Biochemical Pharmacology 78 (2009) 1186–1197

Contents lists available at ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

PPARα activator fenofibrate modulates angiotensin II-induced inflammatory responses in vascular smooth muscle cells via the TLR4-dependent signaling pathway

Yuan-Yuan Ji [a], Jun-Tian Liu [a,*], Na Liu [a], Zhi-Dong Wang [b], Chuan-Hao Liu [a]

[a] Department of Pharmacology, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710061, PR China
[b] Department of General Surgery, The Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710004, PR China

ARTICLE INFO

Article history:
Received 16 April 2009
Accepted 22 June 2009

Keywords:
PPARα
Angiotensin II
Toll-like receptor 4
Inflammation
Vascular smooth muscle cells (VSMCs)
Atherosclerosis

ABSTRACT

Angiotensin II (Ang II) is a crucial contributor to inflammatory processes involved in development and progression of atherosclerotic lesion. Toll-like receptor 4 (TLR4) signaling responsible for the initiation of inflammation also participates in pathogenesis of atherosclerosis. The protective effect of peroxisome proliferator-activated receptor α (PPARα) activators on atherosclerosis may be due to their impact on vascular inflammation, plaque instability and thrombosis. However, mechanisms underlying the inhibitory effects of PPARα activators on Ang II-induced vascular inflammation and the TLR4-dependent signaling pathway involved in vascular smooth muscle cells (VSMCs) remain unclear. The present study demonstrated that PPARα activator fenofibrate decreased Ang II-induced generation of pro-inflammatory mediators such as TLR4, MMP-9 and TNF-α, but enhanced production of anti-inflammatory molecules like PPARα and 6-keto-$PGF_{1\alpha}$ both in vivo and in vitro. Meanwhile, treatment of VSMCs with the TLR4 inhibitor or TLR4 siRNA showed that the inhibitory effects of fenofibrate on Ang II-induced inflammatory responses in VSMCs were dependent on TLR4. Furthermore, fenofibrate depressed Ang II-induced inflammatory responses in VSMCs by intervening the downstream effector molecules of the TLR4-dependent signaling pathway, including interferon-gamma inducible protein 10 (IP-10), protein kinases C (PKC) and nuclear factor κB (NF-κB). Thus, these findings provide the evidence for beneficial effects of PPARα activator fenofibrate to counter-regulate vascular inflammation induced by Ang II. More importantly, anti-inflammatory action of fenofibrate via interfering with the TLR4-dependent signaling pathway (TLR4/IP-10/PKC/NF-κB) works in concert to protect against atherosclerosis.

1. Introduction

Vascular inflammation is closely involved in the initiation and progression of atherosclerosis, and is also present in hypertension- and diabetes-induced vascular complications. Angiotensin II (Ang II), an endogenous vasoconstrictor, plays a crucial role in the regulation of blood pressure and electrolyte homeostasis [1,2]. Accumulating evidences show that Ang II is also capable of inducing vascular inflammation, which has emerged as a major driving force of atherosclerotic lesion development [3–5]. Ang II enhances release of tumor necrosis factor-α (TNF-α) and reduces secretion of prostacyclin (PGI_2) [6,7]. Furthermore, Ang II upregulates expressions of matrix metalloproteinase-9 (MMP-9) and toll-like receptor 4 (TLR4) in vascular smooth muscle cells (VSMCs), which are also relevant for activation of the inflammatory and immunological systems in atherosclerotic diseases [8–10].

TLR4, a pattern-recognizing receptor, recognizes mostly exogenous ligands like bacterial lipopolysaccharides (LPS) and activates inflammatory and innate immune responses, and then initiates intracellular signaling cascades, which ultimately cause the activation of nuclear factor-κB (NF-κB) and the expressions of pro-inflammatory cytokines [11,12]. The TLR4 signaling also leads to activation of mitogen-activated protein kinases and production of interferon-gamma inducible protein 10 (IP-10), which, in turn, activates protein kinases C (PKC) and finally results in the nuclear translocation of NF-κB [13–15]. Recent studies have shown that TLR4 is markedly expressed in human atherosclerotic vessels [16]. Therefore, the TLR4 signaling provides an important link between inflammation, immunity and atherosclerosis.

* Corresponding author at: Postbox 58, Xi'an Jiaotong University School of Medicine, Xi'an, Shaanxi 710061, PR China. Tel.: +86 29 82655188; fax: +86 29 82655188.
E-mail address: ljt@xjtu.edu.cn (J.-T. Liu).

0006-2952/$ – see front matter.
doi:10.1016/j.bcp.2009.06.095

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors which form a subfamily of the nuclear receptor gene family. The PPAR family comprises three members, α, γ and β/δ. PPARα activation by fibric acids regulates lipid metabolism, improves insulin sensibility, and decreases thrombosis and vascular inflammation, thus preventing the development of atherosclerosis [17,18]. PPARα activation also inhibits interleukin-1-stimulated interleukin-6 secretion in human aortic smooth muscle cells and MMP-9 expression in human atheroma [19,20]. In clinical studies, PPARα activator fenofibrate significantly lowers TNF-α level in plasma of patients with hypertriglyceridemia [21]. In animal studies, fenofibrate improves myocardial inflammation and collagen deposition in rats infused with Ang II [22]. In addition, reverse by fenofibrate of the elevated blood pressure response to infusion of Ang II is accompanied by reduced oxidative stress and inflammation in the vascular wall [23]. Fenofibrate thus shows a range of anti-inflammatory effect in vitro and in vivo consistent with clinical benefits on atherosclerosis or its complications [24]. Although PPARα serves anti-inflammatory action by transrepressing inflammatory signaling pathways [25], much less is known about the underlying mechanisms for the inhibitory effects of fenofibrate on Ang II-induced vascular inflammation and the TLR4-dependent signaling pathway involved. Herein, we examined whether fenofibrate was able to inhibit Ang II-mediated inflammatory responses in VSMCs via interfering with the TLR4-dependent signaling pathway so as to elucidate its anti-inflammatory and anti-atherosclerotic mechanisms.

2. Materials and methods

2.1. Reagents

Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin and streptomycin were purchased from Gibco BRL (Carlsbad, CA, USA). Ang II, LPS from *Escherichia coli* 0111:B4, PD123319, PD098059, phorbol 12-myristate-13 acetate (PMA), chelerythrine, bovine serum albumin, pentobarbital and ethidium bromide were produced by Sigma (St. Louis, MO, USA). Losartan was purchased from Merk (Merk Corp., Darmstadt, Germany). Fenofibrate was from Cayman (Ann Arbor, MI, USA). Polyclonal anti-rat TLR4, anti-AT_1, anti-PKC, anti-NF-κB and anti-α-smooth muscle actin antibodies were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against PPARα, PPARγ, IP-10 and MMP-9 were supplied by ABCAM (Cambridge, UK). Antibodies against phospho-ERK1/2 and total ERK1/2 were purchased from Cell Signaling Technology (Beverly, MA, USA). Monoclonal anti-TLR4 antibody (MTS510) was from eBioscience (San Diego, CA, USA). Rat TNF-α ELISA kit was purchased from Bender (Bender MedSystems, CA, USA). Rat 6-keto prostaglandin $F_{1\alpha}$ (6-keto-$PGF_{1\alpha}$) ELISA kit was purchased from Cayman (Ann Arbor, MI, USA). TNF-α and 6-keto-$PGF_{1\alpha}$ Detection kits were produced by Eastern Asia Radioimmunity Research Institute (Beijing, China). siRNA specific for TLR4 (siGENOME SMARTpool, M-090819-00), siRNA specific for IP-10 (siGENOME SMARTpool, M-099124-00), negative control siRNA (siGENOME Non-Targeting siRNA Pool, D-001206-13-05) and DharmaFECT 2 transfection reagent (T-2002-02) were obtained from Dharmacon (Lafayette, CO, USA). TRIzol kit and SuperScript III Platinum SYBR-Green One-Step qRT-PCR kit were provided by Invitrogen Corp. (Carlsbad, CA, USA). Revert Aid™ First Strand cDNA Synthesis kit was produced by Fermentas (St. Leon-Rot, Germany). Agarose gels were from Spainish Biochemicals Corp. (Pronadisa, Madrid, Spain). Reagents for the enhanced chemiluminescence were purchased from Pierce Corp. (Rockford, IL, USA).

2.2. Animal experiments

The study was approved by the appropriate Institutional Animal Care Committee and conformed to NIH guidelines. Male Sprague–Dawley rats (weight 200–220 g) were obtained from the Laboratory Animal Institute of Xi'an Jiaotong University School of Medicine. And the experiment was also granted by the Ethics Review Board of Xi'an Jiaotong University. Rats were subcutaneously infused by use of Alzet osmotic mini-pumps (model 2001, Alza Corp., USA) with Ang II at a dose of 150 ng/(kg min) or the same volume of 0.9% saline for 7 days.

