Suppression of Pro-inflammatory Adhesion Molecules by PPAR-δ in Human Vascular Endothelial Cells

Yanbo Fan, Ying Wang, Zhihui Tang, Hong Zhang, Xiaomei Qin, Yi Zhu, Youfei Guan, Xian Wang, Bart Staels, Shu Chien, Nanping Wang

Objective—Endothelial activation is implicated in atherogenesis and diabetes. The role of peroxisome proliferator-activated receptor-δ (PPAR-δ) in endothelial activation remains poorly understood. In this study, we investigated the anti-inflammatory effect of PPAR-δ and the mechanism involved.

Methods and Results—In human umbilical vein endothelial cells (HUVECs), the synthetic PPAR-δ ligands GW0742 and GW501516 significantly inhibited tumor necrosis factor (TNF)-α–induced expression of vascular cell adhesion molecule-1 and E-selectin (assayed by real-time RT-PCR and Northern blotting), as well as the ensuing endothelial-leukocyte adhesion. Activation of PPAR-δ upregulated the expression of antioxidant genes superoxide dismutase 1, catalase, and thioredoxin and decreased reactive oxygen species production in ECs. Chromatin immunoprecipitation assays showed that GW0742 switched the association of BCL-6, a transcription repressor, from PPAR-δ to the vascular cell adhesion molecule (VCAM)-1 promoter. Small interfering RNA reduced endogenous PPAR-δ expression but potentiated the suppressive effect of GW0742 on EC activation, which suggests that the nonliganded PPAR-δ may have an opposite effect.

Conclusions—We have demonstrated that ligand activation of PPAR-δ in ECs has a potent antiinflammatory effect, probably via a binary mechanism involving the induction of antioxidative genes and the release of nuclear corepressors. PPAR-δ agonists may have a potential for treating inflammatory diseases such as atherosclerosis and diabetes.


Key Words: adhesion molecules ■ nuclear receptor ■ endothelium ■ gene expression ■ reactive oxygen species

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors belonging to the nuclear receptor superfamily. The PPAR family consists of 3 closely related subtypes: PPAR-α, β/δ, and γ.1 PPAR-δ is expressed ubiquitously, but its functions are largely unexplored. Saturated and polyunsaturated fatty acids are known to activate PPAR-δ,2–4 but physiological ligands of PPAR-δ have not been identified. Development of selective PPAR-δ ligands, including GW0742, GW501516, and L-165041, has extended the understanding of the physiological functions of this nuclear receptor. PPAR-δ may play a role in many important biological processes such as placental development,5 wound healing,6,7 oligodendrocyte differentiation,8,9 and carcinogenesis in the colon.10 Activation of PPAR-δ has been shown to raise high-density lipoprotein levels, reduce triglyceride levels, and improve insulin sensitivity.11 PPAR-δ increases fatty acid oxidation in skeletal muscle and ameliorates obesity and insulin resistance.12

The roles of PPAR-δ in atherosclerosis have been explored in mouse models of atherogenesis with the use of a loss-of-function approach and selective PPAR-δ agonists.13,14 The activation of endothelial cells (ECs), characterized by induced expression of pro-inflammatory adhesion molecules such as vascular cell adhesion molecule (VCAM)-1 and E-selectin, is an early step of atherosclerosis and often evoked by various atherogenic risk factors, including increased level of inflammatory cytokines (such as tumor necrosis factor-α [TNF-α] and interleukin [IL]-1), dyslipidemia, hyperinsulinemia, and insulin-resistant (type 2) diabetes.15 Recent studies have demonstrated that PPAR-δ is expressed in vascular smooth muscle cells as well as in ECs and plays potential roles in endothelial survival and proliferation.16–18 The expression of PPAR-δ is induced in response to pro-inflammatory cytokines such as TNF-α and platelet-derived growth factor (PDGF).16,19 However, the role of PPAR-δ in EC activation remains poorly understood. We aimed to elucidate the roles of PPAR-δ in TNF-α–induced expression of pro-inflammatory adhesion molecules and the ensuing EC-leukocyte interaction by using the synthetic PPAR-δ...
agonists GW0742 and GW501516 and knocking down the expression of endogenous PPAR-δ with small interfering RNA (siRNA) in human vascular ECs.

