCs were cultured in GM supplemented with troglitazone for 48 h in an incubator under normal conditions. The supernatant was collected to measure LDH concentration (an index of cell injury). Significant cell injury was only observed when the troglitazone concentration was increased up to 60 and 80 µM (Fig. 1).

The percentage of LDH in the supernatant was 1.2±0.5% when the cells were cultured in GM without troglitazone, while it was 1.5±0.5% with 0.5 µM troglitazone, 1.8±0.4% with 1 µM, 2±0.4% with 2 µM, 1.8±0.6% with 5 µM, 2.1±0.5% with 10 µM, 1.8±0.3% with 20 µM, 2.1±0.5% with 40 µM, 3±0.8% with 60 µM and 3.7±0.6% with 80 µM troglitazone (Fig. 1). These results demonstrated that troglitazone was well tolerated by PASMCs.

Concentration-dependent effects of troglitazone on PASMC proliferation. The proliferation rate of the PASMCs was altered in a concentration-dependent manner by troglitazone in the cells cultured in GM under hypoxic conditions (Fig. 2A). The cell number of PASMCs treated with 0.5 µM troglitazone was reduced to 93.3±1.2% compared with that of the PASMCs not treated with troglitazone (considered as 100%). The percentage cell number of PASMCs cultured with troglitazone was 91±2.7% with 1 µM of troglitazone, 91.1±3.5% with 2 µM, 93.5±2.8% with 5 µM, 84.3±4.2 with 10 µM, 72±5.8% with
20 µM, 70.4±3% with 40 µM, 59.3±8.3% with 60 µM and 64.6±2.1% with 80 µM troglitazone. Starting at the concentration of 20 µM, troglitazone significantly reduced the proliferation rate of PASMCs under hypoxic conditions (P<0.05). Thus, we selected the concentration of 20 µM troglitazone for the remaining experiments.

**Troglitazone increases PTEN gene expression.** Troglitazone significantly increased PTEN gene expression by 2.4±0.1-fold at 2 h as compared with the baseline levels (P<0.05) under hypoxic conditions. Upregulated PTEN gene expression was maintained up to 8 h (1.7±0.02-fold, P<0.05) (Fig. 3A).

**Troglitazone does not alter gene expression of AKT-1 and AKT-2.** Troglitazone did not significantly alter the gene expression of AKT-1 and AKT-2 in the PASMCs between any 2 time points (Fig. 3B and C).
Western blot analysis of cultured PASMCs supplemented with troglitazone. Troglitazone increased PTEN protein expression in PASMCs cultured under hypoxic conditions for 2 h. A significantly increased PTEN protein expression level was observed when the cells were treated with troglitazone at concentrations between 20-80 µM, while no significant changes in p-AKT and AKT expression were observed (Fig. 4A). The PASMCs were then cultured with 20 µM troglitazone for 24 h and DMSO-treated cells (without troglitazone) were used as controls, since troglitazone was diluted in DMSO. In the cells treated with troglitazone, a significantly increased PTEN protein expression was observed within 2 h compared with the cells treated with DMSO alone. The highest PTEN protein expression level was observed within 4 h after the addition of troglitazone, which was significantly higher than that at 0.5 h (Fig. 4B). Troglitazone also progressively reduced p-AKT expression within 2 h; these levels were significantly lower than those from the cells treated with DMSO alone.

To determine whether the upregulated PTEN protein expression induced by troglitazone was mediated by the PPARγ signaling pathway and inhibited by the PTEN inhibitor, PASMCs were pre-treated with 2.5 µM bpV(HOpic) (a PTEN inhibitor) or 1 µM GW9662 (PPARγ antagonist) for 1 h prior to the addition of troglitazone. It was found that the upregulated protein expression of PTEN was reduced by pre-treatment with bpV(HOpic) and GW9662 for up to 24 h (Fig. 4C and D).