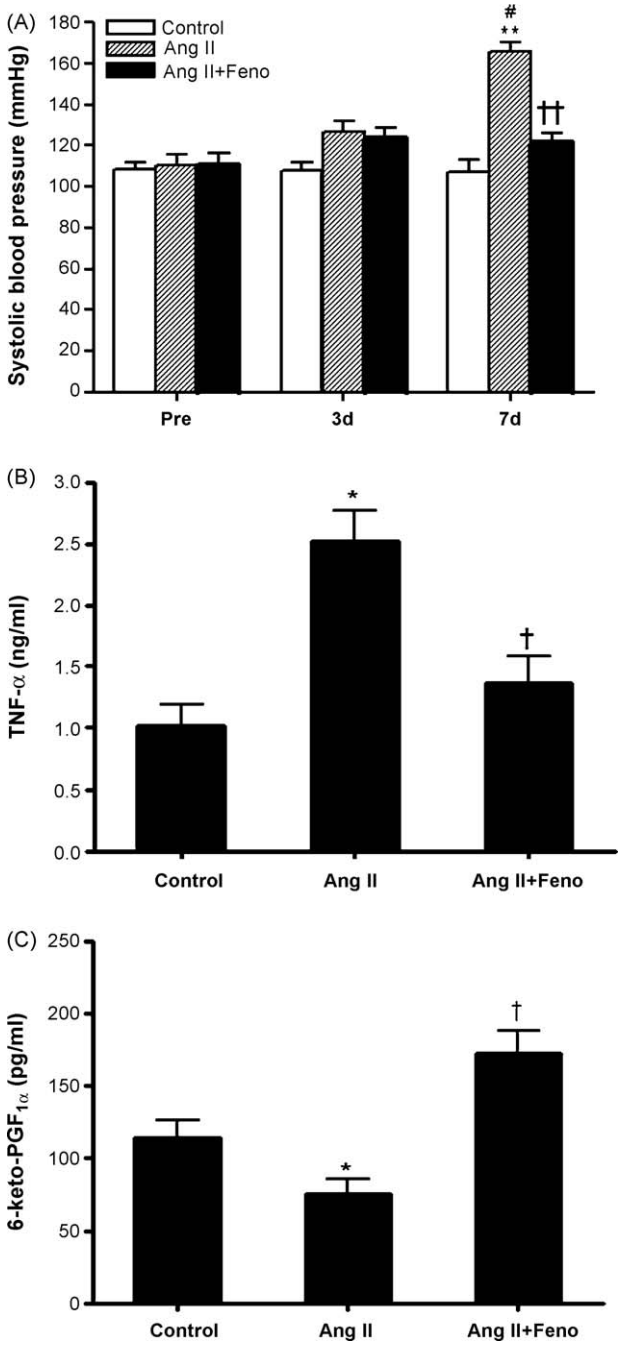

Fig. 1. (A) Systolic blood pressure in Ang II-infused rats treated with or without fenofibrate (Feno) for before (Pre), 3 and 7 days. (B and C) Contents of serum TNF-α and plasma 6-keto-$PGF_{1\alpha}$ in Ang II-infused rats treated with or without fenofibrate (Feno) for 7 days as measured by a radioimmunity method. Values are expressed as means ± S.E.M. ($n = 6$). $^{*}P < 0.05$, $^{**}P < 0.01$ vs. control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. Ang II; $^{\#}P < 0.05$ vs. Pre in the same group.

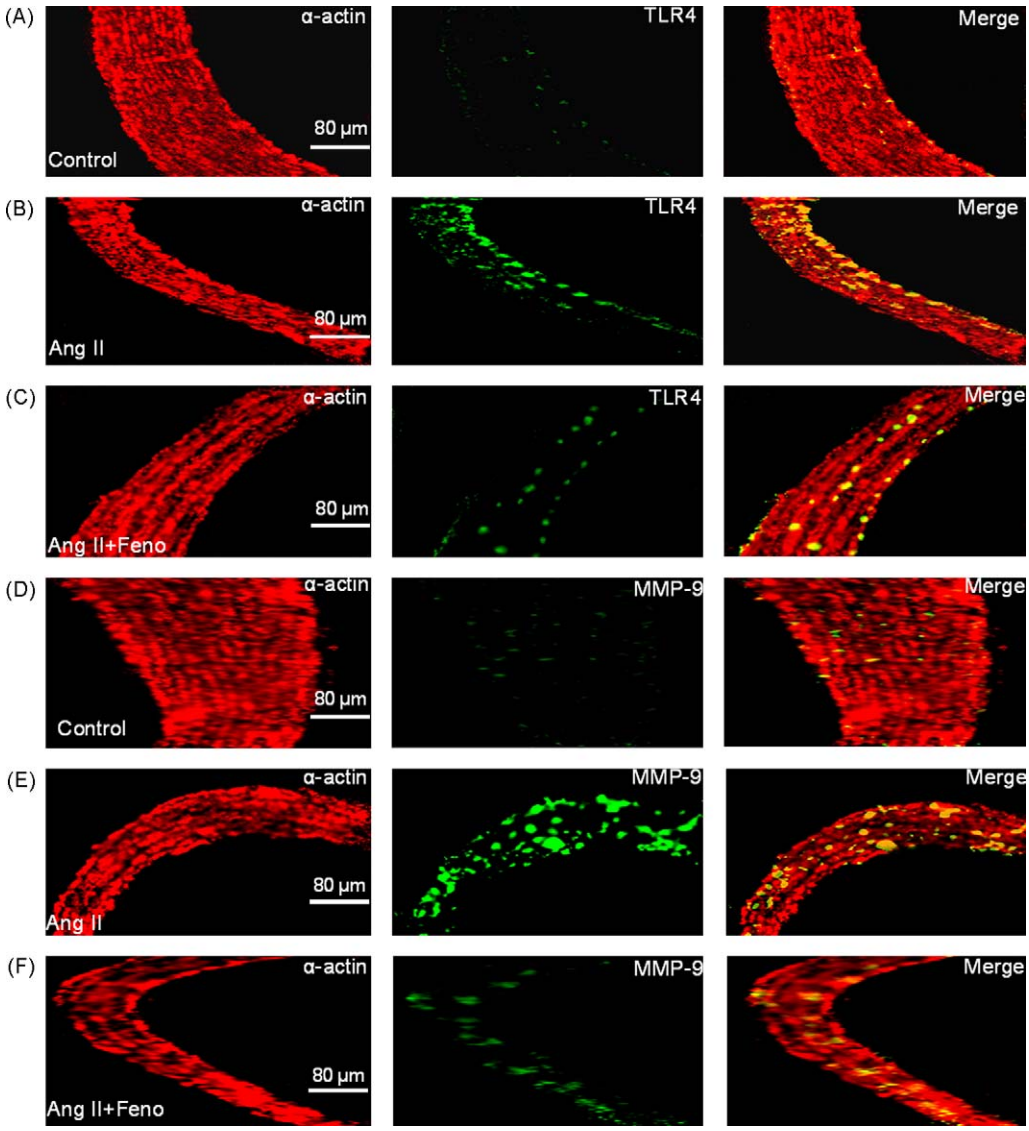

Fig. 2. Immunofluorescent double staining of TLR4 and MMP-9 in vascular smooth muscle tissue in the thoracic aorta of Ang II-infused rats (150 ng/(kg min)) treated with or without fenofibrate (Feno) for 7 days. (A–C) Representative TLR4 expression was shown on the luminal surface in each group. (D–F) Representative MMP-9 expression was observed on the media in each group. TLR4 and MMP-9 were expressed predominantly in smooth muscle cells, as identified by antibody against α-actin. Yellow color in the right is the merged fields of red (for α-actin) and green (for TLR4 or MMP-9). The results show a representative of six animals in each group.

Fenofibrate (150 mg/(kg day)) dissolved in 0.9% saline was intragastrically administrated for 7 days, starting from the day of Ang II infusion. Systolic blood pressure (SBP) was measured by the tail-cuff method. The animals were sacrificed by injecting excess amount of pentobarbital, and blood was collected by abdominal aorta for analysis of serum TNF-α and plasma 6-keto-$PGF_{1\alpha}$ levels. One portion of thoracic aorta was dissected and cleaned of fat, then frozen in liquid nitrogen for western blot assay and RNA extraction. Other portion of thoracic aorta was fixed in 4% formaldehyde solution and embedded in paraffin. Tissue sections (5 μm thick) were prepared for confocal immunohistochemical evaluation. The

Table 1
Primer sequence used for real-time PCR analysis.

Gene	Primer sequence	Accession number	Expected size (bp)
TLR4	5′-GGCATCATCTTCATTGTCCTTG-3′ 5′-AGCATTGTCCTCCCACTCG-3′	NM_019178	111
PPARγ	5′-GGAAGCCCTTTGGTGACTTTATGG-3′ 5′-GCAGCAGGTTGTCTTGGATGTC-3′	NM_013124	174
PPARα	5′-CGGGTCATACTCGCAGGAAAG-3′ 5′-TGGCAGCAGTGGAAGAATCG-3′	NM_013196	155
MMP-9	5′-CCCTACTGCTGGTCCTTCTGAG-3′ 5′-AATTGGCTTCCTCCGTGATTCG-3′	NM_031055	162
β-Actin	5′-ATCGGCAATGAGCGGTTCC-3′ 5′-AGCACTGTGTTGGCATAGAGG-3′	NM_031144	149

details of the procedures were performed according to the methods of Gao et al. [26].

2.3. Radioimmunity analysis

To determine the effect of fenofibrate on serum TNF-α and plasma 6-keto-$PGF_{1\alpha}$ levels in Ang II-infused rats, a radioimmunity method was used to measure the contents with the TNF-α and 6-keto-$PGF_{1\alpha}$ detection kits according to the manufacturer's instruction. Radioiodinated TNF-α was produced by the balance method ranging from (0.3 to 24.3) ng/ml to construct the standard curve. Sensitivity of the assay was 0.3 ng/ml. Intra- and inter-assay coefficients of variation were <5% and <8%, respectively. Concentrations of 6-keto-$PGF_{1\alpha}$ ranging from (25 to 1600) pg/ml were used to construct the standard curve. The sensitivity of the assay was <25 pg/ml. Intra- and inter-assay coefficients of variation were <3.5% and <10%, respectively. Details of the procedures were reported previously [27].

2.4. Immunofluorescent double staining of rat vessels

For immunofluorescent double staining, deparaffinized sections were fixed in 100% cold methanol and non-specific proteins were blocked with 1% bovine serum albumin. The sections were incubated with rabbit anti-rat TLR4 antibody (1:200) or anti-rat MMP-9 antibody (1:400) and mouse anti-rat α-actin antibody (1:100) at 4 °C overnight. After the incubation with appropriate fluorescence-labeled secondary antibody at the room temperature for 1 h, the sections were observed under confocal microscope (Leica TCS SP2-AOB, Germany).

2.5. Cell culture

VSMCs were isolated from the thoracic aorta of male Sprague–Dawley rats by the explant technique as previously described [28]. The cells were grown in DMEM supplemented with 10% FBS, 100 U/ml streptomycin, and 100 U/ml penicillin in 5% CO_2 at 37 °C. The cells were used between passages 3 and 10 for all experiments. The cells exhibited the typical "hill and valley" growth morphology and were confirmed by smooth muscle α-actin immunostaining. When the cells were grown to confluence, the medium was changed to serum free medium for an additional 24 h before the experiments.

2.6. Small-interfering RNA

VSMCs at a density of 5×10^6 cells/well were seeded into 6-well plates and were grown until 60–80% confluent. The cells were transiently transfected with 150 pM of TLR4 small-interfering RNA (siRNA), 100 nM of IP-10 siRNA or negative control siRNA (NC siRNA) using DharmaFECT 2 transfection reagents according to the manufacturer's instructions. After 48 h, TLR4 mRNA levels were detected by quantitative real-time PCR and RT-PCR, and IP-10 protein expression was analyzed by western blot. Transfection rates of 60–70% of the cells were accepted for all the experiments.

