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ORIGINAL ARTICLE

Proinflammatory cytokine and ligands modulate cardiac peroxisome proliferator-activated receptors

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ABSTRACT

Background Peroxisome proliferator-activated receptors (PPAR) mediate inflammatory processes and alter cardiac function. However, it is not clear whether inflammatory cytokines or PPAR ligands regulate PPARs in the cardiomyocytes to modulate cardiac functions. We investigated the effects of tumour necrosis factor-alpha (TNF-α) and PPAR ligands on the expression of PPARs in HL-1 cardiomyocytes.

Materials and methods HL-1 cardiomyocytes were incubated with and without TNF-α (1, 10, 25 and 50 ng mL^{-1}) or PPAR ligands (rosiglitazone, pioglitazone and fenofibrate) at concentrations of 0·1, 1 and 10 μM for 24 h. The cells also received SN-50 (NF-κB inhibitor, 50 μg mL^{-1}), ascorbic acid (100 μM) and coenzyme Q_{10} (10 μM) alone or combined with TNF-α.

Results Using reverse transcriptase–polymerase chain reaction and Western blot, we found that incubation of TNF-α (50 ng mL^{-1}) for 24 h decreased PPAR-α, but increased PPAR-γ without altering PPAR-δ. These effects were not changed by co-administration of SN-50. However, co-administration of ascorbic acid prevented the effect of TNF-α both on PPAR-α and PPAR-γ. Coenzyme Q_{10} partially attenuated the effect of TNF-α on PPAR-γ but did not alter its effect on PPAR-α. The administration of rosiglitazone (10 μM) and pioglitazone (10 μM) for 24 h increased PPAR-γ mRNA, but did not alter PPAR-α or PPAR-δ. Moreover, fenofibrate (0·1, 1 and 10 μM) increased PPAR-γ without any effects on PPAR-α or PPAR-δ.

Conclusions Oxidative stress causes the regulations of PPAR-α and PPAR-γ in the TNF-α-treated cardiomyocytes. The up-regulation of PPAR-γ by PPAR ligands may contribute to their anti-inflammation effects.

Keywords Ascorbic acid, coenzyme Q_{10}, peroxisome proliferator-activated receptors, tumour necrosis factor-alpha.

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Introduction

Peroxisome proliferator-activated receptors (PPAR) are nuclear transcription factors with three isoforms (α, γ and δ) that are expressed in the heart with anti-inflammation activity [1–3]. Previous studies have shown that both natural and synthetic ligands of PPAR-γ have anti-inflammation potentials [4,5]. Moreover, PPAR-α and PPAR-γ ligands, used for treating hyperlipidaemia and hyperglycaemia, also have anti-inflammatory effects [2,4]. These ligands inhibit inflammation by regulating the activities of transcription factor activator protein-1, or nuclear factor-κB, (NF-κB) [6,7]. In contrast, the role of inflammation on PPARs remains unclear. Experimental autoimmune myocarditis up-regulates PPAR-γ protein expression accompanied by concomitant increase in myocardial interleukin-1β and tumour necrosis factor-alpha (TNF-α) expression [8]. These findings lead us to investigate whether TNF-α could directly regulate the expression of PPARs in the cardiomyocytes.

TNF-α is a proinflammatory cytokine that mediates inflammation and plays a vital role in the pathophysiology of cardiovascular diseases [8–10]. TNF-α has been shown to regulate PPAR-γ mRNA level in the hepatic stellate cells during sepsis [11,12]. It is therefore possible that TNF-α can directly regulate PPARs as a consequence of modulating inflammation. TNF-α can activate intracellular NF-κB to regulate the transcription, but it is not clear whether TNF-α alters PPAR isoform expression via this pathway [13].