**Materials and Methods**

**Cells and Reagents**

Human umbilical vein endothelial cells (HUVECs) were cultured as previously described. THP-1 was grown in RPMI 1640 containing 10% FBS. Bovine aortic endothelial cells (BAECs) were harvested from bovine aorta and maintained in DMEM with 10% FBS. Sources of reagents are listed in the supplemental materials (available online at http://atvb.ahajournals.org).

**Northern Blotting**

Fifteen µg of total RNA per lane was fractionated on formaldehyde-agarose gels, transferred to nylon membranes, and hybridized to cDNA probes for human VCAM-1, E-selectin, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes. The cDNA probes were synthesized by RT-PCR and labeled with ³²P-dCTP as previously described. Results of autoradiography were scanned, and the signal intensity was analyzed by use of National Institutes of Health Image J software.

**Real-Time Quantitative RT-PCR**

Two µg of total RNA was reverse transcribed into cDNA with M-MLV reverse transcriptase and oligo-dT used as a primer. Real-time PCR involved SYBR-green dye and Taq polymerase. Please see supplemental materials for the primer sequences.

**Immunoprecipitation and Western Blotting**

Cellular proteins were extracted with lysis buffer (50 mmol/L Tris-HCl, pH 7.5, 15 mmol/L EGTA, 100 mmol/L NaCl, 0.1% [wt/vol] Triton X-100 and complete protease inhibitor cocktail). The supernatants were immunoprecipitated with 1 µg of anti–BCL-6 antibody and protein A/G Plus-Agarose beads. The immunoprecipitates were immunoblotted as previously described. Cytoplasmic and nuclear proteins were extracted with use of high-salt hypotonic lysis buffer (10 mmol/L Tris-HCl, pH 7.5, 1.5 mmol/L MgCl₂, 10 mmol/L KCl, 0.5% NP-40). Nuclear proteins were extracted with use of high-salt buffer (20 mmol/L Tris-HCl, 1.5 mmol/L MgCl₂, 420 mmol/L NaCl, 10% glycerol, 0.2 mmol/L EGTA).

**Chromatin Immunoprecipitation (ChIP) Assay**

Cells were cross-linked with 1% formaldehyde and quenched before harvest and sonication. The sheared chromatin was immunoprecipitated with anti–BCL-6 antibody (or control IgG) and protein A/G Sepharose beads. The eluted immunoprecipitates were digested with proteinase K, and DNA was extracted and underwent PCR with primers specific for the human VCAM-1 promoter region (5’-ACAATGAGTAGAGAGATGCACC as published). Double-strand RNA was transfected into HUVECs by use of Lipofectamine 2000. The siRNA against an irrelevant sequence derived from the Thermotoga maritima genome was used as a control. The Cy3-labeled siRNA against luciferase mRNA (Dharmacon) was transfected and visualized under fluorescence microscopy.

**Statistical Analysis**

Quantitative data are expressed as mean±SEM. Statistical analyses involved Student t test or 1-way ANOVA followed by Newman-Keuls test. A P<0.05 was considered significant. Nonquantitative results were representative of at least 3 independent experiments.

**Results**

**PPAR-δ Inhibits the Expression of Pro-inflammatory Adhesion Molecules in ECs**

To evaluate the ability of GW0742 to activate PPAR-δ in ECs, BAECs were transfected with pPPRE-TK-luc, together with pcDNA-PPAR-δ or the control vector. Promoter reporter assays showed that GW0742 activated PPAR-δ in ECs in a dose-dependent manner, with significant activation at 1 µmol/L (please see supplemental materials). To examine the effect of PPAR-δ on endothelial activation, HUVECs were pretreated with GW0742 (1 µmol/L, 24 hours), then TNF-α (2 ng/mL, 2 hours). As shown in Figure 1, the mRNA expression of pro-inflammatory adhesion molecules VCAM-1 and E-selectin was induced in response to TNF-α. GW0742 significantly inhibited the TNF-α–induced expression of VCAM-1 and E-selectin but not that of GAPDH gene. Similarly, another PPAR-δ agonist, GW501516, inhibited the TNF-α–induced expression of VCAM-1 and E-selectin. In addition, GW0742 and GW501516 significantly suppressed the induction of VCAM-1 and E-selectin stimulated by IL-1β (supplemental materials). To examine whether the PPAR-δ...
agonist decreased the TNF-α–induced mRNA level of VCAM-1 via an increase in mRNA turnover rate, HUVECs were pretreated with GW0742, stimulated with TNF-α, and treated with actinomycin (10 μg/mL, 2 hours), which inhibits RNA polymerase II. GW0742 did not decrease the half-life of VCAM-1 steady-state mRNA (supplemental materials), which indicates that the suppressive effect of GW0742 was probably via a transcriptional mechanism rather than a change in mRNA turnover.