2.7. Western blot analysis

As described previously [26], protein samples (20 μg) were separated on 12% SDS-PAGE gels and transferred onto a polyvinylidene difluoride (PVDF) membrane (Bio-Rad, USA).

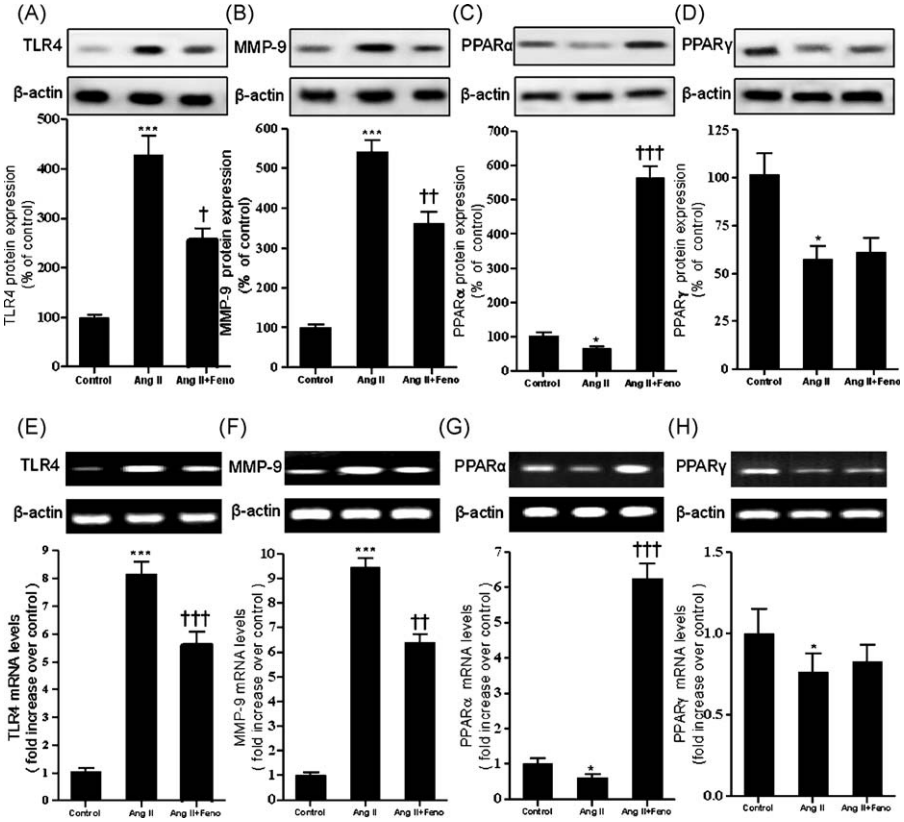

Fig. 3. Protein and mRNA expressions of TLR4, MMP-9, PPARα and PPARγ in the thoracic aorta of Ang II-infused rats (150 ng/(kg min)) treated with or without fenofibrate (Feno) for 7 days. (A) TLR4, (B) MMP-9, (C) PPARα and (D) PPARγ protein expressions were measured by western blot. (E) TLR4, (F) MMP-9, (G) PPARα and (H) PPARγ mRNA levels were analyzed by RT-PCR and quantitative real-time PCR after normalization to β-actin mRNA. Values are expressed as means ± S.E.M. ($n = 6$). $^{*}P < 0.05$, $^{***}P < 0.001$ vs. control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. Ang II.

The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, and incubated with specific antibodies against TLR4 (1:200), MMP-9 (1:400), PPARα (1:100), PPARγ (1:400), IP-10 (1:5000), PKC (1:400), NF-κB (1:400) and β-actin (1:400). The expression of β-actin was used as a loading control. Reagents for the enhanced chemiluminescence were applied to the blots, and the light signals were detected by X-ray film. Optical densities of the bands were scanned and quantified with the Gel Doc 2000 (Bio-Rad, USA).

2.8. Quantitative real-time PCR and RT-PCR

mRNA expression was determined by a modification of our previous method [29]. Total RNA was isolated using TRIzol kit. cDNA was synthesized from 1 μg samples of total RNA using Revert Aid™ First Strand cDNA Synthesis kit following the manufacturer's instructions. Real-time PCR was performed with the SuperScript III Platinum SYBR-Green One-Step qRT-PCR kit on a Mx3000P QPCR System (Stratagene, LaJolla, CA, USA) following the manufacturer's instructions. The samples were run in triplicate. Primers for rat TLR4, MMP-9, PPARα, PPARγ and β-actin were designed with Beacon designer v 4.0 (Premier Biosoft, USA) (see Table 1 for the sequences). β-actin was used as an endogenous control. Traditional PCR was performed according to the manufacturer's instructions. The RT-PCR products were analyzed by electrophoresis through 2% agarose gels containing ethidium bromide. A melting point dissociation curve generated by the instrument was used to confirm that only a single product was present. Quantitation of relative gene expression was calculated by the comparative C_t method ($2^{-\Delta\Delta C_t}$) as described by the manufacturer. Data were normalized to rat β-actin mRNA levels.

2.9. Enzyme-linked immunosorbent assay (ELISA)

VSMCs were seeded into 6-well plates at a density of 5×10^6 cells/well, and untreated or treated for the indicated time with fenofibrate (25, 50 and 100 μM) and Ang II (0.1 μM). In another experiment, the cells were pretreated with anti-TLR4 antibody (1 μg/ml) for 1 h prior to the addition of fenofibrate (100 μM) for 1 h, and subsequently stimulated with Ang II (0.1 μM) or LPS (100 ng/ml) for 24 h. Moreover, after application of NC siRNA or TLR4 siRNA for 48 h, VSMCs were exposed to fenofibrate (100 μM) for another 1 h, and subsequently stimulated with Ang II (0.1 μM) for 24 h. 6-Keto-$PGF_{1\alpha}$ and TNF-α in the culture supernatant of VSMCs were measured by ELISA kits according to the manufacturer's instructions.

2.10. Luciferase assay

To detect the effects of fenofibrate on TLR4 and MMP-9 expressions in Ang II-stimulated VSMCs, the cells were pretreated with fenofibrate (25, 50 and 100 μM) for 1 h, and then exposed to Ang II (0.1 μM) for 24 h. After the treatment, the cells were fixed with 4% formaldehyde–PBS for 15 min. The cell membranes were fenestrated with 0.3% Triton-100–PBS, and non-specific binding sites were blocked with 10% goat serum. The cells were incubated with rabbit anti-rat TLR4 (1:200) or anti-rat MMP-9 antibody (1:400), and then incubated with the secondary antibody conjugated to fluorescein isothiocyanate (FITC). The immunolabeled cells were observed and quantified under fluorescence confocal microscopy (Leica TCS SP2-AOB, Germany). Data were expressed as relative to control (%).

2.11. Statistical analysis

Data were expressed as means ± S.E.M. Differences between two groups were determined either by unpaired Student's *t*-test or by one-way ANOVA followed by post hoc Dunns multiple-comparison test. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. SBP in Ang II-infused rats

SBP of the rat was evaluated at before, 3 and 7 days after infusing Ang II. At the third day, there was no significant difference in SBP among groups (Fig. 1A). After infusing Ang II for 7 days, SBP was increased but, fenofibrate at the dose of 150 mg/(kg day) significantly lowered the rise of SBP.

3.2. Effects of fenofibrate on TNF-α and 6-keto-$PGF_{1\alpha}$ productions in Ang II-infused rats

To evaluate the effects of fenofibrate on TNF-α and 6-keto-$PGF_{1\alpha}$ productions in Ang II-infused rats, we measure serum TNF-α and plasma 6-keto-$PGF_{1\alpha}$ levels by a radioimmunity method. The results indicated that treatment of Ang II-infused rats with fenofibrate obviously decreased TNF-α and increased 6-keto-$PGF_{1\alpha}$ compared with Ang II-treated group (Fig. 1B and C),

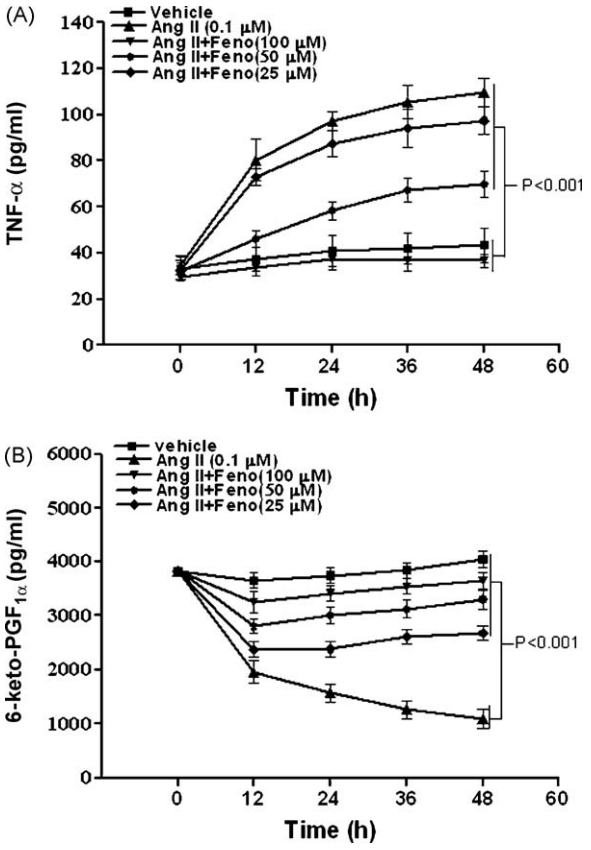

Fig. 4. Effect of fenofibrate (Feno) on TNF-α and 6-keto-$PGF_{1\alpha}$ productions in Ang II-stimulated VSMCs. The cells were pretreated for 1 h with different concentrations of Feno (25, 50 and 100 μM) before exposure to Ang II (0.1 μM) for the indicated time (12, 24, 36 and 48 h). The culture medium was then collected, and TNF-α and 6-keto-$PGF_{1\alpha}$ were determined with ELISA. Concentration-dependent reduction by fenofibrate of TNF-α production (A) and enhancement of 6-keto-$PGF_{1\alpha}$ secretion (B) in Ang II-stimulated VSMCs. Values are expressed as means ± S.E.M. from three independent experiments.

implying that fenofibrate has ability to reduce TNF-α production and to enhance 6-keto-$PGF_{1\alpha}$ release in Ang II-infused rats.