It is known that PPAR ligands as therapeutic targets have contradictory effects in cardiovascular disease [4]. PPARs have cardioprotective effects from ischaemia/reperfusion [4,14]. However, PPAR ligands as therapeutic targets in cardiovascular

disease received a widespread attention due to their potential adverse effects; for example, dual PPAR-α/γ agonist muraglitazar and PPAR-γ agonist rosiglitazone have been reported to increase the risk of death, cardiovascular events and myocardial infarction [15,16]. Nevertheless, information regarding the potential of PPAR ligands on cardiac PPAR isoform expression is unavailable. The objectives of our study were to evaluate the effect of TNF-α on PPAR isoforms and to investigate the role of PPAR agonists on PPAR isoforms in HL-1 cardiomyocytes.

Methods

Cell preparations

HL-1 cells (kindly provided by Dr Claycomb) were derived from mouse atrium with differentiated biochemical and morphological properties of adult atrial cardiomyocytes [17]. HL-1 cells were cultured in a humidified atmosphere of 5% CO_2 at 37 °C in Claycomb medium (SAFC Biosciences, Lenexa, KS, USA) supplemented with penicillin–streptomycin, norepinephrine (Gibco, Grand Island, NY, USA) and 10% fetal bovine serum (JRH Biosciences, Lenexa, KS, USA). The experimental and control cells were incubated with and without TNF-α (1, 10, 25 and 50 ng mL^{-1}) for 24 h. In order to evaluate on the role of NF-κB and antioxidants in TNF-α, cells were pretreated with SN-50 (NF-κB inhibitor, 50 μg mL^{-1}, Calbiochem, La Jolla, CA, USA), coenzyme Q_{10} (10 μM, Sigma-Aldrich, St Louis, MO, USA), or L-ascorbic acid (100 μM, Sigma-Aldrich) for 1 h before incubating with TNF-α for 24 h. The dose of coenzyme Q_{10} and L-ascorbic acid used in this experiment has been shown to contain effective antioxidant potentials [18,19]. To examine the effects of PPAR agonists on the expressions of PPAR isoform mRNA, cells were incubated with PPAR-α ligand (fenofibrate, Synkem, Chenove, France) and PPAR-γ ligands (rosiglitazone, GlaxoSmithKline, Brentford, UK; pioglitazone, Takeda Pharmaceutical, Osaka, Japan) at different concentrations (0·1, 1 and 10 μM) for 24 h.

Reverse transcriptase–polymerase chain reaction

Total mRNA was isolated from HL-1 cells and reverse transcribed by using superscript III cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) into cDNA according to the manufacturer's instructions. The resulting cDNAs were amplified by polymerase chain reaction (PCR) using primer for mouse PPAR isoforms. The primer sequence for PCR products are as follows: PPAR-α (GenBank: NM_011144), forward: 5′-AACATCGAGTGTCGAATATGTGG-3′, reverse: 5′-R-AGCCGAATAGTTCGCCGAAAG-3′; PPAR-γ (GenBank: NM_001127330), forward: 5′-TGTGGGGATAAAGCATCAGGC-3′, reverse: 5′-CCGGCAGTTAAGATCACACCTAT-3′; PPAR-δ (GenBank: NM_0111453), forward: 5′-ACAAGGCCTCGGGCTTCCACTA-3′, reverse: 5′-CAGATCCGATCGCACTTCTCATAC-3′. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, GenBank: NM008084), forward: 5′-GCTACACTGAGGACCAGGTTGTC-3′, reverse: 5′-GAAGGTGGAAGAGTGGGAGTTG-3′ served as a housekeeping gene. After reverse transcriptase-PCR (RT-PCR), reaction products were separated in 1·6% Tris–borate–EDTA–agarose gel containing 0·5 μg mL^{-1} ethidium bromide by electrophoresis. The gel was photographed (Photo Analyst, Visionary, Fotodyne Inc., Hartland, WI, USA), and band density was determined by Image-Pro Plus software (Media Cybernetics Inc., Bethesda, MD, USA). The expressions of PPAR isoforms were normalized with GAPDH as internal control.