**PPAR-δ Suppresses TNF-α–Induced Endothelial-Leukocyte Adhesion**

EC-leukocyte adhesion assays were performed to determine whether GW0742 inhibits the recruitment of leukocytes to activated ECs. TNF-α markedly increased THP-1 adhesion to ECs, and GW0742 significantly suppressed the TNF-α–induced leukocyte adhesion (by 44.6±14.3% versus the solvent control, P<0.05; supplemental materials).

**GW0742 Does Not Prevent NF-κB Nuclear Translocation in ECs**

Because NF-κB plays a central role in the transcriptional regulation of adhesion molecule genes,23 we examined the effect of GW0742 on TNF-α–induced degradation of IκBα, the inhibitor of NF-κB, and the protein level of p65 NF-κB in cytosolic and nuclear portions. HUVECs were pretreated with GW0742 (1 μmol/L) or DMSO for 24 hours, then stimulated with TNF-α. Western blot analyses revealed that TNF-α induced IκBα degradation and the nuclear translocation of p65 NF-κB, which were not inhibited by GW0742 (Figure 2A). Furthermore, GW0742 did not inhibit TNF-α–induced phosphorylation of JNK, p38, and extracellular signal regulated kinase (ERK) (supplemental materials).

**GW0742 Decreases the Association of BCL-6 With PPAR-δ in ECs**

A previous study in 293 cells indicated that PPAR-δ, in the absence of a specific ligand, may bind BCL-6, a transcription repressor.14 Therefore, we performed coimmunoprecipitation (coIP) and ChIP experiments to explore this possibility in HUVECs. CoIP revealed the association of PPAR-δ with Bcl-6 (Figure 2B). GW0742 markedly reduced the amount of BCL-6 associated with PPAR-δ but not the amount of intracellular BCL-6 protein. In contrast, ChIP assays demonstrated that GW0742 increased the association of BCL-6 with the VCAM-1 promoter (Figure 2C), which suggests that GW0742 may suppress EC activation via triggering the relocation of BCL-6 from PPAR-δ to the promoter regions of the pro-inflammatory genes to inhibit their transcriptional activities.

**GW0742 Attenuates TNF-α–Induced ROS via Upregulation of the Antioxidant Genes in ECs**

Because increased generation of ROS is of crucial importance in EC activation,24 we further examined the effect of GW0742 on intracellular generation of ROS. HUVECs were pretreated with GW0742 for 24 hours, then stimulated with TNF-α for 1 hour. TNF-α induced a robust increase in ROS production, which was significantly inhibited by GW0742.

![Figure 2](http://atvb.ahajournals.org/)

**Figure 2.** Effect of GW0742 on NF-κB activation. A, Western blots were detected with antibodies against IκBα, p65 NF-κB, α-tubulin, or histone H3. The bar graph shows the nuclear translocation of p65 expressed as percentage of nuclear (Nuc)/cytoplasmic (Cyto) contents. B, Immunoprecipitation was performed with anti–PPAR-δ or anti–BCL-6 antibody. C, ChIP assays were performed with anti–BCL-6 antibody and amplified with the primers for the VCAM-1 promoter. Bar graph represents the quantified data from 3 independent real-time PCR experiments. *P<0.05 vs control.
To determine whether GW0742 reduced the TNF-α–induced increase in ROS by enforcing the antioxidative mechanism, we examined the expression of several key antioxidant genes. GW0742 significantly increased the expression of SOD1, catalase, and thioredoxin, by 72.8 ± 29%, 31.9 ± 10.5%, and 73 ± 28.9%, respectively (Figure 3B).

**Effect of siRNA-Mediated Knockdown of PPAR-δ on Induction of Adhesion Molecules**

To examine whether the antiinflammatory effect of PPAR-δ agonists depends on an adequate level of PPAR-δ expression, HUVECs were transfected with the PPAR-δ siRNA or control siRNA. The transfection efficiency of siRNA in HUVECs was confirmed with Cy3-labeled siRNA in that most cells showed uptake of siRNA (Figure 4A). PPAR-δ siRNA significantly decreased the mRNA level of endogenous PPAR-δ, by 75%, in ECs. Surprisingly, PPAR-δ siRNA not only did not inhibit the suppressive effects of the PPAR-δ ligand GW0742 on VCAM-1 and E-selectin induction but also enhanced these suppressive effects (Figure 4B and 4C). Thus, the synthetic ligand can robustly suppress the proinflammatory response in ECs at a reduced level of its endogenous cognate PPAR-δ, and the receptor, in the absence of specific ligands, may facilitate EC activation through physical association with anti-inflammatory corepressors such as BCL-6.