3.3. Immunofluorescent double staining of rat vessels for TLR4 and MMP-9

Expressions of TLR4 and MMP-9 in the thoracic aorta were identified with the immunofluorescent double staining and observed by confocal microscope. Representative graphs from each group were shown in Fig. 2. Compared with the control group, stronger TLR4 staining on the luminal surface and MMP-9 expression on the media were seen in the Ang II group, whereas TLR4 and MMP-9 expressions were remarkably impaired by fenofibrate. Identification of smooth muscle cells with the antibody to α-actin showed that TLR4 and MMP-9 were expressed predominantly in smooth muscle cells. These results demonstrate that fenofibrate markedly attenuates TLR4 and MMP-9 expressions in the aortic VSMCs of Ang II-infused rats.

3.4. Effects of fenofibrate on protein and mRNA expressions of TLR4, MMP-9, PPARα and PPARγ in the thoracic aorta of Ang II-infused rats

Under suggestion of the above-mentioned results with the immunofluorescent method, we further examined effects of fenofibrate on protein and mRNA expressions of TLR4, MMP-9, PPARα and PPARγ in the thoracic aorta of Ang II-infused rats. As shown in Fig. 3, fenofibrate downregulated protein and mRNA expressions of TLR4 (Fig. 3A and E) and MMP-9 (Fig. 3B and F), and upregulated PPARα expression (Fig. 3C and G) in the thoracic aorta of Ang II-infused rats. However, fenofibrate had little effect on PPARγ expression (Fig. 3D and H).

3.5. Concentration-dependent effects of fenofibrate on Ang II-induced TNF-α and 6-keto-$PGF_{1\alpha}$ productions in VSMCs

To further validate effects of fenofibrate on Ang II-induced TNF-α and 6-keto-$PGF_{1\alpha}$ productions, VSMCs were pretreated with different concentrations of fenofibrate (25, 50 and 100 μM) for 1 h, and then stimulated with Ang II (0.1 μM) for the indicated time (12, 24, 36 and 48 h). The results showed that fenofibrate concentration-dependently inhibited Ang II-stimulated TNF-α production and promoted 6-keto-$PGF_{1\alpha}$ secretion in VSMCs (Fig. 4).

3.6. Alleviation by fenofibrate of Ang II-induced TLR4 and MMP-9 expressions in VSMCs with luciferase assay

On the basis of the above-mentioned results in vivo, effects of fenofibrate on Ang II-induced TLR4 and MMP-9 expressions in VSMCs were further determined. The cells were pretreated for 1 h with different concentrations of fenofibrate (25, 50 and 100 μM) before exposure to Ang II (0.1 μM) for 24 h, and then TLR4 and MMP-9 expressions were identified with immunocytofluorescence and observed by confocal microscope. The results revealed that

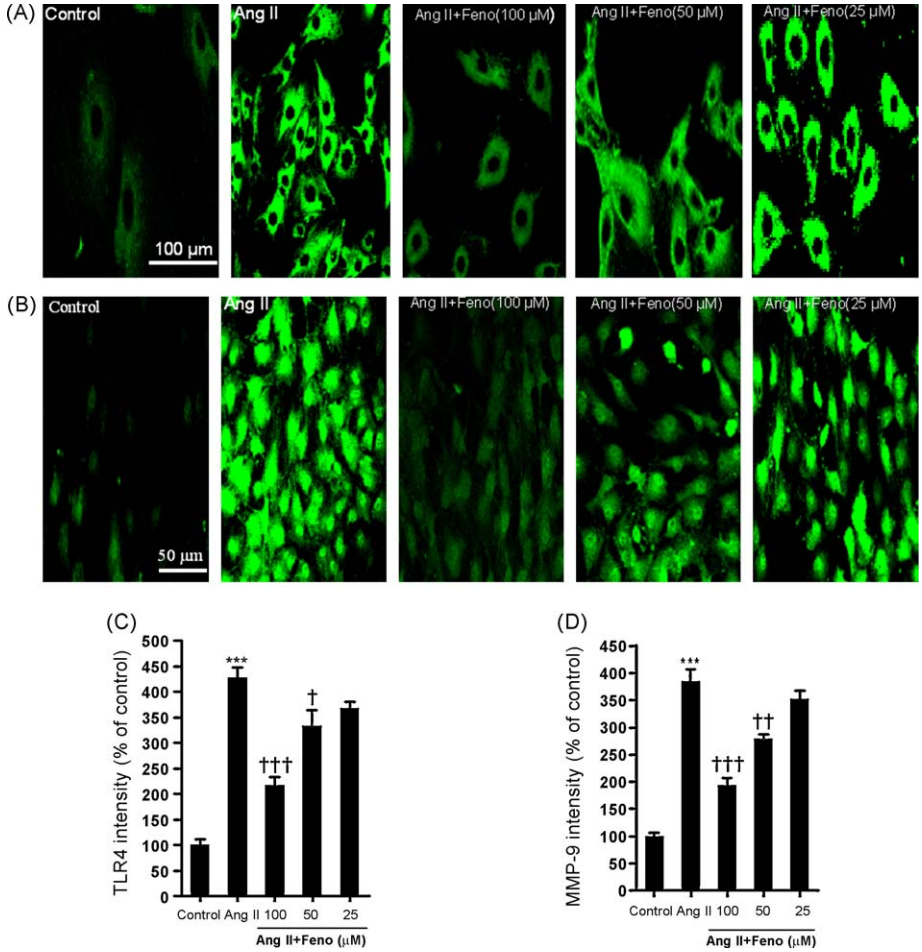

Fig. 5. Immunofluorescence analysis of TLR4 and MMP-9 expressions in Ang II-stimulated VSMCs treated with or without fenofibrate (Feno). VSMCs were pretreated for 1 h with different concentrations of fenofibrate (25, 50 and 100 μM) before exposure to Ang II (0.1 μM) for 24 h, and then expressions of TLR4 (A) and MMP-9 (B) were identified with immunocytofluorescence and observed by confocal microscope. Fluorescence intensity of TLR4 (C) and MMP-9 (D) were also detected. Values are expressed as means ± S.E.M. from three independent experiments. $^{***}P < 0.001$ vs. control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. Ang II.

fenofibrate also attenuated Ang II-induced expressions of TLR4 and MMP-9 in VSMCs in a concentration-dependent fashion (Fig. 5).

3.7. Effects of fenofibrate on expressions of TLR4, MMP-9, PPARα and PPARγ in Ang II-stimulated VSMCs

We further observed the effects of fenofibrate on protein and mRNA expressions of TLR4, MMP-9, PPARα and PPARγ in Ang II-stimulated VSMCs. As shown in Fig. 6, fenofibrate concentration-dependently downregulated protein and mRNA expressions of TLR4 and MMP-9, upregulated protein and mRNA expressions of PPARα but, showed little effect on PPARγ expression in Ang II-stimulated VSMCs.

3.8. Relationship between effect of fenofibrate on Ang II-induced inflammatory responses in VSMCs and TLR4

As mentioned above, fenofibrate is able to decrease TNF-α production and increase 6-keto-$PGF_{1\alpha}$ release, and downregulate TLR4 expression in Ang II-stimulated VSMCs in vivo and in vitro. To confirm whether fenofibrate suppresses Ang II-induced inflammatory responses via TLR4, VSMCs were pretreated with anti-TLR4 antibody (1 μg/ml) for 1 h prior to the addition of fenofibrate (100 μM) for 1 h, and subsequently stimulated with Ang II (0.1 μM) or LPS (100 ng/ml) for 24 h. As shown in Fig. 7A and B, compared with the control, stimulating the cells with Ang II or LPS leaded to TNF-α elevation and 6-keto-$PGF_{1\alpha}$ reduction, whereas the TLR4 inhibitor and fenofibrate partially reversed the Ang II- and LPS-induced effects on TNF-α and 6-keto-$PGF_{1\alpha}$ in VSMCs. Moreover, treatment of the cells with the combination of the TLR4 blocker and fenofibrate synergistically reversed the effects induced by Ang II in comparison with the treatment of the TLR4 blocker or fenofibrate alone. Considering that the TLR4 blocker antagonizes effects of Ang II and LPS on TNF-α and 6-keto-$PGF_{1\alpha}$ and fenofibrate also downregulates TLR4 expression in VSMCs, the modulatory effects of fenofibrate on TNF-α production and 6-keto-$PGF_{1\alpha}$ release in Ang II-stimulated VSMCs are related to TLR4.

The experiment also found that the TLR4 inhibitor reduced MMP-9 expression and enhanced PPARα and PPARγ expressions in Ang II- and LPS-stimulated VSMCs. Fenofibrate also exerted the similar effects on MMP-9 and PPARα expressions to the TLR4 inhibitor but, had little effect on PPARγ expression. Treatment of the cells with the combination of the TLR4 blocker and fenofibrate also synergistically reversed Ang II-induced changes of MMP-9 and PPARα expressions (Fig. 7C). Therefore, the effects of fenofibrate on MMP-9 and PPARα protein expressions in Ang II-stimulated VSMCs are also associated with TLR4. Considered together, these imply that the inhibitory effect of fenofibrate on Ang II-induced inflammatory responses in VSMCs are dependent on TLR4.