Effects of bpV(HOpic) and GW9662 on proliferation of PASMCs treated with troglitazone. Typical images of the PASMC growth profile are shown in Fig. 5A-G. Troglitazone significantly reduced the proliferation rate of the PASMCs under hypoxic conditions. bpV(HOpic) was used to determine whether the PTEN inhibitor reversed the inhibitory effects of troglitazone on PASMC proliferation. It was found that troglitazone did not reduce the proliferation rate of the PASMCs that were pre-treated with bpV(HOpic). These results suggest that troglitazone inhibits PASMC proliferation by upregulating PTEN expression, which may be reversed by the PTEN inhibitor (Fig. 5H).

GW9662 was also used to determine whether the upregulation in PTEN expression stimulated by troglitazone was mediated by the PPARγ signaling pathway. It was found that GW9662 reversed the inhibitory effects of troglitazone on PASMC proliferation. These results suggest that troglitazone upregulates PTEN expression through the PPARγ signaling pathway (Fig. 5).
Figure 4. Western blot analysis of pulmonary artery smooth muscle cells (PASMCs) cultured in GM supplemented with troglitazone for 2 h under hypoxic conditions. (A1-3) Effects of various concentrations of troglitazone on the protein expression of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) and p-AKT in PASMCs cultured under hypoxic conditions. A significantly increased PTEN protein expression was observed with concentrations of troglitazone starting from 20 µM, while no significant change in phospho-AKT (p-AKT) expression was observed. (*P<0.05 vs. 0 µM troglitazone). (B1-4) Effects of troglitazone on PTEN protein expression and p-AKT expression in PASMCs cultured with 20 µM troglitazone under hypoxic conditions for various periods of time. PASMCs treated with DMSO (without troglitazone) were used as controls. Troglitazone significantly increased PTEN protein expression starting from 2 h as compared with DMSO treatment only, while troglitazone progressively reduced p-AKT expression. (*P<0.05 vs. 0.5 h troglitazone; #P<0.05 vs. without troglitazone at 2, 4, 8 and 24 h). (C1) GW9662 (PPARγ antagonist) inhibited PTEN protein expression in PASMCs. GW9662 was added 1 h prior to the addition of 20 µM troglitazone. (C2) GW9662 (PPARγ antagonist) inhibited PTEN protein expression in PASMCs. GW9662 was added 1 h prior to the addition of 20 µM troglitazone. (D1-2) bpV(HO)pic (PTEN inhibitor) inhibited PTEN protein expression in PASMCs. Trog, troglitazone. The experiment was performed in duplicate.
Troglitazone increases caspase-3, -8 and -9 activities. A significantly increased caspase-3 activity was observed when the PASMCs were cultured in GM (6.5±0.15 FI/min/ml) supplemented with troglitazone for 8 h (Fig. 6A). Similarly, a significant increase in caspase-8 and -9 activities was observed when the PASMCs were cultured in GM (5.3±0.15 and 6.1±0.27 FI/min/ml) supplemented with troglitazone for 8 h (Fig. 6B and C).

Troglitazone increases the apoptosis of PASMCs under hypoxic conditions. TUNEL staining revealed that the apoptosis of PASMCs cultured in BM increased after 72 h under hypoxic conditions (Fig. 6D and E). Quantification of TUNEL+ cells suggested that troglitazone significantly increased the apoptotic PASMC number compared with the cells cultured in BM alone (P<0.05).

Discussion

The present study demonstrates that troglitazone, a high-affinity synthetic ligand of PPARγ upregulates PTEN gene and protein expression levels in PASMCs under hypoxic conditions in a concentration-dependent manner. In addition, we demonstrate that troglitazone increases the apoptosis of PASMCs under hypoxic conditions.

Pulmonary vascular remodelling in pulmonary arterial hypertension is characterized by changes in pulmonary vascular structure (1,2). This is partially caused by an imbalance in PASMC proliferation and apoptosis (10). The increased proliferation and decreased apoptosis of PASMCs result in the thickening of the pulmonary vasculature, which subsequently increases pulmonary vascular resistance and pulmonary arterial pressure (11).