Quantitative real-time PCR

PPAR isoform expression analysis were performed by quantitative PCR with ABI PRISM 7300 system (Applied Biosystems, Foster City, CA, USA) using SYBER Green (Applied Biosystems). Primer sequences for PPAR isoforms were as follows: PPAR-α: forward: 5′-AACATCGAGTGTCGAATATGTGG-3′, reverse: 5′-AGCCGAATAGTTCGCCGAAAG-3′; PPAR-γ: forward: 5′-CCACCAACTTCGGAATCAGC-3′, reverse: 5′-AGTGGTCTTCCATCACGGAGGA-3′; PPAR-δ: forward: 5′-CCCACGAGTTCTTGCGAAGT-3′, reverse: 5′-TTGGGCTCAATGATGTCACTG-3′; GAPDH: forward: 5′-GAAGGTGGAAGAGTCGGAGTTC-3′, reverse: 5′-GCTACACTGAGGACCAGGTTGTC-3′. The threshold cycle (Ct) values for PPAR-α, PPAR-γ and PPAR-δ were first normalized with their respective Ct value for GAPDH and subsequently to a control sample.

Nuclear and total protein Isolation

Nuclear protein isolation was performed by scraped off cells with phosphate-buffered saline resuspended in 200 μL of ice-cold buffer A (10 mM HEPES, pH 7·9, 10 mM KCl, 0·1 mM EDTA, 0·1 mM EGTA, 1 mM DTT, protease inhibitor cocktail) after centrifugation. The cells were lysed by adding Nonidet P-40 (12·5 μL of 10%) followed by centrifugation (12 000 *g*) for 30 s. Pellets were resuspended in ice-cold buffer B (50 μL), containing HEPES (20 mM), NaCl (400 mM), EDTA (1 mM), EGTA (1 mM), DTT (1 mM), and protease inhibitor cocktail. After centrifugation at 14 000 *g* for 5 min, the supernatants were saved as nuclear fraction and stored at –80 °C. Total protein extraction was derived from cells lysed in 200 μL of ice-cold Triton-based lysis buffer (1% Triton X-100, 200 mM Tris pH 7·4, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 2·5 mM sodium pyrophosphate, 1 mM β-glycerophosphate). After centrifugation at 14 000 *g* for 30 min, soluble supernatants were stored at –80 °C for further study.

Western blot analysis

Equal amounts of nuclear or total proteins from HL-1 cell were resolved by sodium dodecyl sulphate–polyacrylamide gel electrophoresis followed by electrophoretic transfer of proteins onto nitrocellulose membranes. Blots were probed with antibodies

against PPAR-α (Santa Cruz Biotechnology, Santa Cruz, CA, USA), PPAR-γ (Santa Cruz Biotechnology), PPAR-δ (Affinity Bio Reagent, Golden, CO, USA), NF-κB p65 (Santa Cruz Biotechnology) and a secondary antibody conjugated with horseradish peroxidase-linked immunoglobulin G (Leinco Technology, St. Louis, MO, USA). Bound antibodies were detected with ECL detection system (Millipore, St. Louis, MO, USA) and analysed with Image-Pro Plus software (Media Cybernetics Inc.). Targeted bands were normalized to cardiac α-sarcomeric actin (Sigma-Aldrich) to confirm equal protein loading.

Statistical analysis

All quantitative data are expressed as mean ± standard error of the mean. Statistical analysis were performed using one-way analysis of variance with a post hoc of LSD or paired *t*-test was used to compare the differences before and after the drug administration of HL-1 cells. A *P*-value of less than 0·05 was considered to be statistically significant.

Results

Expression of PPAR isoforms in HL-1 cell

Figure 1 shows expressions of different PPAR isoforms in HL-1 cardiomyocytes. Through RT-PCR, PPAR-γ has less expression than PPAR-α and PPAR-δ. The percentages of PPAR-α, PPAR-γ and PPAR-δ were 36 ± 8%, 23 ± 2% and 41 ± 1%, respectively (Fig. 1).