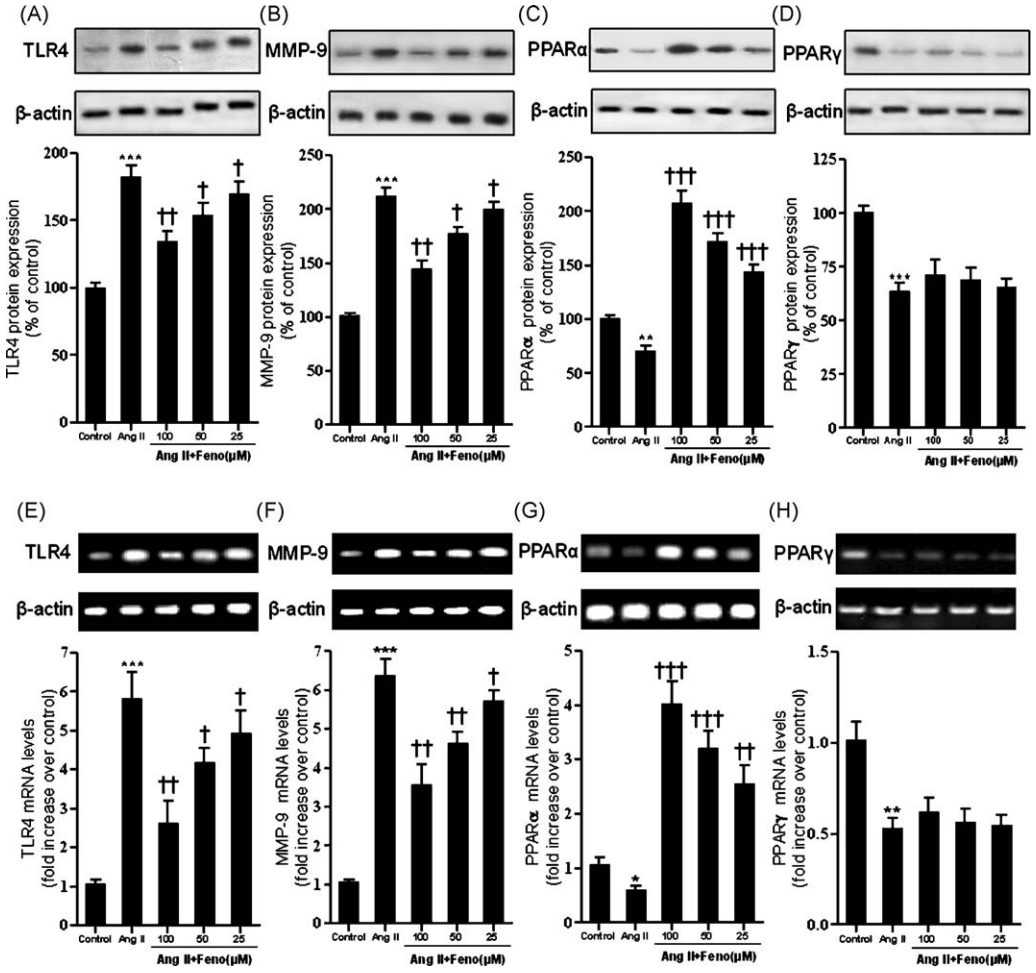

Fig. 6. Effects of fenofibrate (Feno) on Ang II-induced protein and mRNA expressions of TLR4, MMP-9, PPARα and PPARγ in VSMCs. VSMCs were pretreated with different concentrations of fenofibrate (25, 50 and 100 μM) for 1 h, and stimulated with Ang II (0.1 μM) for 24 h. Protein expressions of TLR4 (A), MMP-9 (B), PPARα (C), PPARγ (D) were measured by western blot, and mRNA levels of TLR4 (E), MMP-9 (F), PPARα (G), PPARγ (H) were analyzed by RT-PCR and quantitative real-time PCR after normalization to β-actin mRNA. Values are expressed as means ± S.E.M. from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. Ang II.

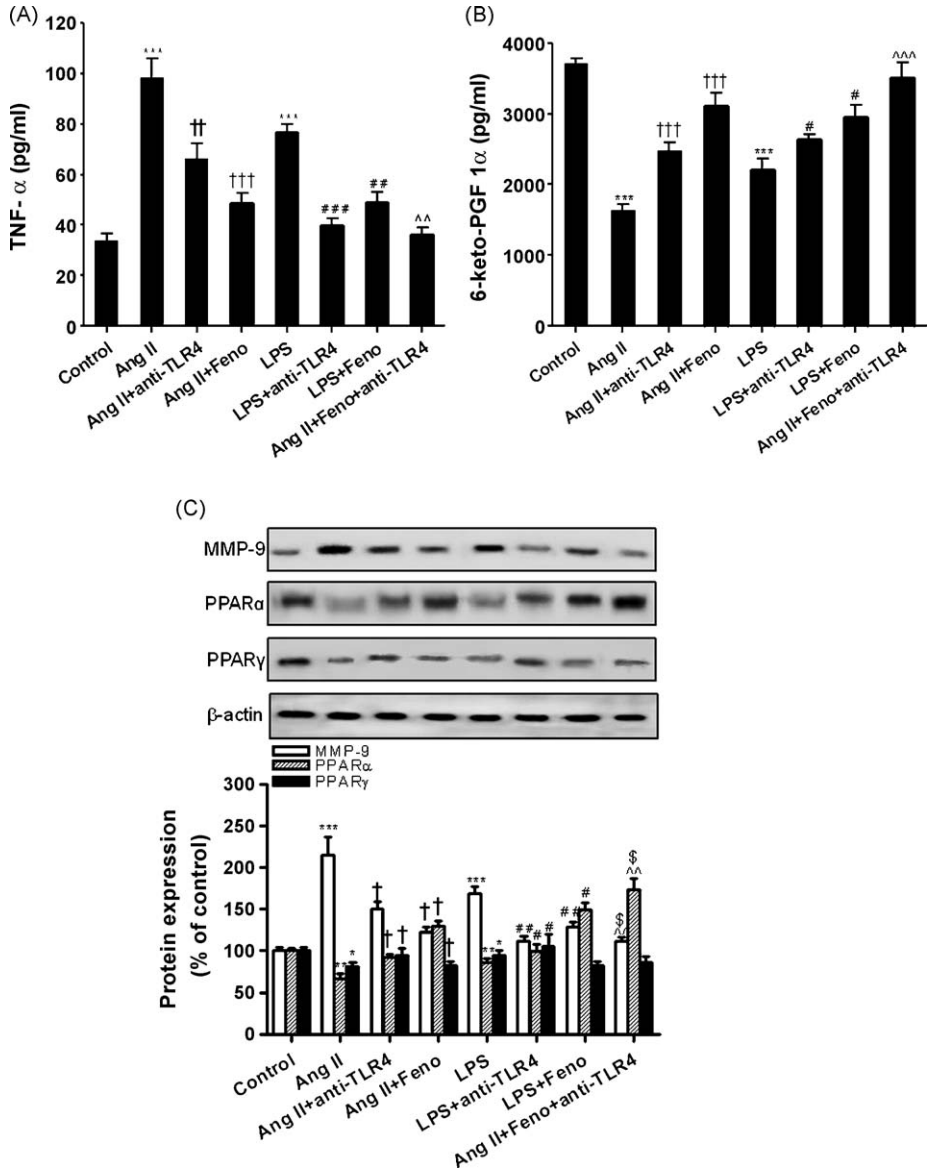

Fig. 7. Relationship between effects of fenofibrate (Feno) on Ang II-mediated inflammatory responses in VSMCs and TLR4. VSMCs were pretreated with or without anti-TLR4 antibody (1 μg/ml) for 1 h prior to the addition of fenofibrate (100 μM) for 1 h, and subsequently stimulated with Ang II (0.1 μM) or LPS (100 ng/ml) for 24 h. The conditioned media were collected and concentrations of TNF-α (A) and 6-keto-$PGF_{1\alpha}$ (B) were determined with ELISA. Protein expressions of MMP-9, PPARα and PPARγ were analyzed by western blot (C). Values are expressed as means ± S.E.M. from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. Ang II; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. LPS; $^{\wedge\wedge}P < 0.01$, $^{\wedge\wedge\wedge}P < 0.001$ vs. Ang II + anti-TLR4; $^{\$}P < 0.05$ vs. Ang II + Feno.

3.9. *Effect of TLR4 siRNA on anti-inflammatory action of fenofibrate in Ang II-stimulated VSMCs*

TLR4 siRNA was applied to VSMCs to further ascertain the role of this membrane receptor in Ang II-induced inflammatory responses in VSMCs and in anti-inflammatory mechanisms of fenofibrate. After VSMCs were transiently transfected with TLR4 siRNA for 48 h, TLR4 mRNA levels were remarkably declined (Fig. 8A). Knock down efficiency of TLR4 was 69% as determined by quantitative real-time PCR. The transfected VSMCs were pretreated with fenofibrate (100 μM) for 1 h prior to stimulation with Ang II (0.1 μM) for 24 h. Lack of TLR4 decreased Ang II-induced TNF-α production and increased 6-keto-$PGF_{1\alpha}$ release compared with negative control, demonstrating that Ang II regulates TNF-α and 6-keto-$PGF_{1\alpha}$ productions through TLR4. Fenofibrate reduced Ang II-induced TNF-α production and enhanced Ang II-decreased 6-keto-$PGF_{1\alpha}$ release in negative control but, the effects of fenofibrate were almost abolished in TLR4 siRNA control (Fig. 8B and C), suggesting that regulation by fenofibrate of TNF-α and 6-keto-$PGF_{1\alpha}$ productions depends on the existence of TLR4. The results also showed that fenofibrate did not change basal TNF-α level, but significantly increased 6-keto-$PGF_{1\alpha}$ secretion in the unstimulated VSMCs. The similar results to TNF-α were achieved for mRNA expression of MMP-9 (Fig. 8D). Furthermore, we found that TLR4 siRNA also abolished Ang II-induced decrease of mRNA expressions of PPARα and PPARγ compared to negative control, demonstrating that Ang II inhibits PPARα and PPARγ expressions through TLR4. Fenofibrate increased PPARα mRNA levels in negative control both in the normal and Ang II-stimulated VSMCs, but TLR4 siRNA did not remarkably change the increased effect of fenofibrate on PPARα mRNA levels (Fig. 8E), indicating that increase of PPARα expression by fenofibrate does not completely depend on TLR4. In addition, fenofibrate did not produce significant effect on PPARγ mRNA levels in negative control and TLR4 siRNA control (Fig. 8F).