**Discussion**

Our results show that the PPAR-δ agonists GW0742 and GW501516 suppressed the TNF-α–induced expression of proinflammatory adhesion molecules VCAM-1 and E-selectin in ECs, as well as the ensuing leukocyte recruitment. We demonstrate for the first time that the suppressive effect on EC activation is associated with an antioxidative mechanism but does not prevent the nuclear translocation of NF-κB. Furthermore, knockdown of PPAR-δ potentiated the anti-inflammatory effect of PPAR-δ agonists in ECs.
Recent studies suggested a role of PPAR-δ in inflammatory processes and atherosclerosis. In macrophages, the PPAR-δ agonist GW0742 inhibited lipopolysaccharide (LPS)-induced expression of iNOS and COX2.14,25 However, in other cell types such as epithelial cells, eosinophils, neutrophils, and lymphocytes, the PPAR-δ agonist was ineffective in inhibiting inflammatory processes,28 which indicates that the effect is cell-type specific. GW0742 reduced atherosclerotic lesions and decreased the expression of MCP-1 and intercellular adhesion molecule-1 (ICAM-1) in the aorta of LDLR-/- mice.27 However, whether PPAR-δ agonists have a direct effect on endothelial activation, an initial step in atherogenesis, remains unclear. The effect of the PPAR-δ agonists GW0742 and GW501516 inhibiting TNF-α-induced expression of VCAM-1 and E-selectin in primary-cultured HUVECs and attenuating EC-leukocyte adhesion is PPAR-δ specific: both of these highly selective ligands for PPAR-δ exert potent effects when used at a concentration (0.1–1 μmol/L) lower than the EC50 for PPAR-α and -γ isomers. In light of a critical role of EC activation in atherogenesis and type 2 diabetes, our finding is of importance in understanding the pathogenesis of these diseases. PPAR-δ activation, together with a lipid-modifying action11 and a role in protecting the survival of ECs,17 may have promising therapeutic application.

Excessive production of intracellular ROS in response to various stimuli (including TNF-α) can induce oxidative stress and has been implicated in the pathogenesis of cardiovascular and metabolic disorders, including atherosclerosis and diabetes.28,29,30 In this study we provide novel evidence that PPAR-δ agonists inhibited the TNF-α–induced ROS production in ECs. This finding is in agreement with a recent report describing a role of PPAR-δ in protecting against H2O2-induced apoptosis in ECs.17 In addition, we found that GW0742 increased the gene expression of SOD1, catalase, and thioredoxin, 3 important antioxidative molecules involved in the elimination of ROS.31 SODs convert superoxide anion into H2O2, which in turn can be reduced into water by catalase. Thioredoxin, via a series of coupled reactions, also functions as an antioxidant molecule in the cytosol of ECs.32 Coordinated induction of these key antioxidant genes can prevent intracellular accumulation of ROS and may account for the anti-inflammatory effect of PPAR-δ. Although the mRNA expression of SOD1 and catalase can be increased by ligands for PPAR-α and PPAR-γ,33–35 our results demonstrate for the first time a role of PPAR-δ in regulation of these antioxidant genes. Because functional PPREs have been found in the 5′-flanking regions of these genes,36 PPAR-δ may execute its antiinflammatory effect via direct activation of these antioxidant targets. Many transcription factors, including NF-κB, activator protein-1 and Ets-1, are known to be oxidant responsive and can modulate intracellular redox states.28,37 Because ROS activate complex signaling pathways,38–41 PPAR-δ may exert its anti-inflammatory effect, at least in part, by positively regulating the antioxidant genes and eliminating excessive production of ROS.