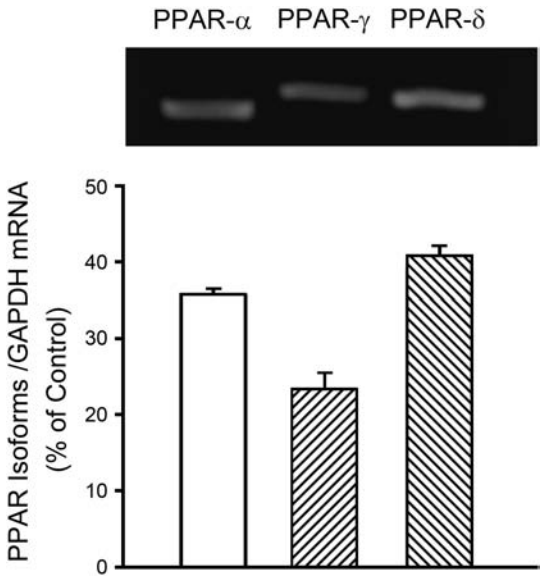

Figure 1 Differential expressions of peroxisome proliferator-activated receptor (PPAR) isoforms in HL-1 cardiomyocytes. The mRNA expressions of PPAR-α, PPAR-γ and PPAR-δ were performed by RT-PCR. The expressions of total PPAR isoforms were calculated as 100% and normalized with GAPDH as internal control. Results were from three independent experiments.

TNF-α regulates PPAR isoforms in HL-1 cell

To assess on the regulation of PPAR isoform expression in HL-1 cells, we performed both mRNA and protein analyses. Treatment of HL-1 cells with TNF-α (50 ng mL^{-1}) decreased mRNA expression of PPAR-α (Fig. 2a) but enhanced the expression of PPAR-γ mRNA level (Fig. 2b). However, TNF-α (1, 10, 25 and 50 ng mL^{-1}) did not result in significant change in PPAR-δ mRNA expression (Fig. 2c). To ascertain whether expression of PPAR isoform mRNA level is accompanied by increased or decreased in protein expression of PPAR isoforms, we examined PPAR protein levels by Western blot. TNF-α (50 ng mL^{-1}) decreased PPAR-α protein (Fig. 3a) but increased PPAR-γ protein level (Fig. 3b). In contrast, treatment with TNF-α (1, 10, 25 and 50 ng mL^{-1}) did not result in significant changes in PPAR-δ protein level (Fig. 3c).

Oxidative stress mediates the effects of TNF-α on PPARs

We further investigated whether TNF-α could regulate the expression of PPARs through NF-κB or oxidative stress. In the presence of SN-50, TNF-α still has a significant effect on the mRNA level of PPAR-α and PPAR-γ in HL-1 cell. Therefore, the regulation of TNF-α on PPARs was not modulated by the NF-κB pathway (Fig. 4a,c). Figure 4b shows that TNF-α-induced NF-κB p65 nuclear translocation was inhibited by SN-50 and this confirmed that SN-50 at 50 μg mL^{-1} can efficiently inhibit the effects of NF-κB. However, TNF-α-mediated PPAR-α down-regulation was prevented by the administration of ascorbic acid (100 μM). The PPAR-γ up-regulation was attenuated by 51% in the presence of ascorbic acid (100 μM). In addition, TNF-α-mediated PPAR-γ up-regulation was also attenuated by 46% in the presence of coenzyme Q_{10} (10 μM). Nevertheless, coenzyme Q_{10} (10 μM) did not alter the effects of TNF-α on PPAR-α (Fig. 4a,c).

Effects of PPAR ligands on PPAR isoforms in HL-1 cell

Through quantitative real-time PCR, we found that rosiglitazone (10 μM) could increase the expression of PPAR-γ mRNA by 41 ± 13% (Fig. 5a), but did not change PPAR-α (Fig. 6a) and PPAR-δ mRNA expression (Fig. 7a). Similarly, pioglitazone (10 μM) could increase the expression of PPAR-γ by 106 ± 33% (Fig. 5b), but did not change PPAR-α (Fig. 6b) and PPAR-δ mRNA expression (Fig. 7b). Moreover, fenofibrate (0·1 μM, 1 μM and 10 μM) can increase expression of PPAR-γ mRNA by 68 ± 20%, 137 ± 34% and 83 ± 20% in a biphasic concentration-dependent manner (Fig. 5c). Fenofibrate has little effect on PPAR-α (Fig. 6c) and PPAR-δ mRNA expression (Fig. 7c).