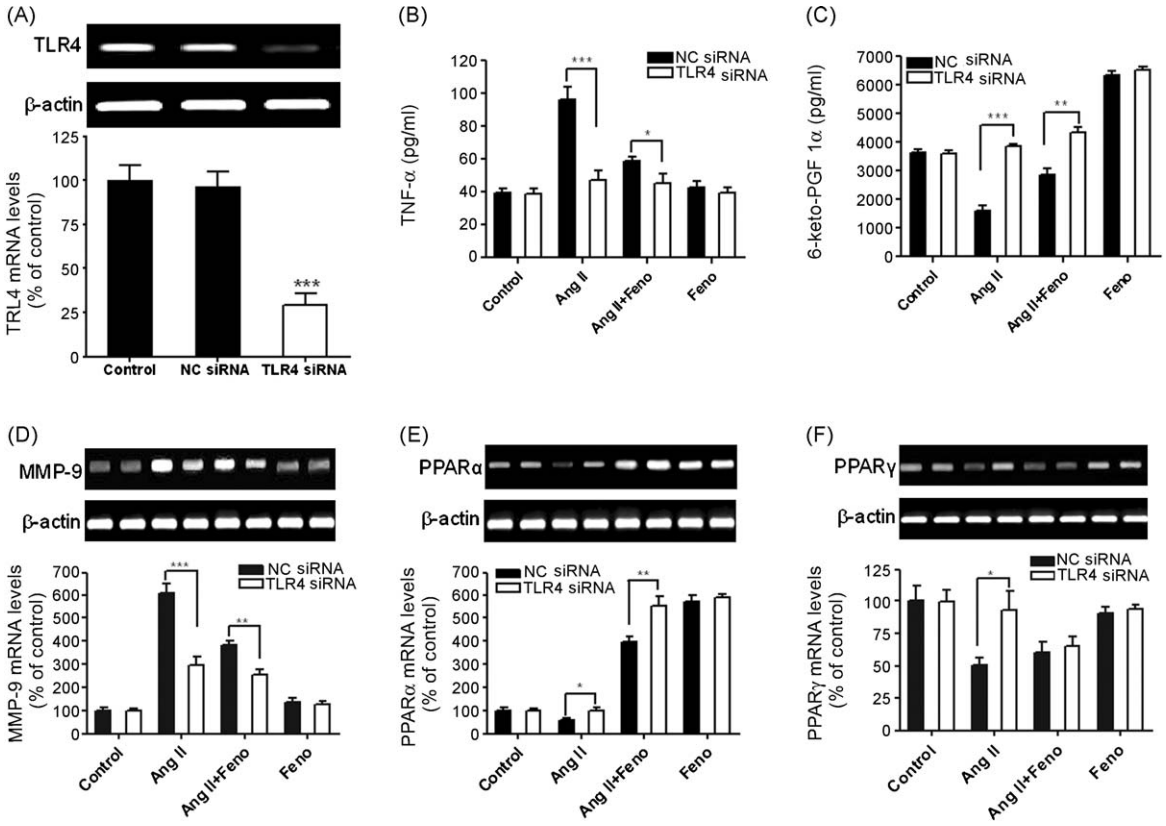

Fig. 8. Effects of TLR4 siRNA on anti-inflammatory action of fenofibrate (Feno) in Ang II-induced VSMCs. After application of negative control siRNA (NC siRNA) or TLR4 siRNA for 48 h, VSMCs were exposed to fenofibrate (100 μM) for another 1 h, and subsequently stimulated with Ang II (0.1 μM) for 24 h. (A) TLR4 mRNA levels in VSMCs were detected by RT-PCR and quantitative real-time PCR at 48 h post-transfection. (B and C) Concentrations of TNF-α and 6-keto-$PGF_{1\alpha}$ were determined by ELISA. mRNA levels of MMP-9 (D), PPARα (E) and PPARγ (F) were analyzed by RT-PCR and quantitative real-time PCR after normalization to β-actin mRNA. Values are expressed as means ± S.E.M. from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. NC siRNA.

3.10. Inhibitory effect of fenofibrate on Ang II-induced TLR4 expression in VSMCs without blockade of AT_1/ERK1/2 signaling

To clarify whether blockade of the AT_1/ERK1/2 signaling was involved in the inhibitory effect of fenofibrate on Ang II-induced TLR4 expression in VSMCs, VSMCs were subjected to losartan (1 μM), PD123319 (10 μM) and PD098059 (1 μM) for 30 min, followed by treatment of fenofibrate (100 μM) for further 1 h, and finally stimulated with Ang II (0.1 μM) for 24 h. As seen from Fig. 9A, AT_1 antagonist losartan and ERK1/2 antagonist PD098059 inhibited Ang II-induced TLR4 expression in VSMCs, whereas the AT_2 antagonist PD123319 had no effect. Although fenofibrate alone also inhibited Ang II-induced TLR4 expression in VSMCs, it did not have any additional effects to AT_1, AT_2 and ERK1/2 blockades, suggesting that blockade of the AT_1/ERK1/2 signaling was not involved in the inhibitory effect of fenofibrate on Ang II-induced TLR4 expression in VSMCs. Furthermore, ERK1/2 phosphorylation induced by Ang II (0.1 μM) treatment for 5 min was slightly impaired by fenofibrate (Fig. 9B). Meanwhile, treatment of VSMCs with Ang II (0.1 μM) for 24 h significantly downregulated AT_1 protein expression. But, fenofibrate did not produce an additional inhibitory effect on AT_1 expression in Ang II-induced cells (Fig. 9C). These results indicate that the inhibitory effect of fenofibrate on Ang II-induced TLR4 expression in VSMCs is not through blockade of AT_1/ERK1/2 signaling.

3.11. Inhibition by fenofibrate of Ang II-induced inflammatory responses in VSMCs via interference with the TLR4-dependent signaling pathway

Effect of TLR4 siRNA on Ang II-induced IP-10 expression in VSMCs pretreated with fenofibrate was examined. As described in Fig. 10A, lack of TLR4 reduced Ang II-induced protein expression of IP-10 compared with negative control. Fenofibrate also potently inhibited Ang II-induced protein expression of IP-10 in negative control, but did not further downregulate Ang II-induced protein expression of IP-10 in TLR4 siRNA control, demonstrating inhibition by fenofibrate of Ang II-induced IP-10 protein expression via interference with the TLR4-dependent signaling pathway. After VSMCs were transfected with IP-10 siRNA for 48 h, IP-10 protein expression was obviously declined in comparison with negative control (Fig. 10B). Fig. 10C displayed that IP-10 siRNA diminished Ang II-induced protein expression of PKC. Although fenofibrate depressed Ang II-induced protein expression of PKC in negative control, lack of IP-10 abolished the inhibitory effect of fenofibrate. Finally, we observed whether the blockade of PKC was required for the inhibitory effect of fenofibrate on Ang II-induced protein expression of NF-κB. VSMCs were pretreated with the specific PKC inhibitor chelerythrine (CH, 5 μM) for 1 h prior to the addition of fenofibrate (100 μM) for 1 h, and subsequently stimulated with Ang II (0.1 μM) or the specific PKC activator PMA (100 nM) for 24 h. As illustrated in Fig. 10D, fenofibrate significantly suppressed Ang II- and PMA-induced protein expression of NF-κB in VSMCs, and potentiated the inhibitory effect of the specific PKC inhibitor. It has been demonstrated that the PKC is able to mediate nuclear translocation of NF-κB, and increased NF-κB activation is associated with increased p65 protein level. Thus, the inhibitory effect of fenofibrate on NF-κB protein expression in VSMCs involves PKC. Taken together, all results in the present study suggest that fenofibrate exerts its inhibitory action on Ang II-induced inflammatory responses in VSMCs via interfering with TLR4/IP-10/PKC/NF-κB signaling pathway.

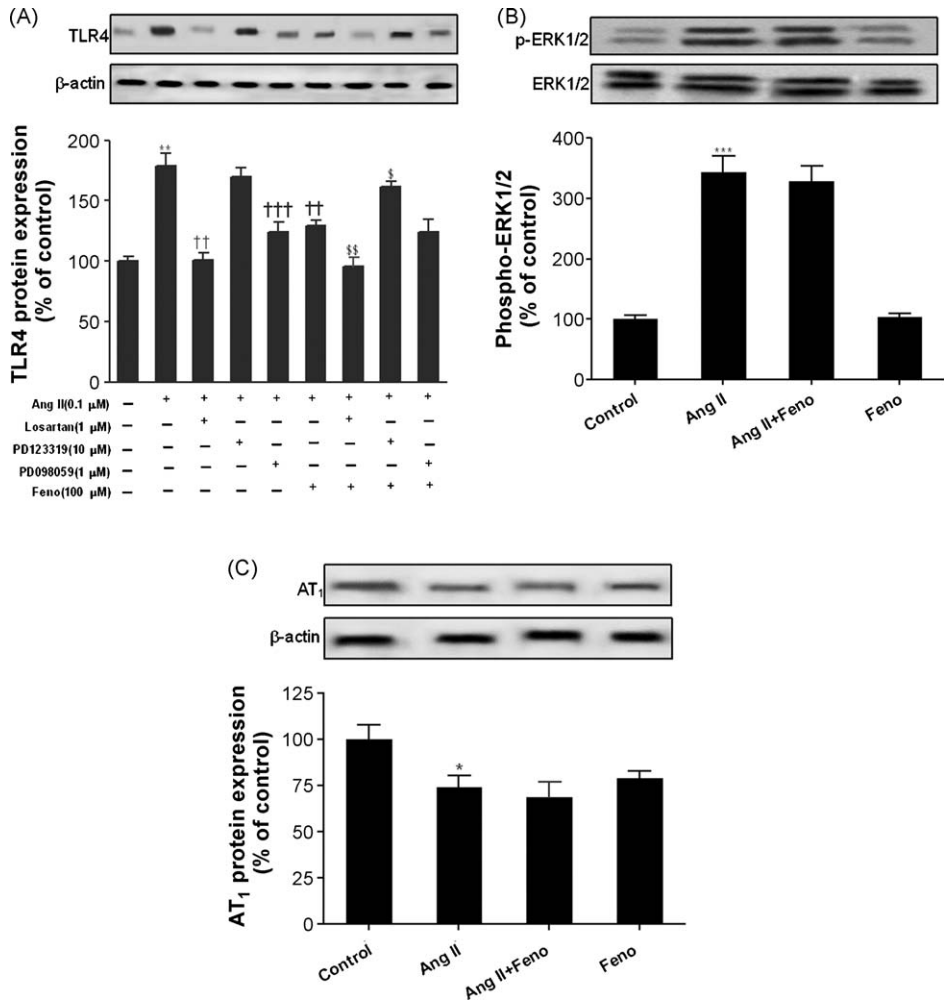

Fig. 9. Inhibitory effect of fenofibrate (Feno) on Ang II-induced TLR4 expression not through blockade of AT_1/ERK1/2 signaling. (A) Blockade of AT_1/ERK1/2 signaling was not involved in the inhibitory effect of fenofibrate on Ang II-induced TLR4 protein expression in VSMCs. (B) Effect of fenofibrate on Ang II-induced activation of ERK1/2 in VSMCs. (c) Effect of fenofibrate on AT_1 protein expression in Ang II-stimulated VSMCs. Values are expressed as means ± S.E.M. from three independent experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control; $^{\dagger\dagger}P < 0.01$, $^{\dagger\dagger\dagger}P < 0.001$ vs. Ang II; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$ vs. Ang II + Feno.