The 5′-flanking regulatory regions of VCAM-1 and E-selectin genes lack PPRE. In this situation, PPAR-δ may downregulate these genes via trans-suppression of other transcription factors such as NF-κB, which plays an important role in regulating the mRNA expression of various pro-inflammatory adhesion molecules. In quiescent ECs, NF-κB is kept inactive by association with IκBα in the cytosol. On stimulation, IκB is phosphorylated and degraded, thus allowing subsequent nuclear translocation and activation of NF-κB. We found that GW0742, while inhibiting the inflammatory action of TNF-α, did not prevent the TNF-α–induced degradation of IκBα and subsequent nuclear translocation of NF-κB (Figure 2). EMSA results also showed no decrease in either basal or TNF-α-stimulated NF-κB DNA binding activity (data not shown). In addition, GW0742 showed no inhibitory effect on the TNF-α–stimulated activation of mitogen-activated protein kinase pathways in ECs. PPAR-δ probably interferes with transcription of the inflammatory genes via a corepressor-related mechanism at the chromatin level.

Another novel finding in this study is that the PPAR-δ agonist GW0742 potently inhibited EC activation, and the inhibitory effect not only persisted but was further enhanced, after the decrease of PPAR-δ expression by siRNA (Figure 4). In fact, knocking down PPAR-δ per se attenuated the induction of VCAM-1 and E-selectin in response to TNF-α (Figure 4B and 4C). Therefore, our findings suggest that the endogenous PPAR-δ, in the absence of specific ligands, may facilitate expression of these pro-inflammatory genes and the ligand robustly suppressed the pro-inflammatory response in ECs even when the level of its cognate receptor had been reduced. This seemingly paradoxical result agrees with observations for other members of the PPAR family such as PPAR-γ. Although the PPAR-γ agonists thiazolidinediones effectively sensitize insulin action, mice with heterozygous deficiency of PPAR-γ actually have improved insulin sensitivity. Unlike other PPAR isomers, PPAR-δ is known to be a transcription repressor rather than an activator, when not liganded by specific agonists, through its ability to recruit...
potent repressors such as SMRT, SHARP, and class I histone deacetylases. A recent study showed that treatment with the PPAR-δ agonist GW501516 decreased the expression of pro-inflammatory cytokines MCP-1 and IL-1β in macrophages, and transplantation of bone marrow from the PPAR-δ-null mice also reduced atheroma formation in LDLR-null mice. To reconcile these observations, Lee and colleagues proposed an unconventional ligand-dependent transcriptional pathway whereby PPAR-δ controls an inflammatory switch through its association and dissociation with transcriptional repressors. In our study, we provide clear evidence that in ECs the ligand binding caused the dissociation of BCL-6 from PPAR-δ and the subsequent association of BCL-6 with the VCAM-1 promoter region (Figure 2B and 2C). Because the PPAR-δ agonist GW0742 also elicits a coordinated expression of a panel of antioxidant genes (Figure 3B), PPAR-δ agonists may suppress EC activation via a binary mechanism, that is, the synthetic ligand binds to PPAR-δ and recruits the coactivators to replace the corepressors such as BCL-6. The released corepressors relocate to repress the transcription of pro-inflammatory genes such as VCAM-1 and E-selectin and, thus, contribute to the vascular protection. In addition, the ligand-activated PPAR-δ is able to induce its target genes, among which are those encoding antioxidative enzymes (SOD1, catalase, and thioredoxin, etc), and to reduce the TNF-α-triggered oxidative stress. Such a synergistic action leads to a potent inhibition of endothelial activation and the ensuing leukocyte activation (Figure 5). This notion is supported by our siRNA results. Reduction of PPAR-δ abundance may also cause the translocation of the repressors from the PPAR-δ targets into the transcriptional complexes at the inflammatory genes. Because PPAR-δ is an abundantly expressed isoform in many types of tissues and cells (including ECs), even at a reduced level, the receptor would still be sufficient to cause the transcription of its target genes in the presence of the specific agonist.

In summary, we have demonstrated in HUVECs that PPAR-δ agonists have a strong anti-inflammatory effect potentiated by the siRNA-mediated reduction of endogenous PPAR-δ. PPAR-δ may be a potential therapeutic target for many cardiovascular and metabolic disorders such as atherosclerosis and diabetes, in which aberrant endothelial activation plays a significant pathophysiological role.

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Disclosures

None.