Our results demonstrated that troglitazone at the concentration of 20-80 µM effectively inhibited PASMC proliferation under hypoxic conditions. The anti-proliferative effects of troglitazone may be related to its role in the upregulation of PTEN and the induction of the apoptosis of PASMCs under hypoxic conditions. PPARγ expression has been found in pulmonary vascular endothelial cells and SMCs (12,13). However its expression is reduced in lung vascular cells of rats with pulmonary hypertension and in patients with advanced primary or secondary pulmonary hypertension (10). The targeted depletion of PPARγ from SMCs has been shown to result in spontaneous pulmonary hypertension in mice (4). However, the activation of PPARγ with thiazolidinediones attenuates vascular remodeling and pulmonary hypertension caused by monocrotaline (14) or hypobaric hypoxia (15) in rats. Thiazolidinediones also reduce pulmonary hypertension in ApoE-deficient mice fed high-fat diets (16) and attenuate hypoxia-induced pulmonary hypertension and Nox4 expression and activity in mice (5).

However, the role of PPARγ in the regulation of PTEN expression of PASMCs under hypoxic conditions has not
Figure 6. Troglitazone increases caspase-3, -8, and -9 activity. Troglitazone increased (A) caspase-3, (B) caspase-8, and (C) caspase-9 activity in pulmonary artery smooth muscle cells (PASMCs) cultured under hypoxic conditions after 72 h (*P<0.05 vs. all other samples). TUNEL staining to detect the apoptosis of PASMCs cultured in basal medium (BM) under hypoxic conditions for 72 h. (D1 and E1) The apoptotic nuclei of the PASMCs are shown in red colour. (D2 and E2) The cells were counterstained with DAPI to show cell nuclei. Magnification, ×100. (F) Quantification of TUNEL+ cells suggested that troglitazone significantly increased the apoptotic PASMC number as compared with those cultured in BM only (*P<0.05 vs. BM). Trog, troglitazone. The experiment was performed in duplicate.
been examined yet. Our results demonstrate that PPARγ upregulates PTEN gene and protein expression levels. Studies have shown that PPARγ agonists upregulate the transcription of PTEN in cancer cells. PPARγ agonists have been used as tumor suppressors in breast cancer by upregulating PTEN gene expression (17), while cells lacking PTEN or PPARγ are unable to induce PTEN-mediated cellular events in the presence of lovastatin or rosiglitazone. Zhang et al (7) showed that PTEN is required for the rosiglitazone-induced inhibition of BEL-7404 cells. Rosiglitazone upregulated PTEN expression in a concentration- and time-dependent manner, which was mediated by PPARγ. Furthermore, PTEN overexpression resulted in the inhibition of BEL-7404 cell migration, and PTEN knockdown blocked the effects of rosiglitazone on cell migration.

It is known that PPARγ agonists can stimulate the production and secretion of ApoE, which has anti-proliferative effects on human PASMCs (4). Nisbet et al (5) found that rosiglitazone attenuated hypoxia-induced pulmonary vascular remodelling and hypertension by suppressing oxidative and proliferative signals. They found that rosiglitazone attenuated the reduction in PTEN expression. However, the present study demonstrates that troglitazone upregulates PTEN gene and protein expression levels in human PASMCs under hypoxic conditions. This may provide new insight into the mechanisms behind the anti-proliferative effects of PPARγ on PASMCs under hypoxic conditions.

The data from the present study also suggest that troglitazone increases the apoptosis of PASMCs under hypoxic conditions. It is known that chronic hypoxia can prolong the growth of human vascular SMCs by inducing telomerase activity and telomere stabilization (18). The upregulation of PTEN negatively regulates the AKT/PKB signaling pathway and leads to a decrease in p-AKT levels and, consequently, to a decrease in AKT-mediated proliferation (19). Thus, a reduced p-AKT expression in PASMCs was found after the addition of troglitazone in this study. By reducing the cellular levels of p-AKT, PTEN downregulates cell division and increases apoptosis. This may result in increased activities of caspase-3, -8 and -9, thus inducing the apoptosis of PASMCs under hypoxic conditions.

In conclusion, the present study highlights that troglitazone increases PTEN gene expression at the transcriptional level under hypoxic conditions in a concentration-dependent manner. Troglitazone increases the apoptosis of PASMCs under hypoxic conditions. The increased PTEN expression and apoptosis of PASMCs provide new insight into the mechanisms behind the anti-proliferative effects of troglitazone on PASMCs.

References