4. Discussion

Accumulating evidences point to inflammation as a key driving force of atherogenesis [30,31]. Recent studies indicate that Ang II and TLR4 are pivotal contributors to inflammatory processes involved in atherosclerotic lesion development [12,32]. The present study show that PPARα activator fenofibrate exhibits a significant and exact anti-inflammatory effect through decreasing TNF-α, TLR4, MMP-9, and increasing 6-keto-$PGF_{1\alpha}$ and PPARα in Ang II-stimulated VSMCs in vivo and in vitro. The further investigations verify that the inhibitory effect of fenofibrate on Ang II-mediated inflammatory responses in VSMCs is dependent on TLR4. Another novel finding in the study is the ability of fenofibrate to inhibit Ang II-mediated inflammatory responses in VSMCs via interfering with TLR4/IP-10/PKC/NF-κB signaling pathway. In addition, fenofibrate is also able to prevent rise of blood pressure caused by infusing Ang II in rats.

Atherosclerosis is increasingly recognized as a chronic inflammatory disease. Pro-inflammatory mediators, such as TNF-α and MMP-9, play a critical role in the inflammatory process associated with atherogenesis [33]. PGI_2, a strong vasodilator, has been demonstrated to relieve inflammatory response [34,35]. In the present study, in vivo treatment of rats or in vitro treatment of VSMCs with fenofibrate reduced Ang II-induced productions of TNF-α and MMP-9 and augmented release of 6-keto-$PGF_{1\alpha}$ (a stable degraded product of PGI_2), further supporting a potential role of PPARα activation in the prevention of vascular inflammation and atherosclerotic diseases. These results seems to be consistent with the fact that treatment with fenofibrate can repress the generations of TNF-α and MMP-9 in the vascular cells [33,36]. It is well known that Ang II-mediated NF-κB activation may lead to expression of pro-inflammatory genes and downregulation of PPARα and PPARγ, which promotes vascular inflammation and acceleration of atherosclerosis [37,38]. In the present study, treatment with fenofibrate conspicuously antagonized Ang II-decreased PPARα expression but, showed little effect on PPARγ expression in VSMCs. Therefore, PPARα activators like fenofibrate prevent the development of atherosclerosis via their normolipidemic activities such as suppressing vascular inflammation [19].

TLR4 existing in VSMCs is critically involved in the pathogenesis of arteriosclerosis, and therefore may make a fundamentally significant contribution to the pathophysiological relationship between inflammation and cardiovascular disorders [39]. Furthermore, a recent report has shown that TLR4 in VSMCs is also activated by Ang II [9]. PPARα activators may depress inflammatory responses in the vasculature by antagonizing Ang II effects [23,40]. Importantly, the present study provides the first evidence that PPARα activator fenofibrate suppresses Ang II-induced TLR4 expression in VSMCs, thus suggesting that activation of PPARα

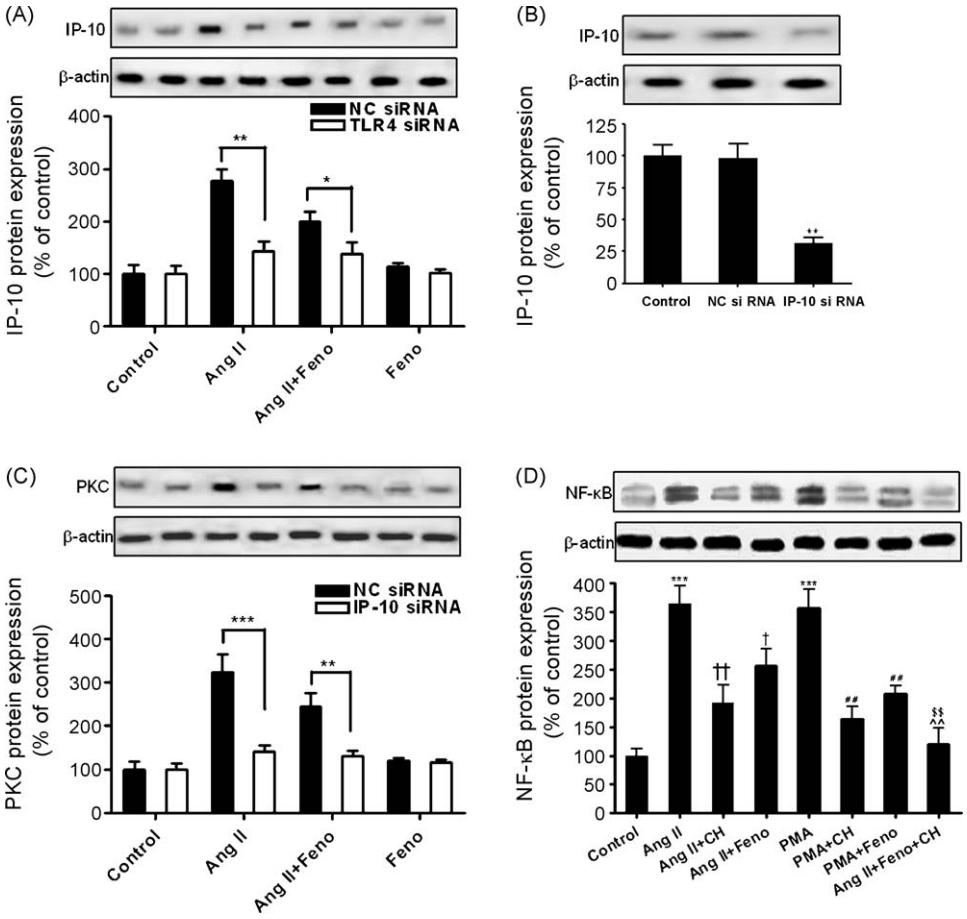

Fig. 10. Inhibition by fenofibrate (Feno) of Ang II-induced inflammatory responses in VSMCs via interference with the TLR4-dependent signaling pathway. (A) TLR4 siRNA abrogated effect of fenofibrate on Ang II-induced IP-10 expression in VSMCs. (B) Effect of IP-10 siRNA on IP-10 protein expression in VSMCs at 48 h post-transfection. (C) IP-10 siRNA abolished effect of fenofibrate on Ang II-induced PKC protein expression in VSMCs. (D) Inhibition by fenofibrate of Ang II-induced NF-κB protein expression via blockade of PKC. Values are expressed as means ± S.E.M. from three independent experiments. CH and PMA indicate chelerythrine and phorbol 12-myristate-13 acetate, respectively. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. NC control or control; $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$ vs. Ang II; $^{\#\#}P < 0.01$ vs. PMA; $^{\wedge\wedge}P < 0.01$ vs. Ang II + CH. $^{\$\$}P < 0.01$ vs. Ang II + Feno.

may intervene the impact of Ang II on the TLR4. So far, the relationship between effect of fenofibrate on Ang II-induced inflammatory responses in VSMCs and TLR4 remains elusive. Consequently, both the TLR4 inhibitor and TLR4 siRNA were applied to VSMCs to clarify whether TLR4 is involved in the inhibitory effect of fenofibrate on Ang II-induced inflammatory responses in VSMCs. The present results indicated that the TLR4 specific monoclonal antibody partially antagonized the Ang II-induced inflammatory responses in VSMCs, which were potentiated by fenofibrate. In addition, inflammatory responses in VSMCs elicited by Ang II require TLR4 at least in part, as productions of MMP-9, PPARα, PPARγ, TNF-α and PGI_2 were modulated by TLR4 siRNA. As LPS is a special TLR4 ligand, fenofibrate also ameliorated LPS-mediated inflammatory responses and directly downregulated Ang II-induced TLR4 expression in VSMCs, further suggesting that TLR4 is involved in the inhibitory effect of fenofibrate on Ang II-induced inflammatory responses in VSMCs. In combination with the finding that lack of TLR4 almost abolished the inhibitory effect of fenofibrate on Ang II-mediated inflammatory responses, TLR4 is a new target for PPARα activators in the inhibition of Ang II-induced inflammatory responses in VSMCs.

On the basis of the results mentioned above, we further investigated the TLR4-dependent signaling pathway involved in anti-inflammatory effect of fenofibrate in Ang II-induced inflammation in VSMCs. Despite limiting the presence of Ang II and the action of its AT_1 receptor has been proven to be important in the treatment of cardiovascular diseases such as systemic and pulmonary hypertension, atherosclerosis and heart failure [41,42], our results do not support that fenofibrate inhibits Ang II-induced inflammatory responses in VSMCs via interfering with the AT_1/ERK1/2 signaling or AT_2 receptor. Accordingly, there is a suggestion that fenofibrate depresses Ang II-induced inflammatory responses in VSMCs possibly by intervening the downstream effector molecules of the TLR4-dependent signaling pathway. Moreover, TLR4-mediated activation of the myeloid differentiation factor 88 (MyD88)-independent signaling results in the rapid productions of interferon beta (IFN-β) and consequent IFN-α and other interferon-inducible genes such as IP-10 [43]. In the present study, TLR4 siRNA decreased Ang II-induced IP-10 protein expression, and fenofibrate remarkably downregulated Ang II-induced IP-10 expression, thus suggesting that anti-inflammatory effects of fenofibrate involve the blockade of TLR4/IP-10 pathway. We also verify that IP-10 may lie upstream from PKC in the signal cascades because IP-10 siRNA almost completely abolished the increase of Ang II-induced PKC expression. Fenofibrate also significantly inhibited Ang II-induced PKC expression. In addition, PKC can mediate NF-κB activation that is potential in the control of immunity and inflammation [44]. Our study further showed that fenofibrate evidently suppressed protein expression of NF-κB in Ang II-stimulated VSMCs, suggesting that NF-κB-p65 translocation is inhibited by fenofibrate, and that fenofibrate exerted its anti-inflammatory action by interfering with the PKC/NF-κB signaling, which is also firmly supported by

the previous reports [45,46]. In combination, the present results clearly establish a role of fenofibrate in inhibiting the signaling pathway (TLR4/IP-10/PKC/NF-κB).

In summary, these findings provide the evidence for beneficial effects of PPARα activator fenofibrate to counter-regulate vascular inflammation induced by Ang II. More importantly, anti-inflammatory action of fenofibrate via interfering with the TLR4-dependent signaling pathway (TLR4/IP-10/PKC/NF-κB) work in concert to protect against atherosclerosis.