References


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Supplemental Data

Expanded Materials and Methods

**Cells and Reagents**—Human umbilical vein endothelial cells (HUVECs) were harvested by collagenase treatment of umbilical cord veins and cultured on collagen-coated plates. Cells were maintained in M199 supplemented with 20% fetal bovine serum (FBS), 20 mM HEPES (pH 7.4), 1 ng/ml of recombinant human fibroblast growth factor (Sigma), and 90 µg/ml of heparin and antibiotics. In all experiments, HUVECs within three passages were used. THP-1, a human monocyte cell line (ATCC, Manassas, VA, USA), was grown in RPMI 1640 containing 10% FBS. Bovine aortic endothelial cells (BAECs) were harvested from bovine aorta and maintained in DMEM with 10% FBS. Recombinant human TNF-α and IL-1β were from R&D systems (Minneapolis, MN, USA). GW0742 was a generous gift of Dr. Timothy Willson (GlaxoSmithKline, North Carolina, USA) and Sigma-Aldrich. GW501516 was purchased from Caymen Chemical (Ann Arbor, MI, USA). Antibodies against PPAR-δ, IκBα, p65 and BCL-6 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against JNK, phospho-JNK (Thr183/Tyr185), ERK, phospho-ERK (Thr202/Tyr204), p-p38 (Thr180/Tyr182) and histone H3 were from Cell Signaling (Danvers, MA, USA).

*The sequences of specific primers:*

**VCAM-1:**
- Forward: 5'-TTCATTGACTTGCAGCACC-3’
- Reverse: 5'-TTCTTGCAGCTTTGTGGATG-3’;

**E-selectin:**
- Forward: 5'-AGCCCAGAGCCTCCAGTGTA-3’
- Reverse: 5'-CTCCAATAGGGGAATGAGCA-3’;
GW0742 activated PPAR-δ in a dose-dependent manner. BAECs were co-transfected with pPPRE-TK-luciferase with either pcDNA-PPAR-δ or pcDNA3.1 and treated with GW0742 or DMSO for 48 h. Luciferase activity was measured, normalized against β-galactosidase activity and expressed as folds induction of the basal activity. *, p<0.05; **, p<0.01.

Effects of GW0742 and GW501516 on IL-1β induced VCAM-1 Expression. HUVECs were pretreated with DMSO, GW0742 (1 µM) or GW501516 (0.1 µM) for 24 h before exposure to IL-1β (10 ng/ml, 6 h). Gene expression of VCAM-1 was determined by quantitative RT-PCR, expressed as folds induction as compared with the control after normalization to GAPDH. Bars represent mean ± SEM of three independent experiments. *, p<0.05.
**Effect of GW0742 on mRNA stability.** Cells were treated with actinomycin D after TNF-α stimulation. At 2, 4 and 8 h, RNA was extracted and hybridized for VCAM-1. The result is expressed as percentage of the mRNA abundance at 0 h of actinomycin D treatment.

**GW0742 inhibited endothelial-leukocyte adhesion.** Confluent HUVECs were treated with GW0742 for 24 h, then with TNF-α or control for 18 h, and incubated with GFP-expressing THP-1 cells for 30 min. THP-1 cells bound to ECs were visualized on fluorescence microscopy. The number of bound THP-1 cells was quantified by counting four microscopic fields per well in triplicates. Bars represent mean ± SEM of three independent experiments. **, p<0.01.
Effects of GW0742 on mitogen-activated protein kinase pathways. HUVECs were treated with GW0742 (1 µM) or control for 24 h before exposure to TNF-α (2 ng/ml) for indicated times. The immunoblots were probed with antibodies against phosphorylated JNK (p-JNK), JNK, phosphorylated ERK (p-ERK), ERK, phosphorylated p38 (p-p38) or α-tubulin. Data are representative of three independent experiments.
GAPDH:  Forward: 5'-ACCACAGTCCATGCCATCAC-3'
        Reverse: 5'-TCCACCACCCTGTTGCTGTA-3';
SOD1:    Forward: 5'-AGGGCATCA TCAATTTGAGGACTACC-3'
        Reverse: 5'-GGGCCTCAGACTACATCCAA-3';
Catalase: Forward: 5'-TTGGCCTCAACAGGACTACC-3'
        Reverse: 5'-TTGACCGCTTTCTTCTGGAT-3';
Thioredoxin: Forward: 5'-TTTCCATCGGCTTTTACG-3'
           Reverse: 5'-TTGGCTCCAGAATTTCACC-3';
PPARδ:  Forward: 5'-TGCAGGCTTAGGTCCTCACT-3'
        Reverse: 5'-GAGGCATCTCGGCTCTACTG-3'.