Conflict of interest

None.

Acknowledgement

This study was supported by a grant from National Natural Science Foundation of China to Juntian Liu (No. 30772567).

References

[1] Cheng ZJ, Vapaatalo H, Mervaala E. Angiotensin II and vascular inflammation. Med Sci Monit 2005;11:RA155–62.
[2] Miyazaki M, Takai S. Tissue angiotensin II generating system by angiotensin-converting enzyme and chymase. J Pharmacol Sci 2006;100:391–7.
[3] Brasier AR, Recinos 3rd A, Eledrisi MS. Vascular inflammation and the renin–angiotensin system. Arterioscler Thromb Vasc Biol 2002;22:1257–66.
[4] Kranzhöfer R, Schmidt J, Pfeiffer CA, Hagl S, Libby P, Kübler W. Angiotensin induces inflammatory activation of human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1999;19:1623–9.
[5] Tummala PE, Chen XL, Sundell CL, Laursen JB, Hammes CP, Alexander RW, et al. Angiotensin II induces vascular cell adhesion molecule-1 expression in rat vasculature: a potential link between the renin–angiotensin system and atherosclerosis. Circulation 1999;100:1223–9.
[6] Brasier AR, Jamaluddin M, Han Y, Patterson C, Runge MS. Angiotensin II induces gene transcription through cell-type dependent effects on the nuclear factor-κB (NF-κB) transcription factor. Mol Cell Biochem 2001;212:155–69.
[7] Kim S, Whelan J, Claycombe K, Reath DB, Moustaid-Moussa N. Angiotensin II increases leptin secretion by 3T3-L1 and human adipocytes via a prostaglandin-independent mechanism. J Nutr 2002;132:1135–40.
[8] Guo RW, Yang LX, Wang H, Liu B, Wang L. Angiotensin II induces matrix metalloproteinase-9 expression via a nuclear factor-kappaB-dependent pathway in vascular smooth muscle cells. Regul Pept 2008;147:37–44.
[9] Otsui K, Inoue N, Kobayashi S, Shiraki R, Honjo T, Takahashi M, et al. Enhanced expression of TLR4 in smooth muscle cells in human atherosclerotic coronary arteries. Heart Vessels 2007;22:416–22.
[10] Nicoletti A, Caliguri G, Paulosson G. Functionality of specific immunity in atherosclerosis. Am Heart J 1999;138:438–43.
[11] Doyle SL, O'Neill LA. Toll-like receptors: from the discovery of NFkappaB to new insights into transcriptional regulations in innate immunity. Biochem Pharmacol 2006;72:1102–13.
[12] Unterholzner L, Bowie AG. The interplay between viruses and innate immune signaling: recent insights and therapeutic opportunities. Biochem Pharmacol 2008;75:589–602.
[13] Palsson-McDermott EM, O'Neill LA. Signal transduction by the lipopolysaccharide receptor Toll-like receptor-4. Immunology 2004;113:153–62.
[14] Re F, Strominger JL. IL-10 released by concomitant TLR2 stimulation blocks the induction of a subset of Th1 cytokines that are specifically induced by TLR4 or TLR3 in human dendritic cells. J Immunol 2004;173:7548–55.
[15] Lucas PC, McAllister-Lucas LM, Nunez G. NF-kappaB signaling in lymphocytes: a new cast of characters. J Cell Sci 2004;117(Pt 1):31–9.
[16] Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. Circulation 2002;105:1158–61.
[17] Neve BP, Fruchart JC, Staels B. Role of the peroxisome proliferator-activated receptors (PPAR) in atherosclerosis. Biochem Pharmacol 2000;60:1245–50.
[18] Fruchart JC, Duriez P, Staels B. Peroxisome proliferator-activated receptor-alpha activators regulate genes governing lipoprotein metabolism, vascular inflammation and atherosclerosis. Curr Opin Lipidol 1999;10:245–57.
[19] Staels B, Koenig W, Habib A, Merval R, Lebret M, Torra IP, et al. Activation of human aortic smooth-muscle cells is inhibited by PPARalpha but not by PPARgamma activators. Nature 1998;393:790–3.
[20] Marx N, Sukhova G, Murphy C, Libby P, Plutzky J. Macrophages in human atheroma contain PPARgamma: differentiation-dependent peroxisomal proliferator-activated receptor gamma(PPARgamma) expression and reduction of MMP-9 activity through PPARgamma activation in mononuclear phagocytes in vitro. Am Pathol 1998;153:17–23.
[21] Koh KK, Ahn JY, Han SH, Jin DK, Kim HS, Lee KC, et al. Effects of fenofibrate on lipoproteins, vasomotor function, and serological markers of inflammation, plaque stabilization, and hemostasis. Atherosclerosis 2004;174:379–83.
[22] Diep QN, Benkirane K, Amiri F, Cohn JS, Endemann D, Schiffrin EL. PPAR alpha activator fenofibrate inhibits myocardial inflammation and fibrosis in angiotensinα-infused rats. J Mol Cell Cardiol 2004;36:295–304.
[23] Diep QN, Amiri F, Touyz RM, Cohn JS, Endemann D, Neves MF, et al. PPAR alpha activator effects on Angα-induced vascular oxidative stress and inflammation. Hypertension 2002;40:866–71.
[24] Libby P, Plutzky J. Inflammation in diabetes mellitus: role of peroxisome proliferator-activated receptor-alpha and peroxisome proliferator-activated receptor-gamma agonists. Am J Cardiol 2007;99:27B–40B.
[25] Duval C, Chinetti G, Trottein F, Fruchart JC, Staels B. The role of PPARs in atherosclerosis. Trends Mol Med 2002;8:422–30.
[26] Gao DF, Niu XL, Hao GH, Peng N, Wei J, Ning N, et al. Rosiglitazone inhibits angiotensin II-induced CTGF expression in vascular smooth muscle cells—role of PPAR-gamma in vascular fibrosis. Biochem Pharmacol 2007;73:185–97.
[27] Wen SJ, Zhao LM, Li P, Li JX, Liu Y, Liu JL, et al. Blood vessel tissue engineering: seeding vascular smooth muscle cells and endothelial cells sequentially on biodegradable scaffold in vitro. Zhonghua Yi Xue Za Zhi 2005;85:816–8.
[28] Griendling KK, Taubman MB, Akers M, Mendlowitz M, Alexander RW. Characterization of phosphatidylinositol-specific phospholipase C from cultured vascular smooth muscle cells. J Biol Chem 1991;266:15498–504.
[29] Peng N, Liu JT, Gao DF, Lin R, Li R. Angiotensin II-induced C-reactive protein generation: inflammatory role of vascular smooth muscle cells in atherosclerosis. Atherosclerosis 2007;193:292–8.
[30] Libby P, Aikawa M. Stabilization of atherosclerotic plaques: new mechanisms and clinical targets. Nat Med 2002;8:1257–62.
[31] Steinberg D. Atherogenesis in perspective: hypercholesterolemia and inflammation as partners in crime. Nat Med 2002;8:1211–7.
[32] Lucas AR, Korol R, Pepine CJ. Inflammation in atherosclerosis: some thoughts about acute coronary syndromes. Circulation 2006;113:e728–32.
[33] Marx N, Kehrle B, Kohlhammer K, Grüb M, Koenig W, Hombach V, et al. PPAR activators as anti-inflammatory mediators in human T lymphocytes: implications for atherosclerosis and transplantation-associated arteriosclerosis. Circ Res 2002;90:703–10.
[34] Wang M, Song WL, Cheng Y, FitzGerald GA. Microsomal prostaglandin E synthase-1 inhibition in cardiovascular inflammatory disease. J Intern Med 2008;263:500–5.
[35] Bezdecny SA, Cao J, Li X. Antiasthma herbal medicine intervention (ASHMI) modulates prostanoid release from human tracheal airway smooth muscle cells (HASMC). J Allergy Clin Immunol 2008;121:S49.
[36] Chinetti G, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors: new targets for the pharmacological modulation of macrophage gene expression and function. Curr Opin Lipidol 2003;14:459–68.
[37] Schupp M, Janke J, Clasen R, Unger T, Kintscher U. Angiotensin type 1 receptor blockers induce peroxisome proliferators activated receptor-gamma activity. Circulation 2004;109:2054–7.
[38] Tham DM, Martin-McNulty B, Wang YX, Wilson DW, Vergona R, Sullivan ME, et al. Angiotensin II is associated with activation of NF-kappa B mediated genes and downregulation of PPARs. Physiol Genomics 2002;11:21–30.
[39] Yang X, Coriolan D, Murthy V, Schultz K, Golenbock DT, Beasley D. Proinflammatory phenotype of vascular smooth muscle cells: role of efficient toll-like receptor 4 signaling. Am J Physiol Heart Circ Physiol 2005;289:H1069–76.
[40] Schiffrin EL. Peroxisome proliferator-activated receptors and cardiovascular remodeling. Am J Physiol Heart Circ Physiol 2005;288:H1037–43.
[41] Conlin PR. Redefining efficacy of antihypertensive therapies beyond blood pressure reduction—the role of angiotensin II antagonists. Int J Clin Pract 2005;59:214–24.
[42] Ribeiro AB. Angiotensin II antagonists—therapeutic benefits spanning the cardiovascular disease continuum from hypertension to heart failure and diabetic nephropathy. Curr Med Res Opin 2006;22:1–16.
[43] Fitzgerald KA, Rowe DC, Barnes BJ, Caffrey DR, Visintin A, Latz E, et al. LPS-TLR4 signaling to IRF-3/7 and NF-kappa B involves the toll adapters TRAM and TRIF. J Exp Med 2003;198:1043–55.
[44] Manicassamy S, Gupta S, Sun Z. Selective function of PKC-theta in T cells. Cell Mol Immunol 2006;3:263–70.
[45] Duez H, Chao YS, Hernandez M, Torpier G, Poulain P, Mundt S, et al. Reduction of atherosclerosis by the peroxisome proliferator-activated receptor agonist fenofibrate in mice. J Biol Chem 2002;277:48051–7.
[46] Delerive P, De Bosscher K, Besnard S, Vanden Berghe W, Peters JM, Gonzalez FJ, et al. Peroxisome proliferator-activated receptor alpha negatively regulates the vascular inflammatory gene response by negative cross-talk with transcription factors NF-kappa B and AP-1. J Biol Chem 1999;274:32048–54.