Discussion

Previous studies have shown that PPARs are expressed in various types of cells [12,20]. However, the expressions of PPAR isoforms in atrial myocytes have not been elucidated. In this study, for the first

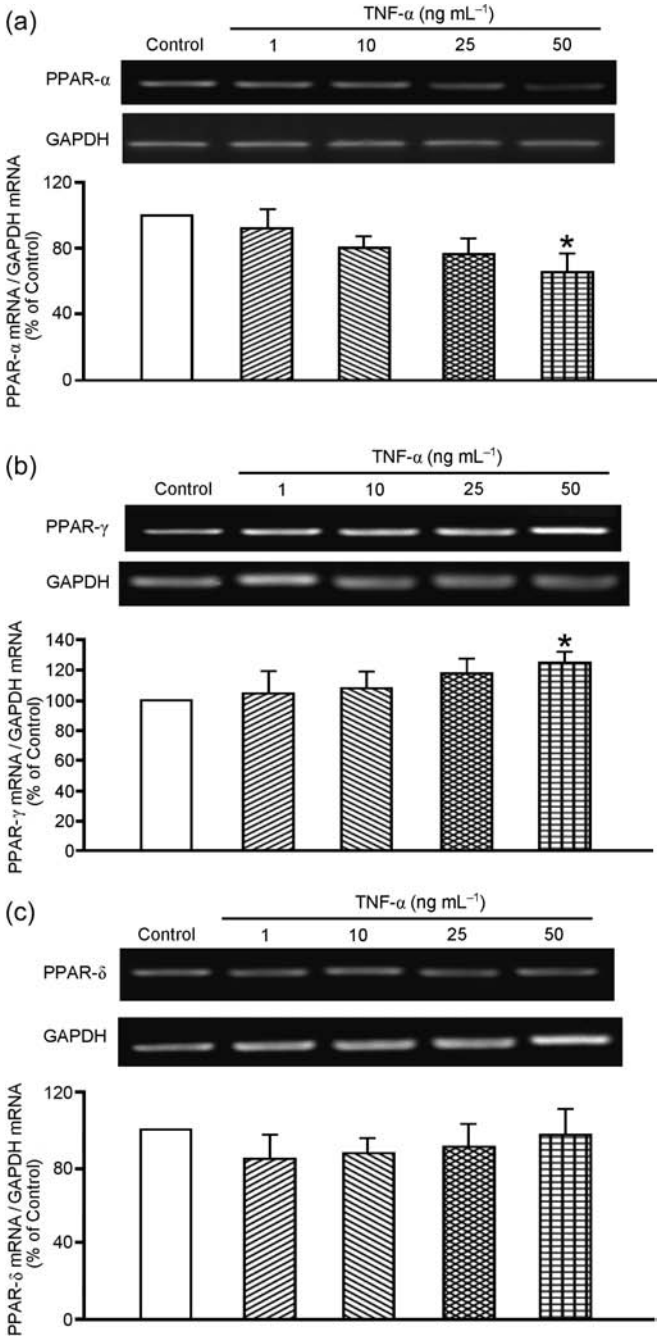

Figure 2 Effects of tumour necrosis factor-alpha (TNF-α) on peroxisome proliferator-activated receptor (PPAR) isoform mRNA expressions in HL-1 cardiomyocytes by RT-PCR. (a) Expressions of PPAR-α mRNA was decreased by TNF-α (50 ng mL^{-1}). (b) TNF-α (50 ng mL^{-1}) increased PPAR-γ mRNA expressions. (c) TNF-α (1, 10, 25 and 50 ng mL^{-1}) did not alter PPAR-δ expressions. Results were from five independent experiments. The mRNA expressions of PPAR isoforms were normalized with GAPDH as internal control. *$P < 0{\cdot}05$ vs. the control group.

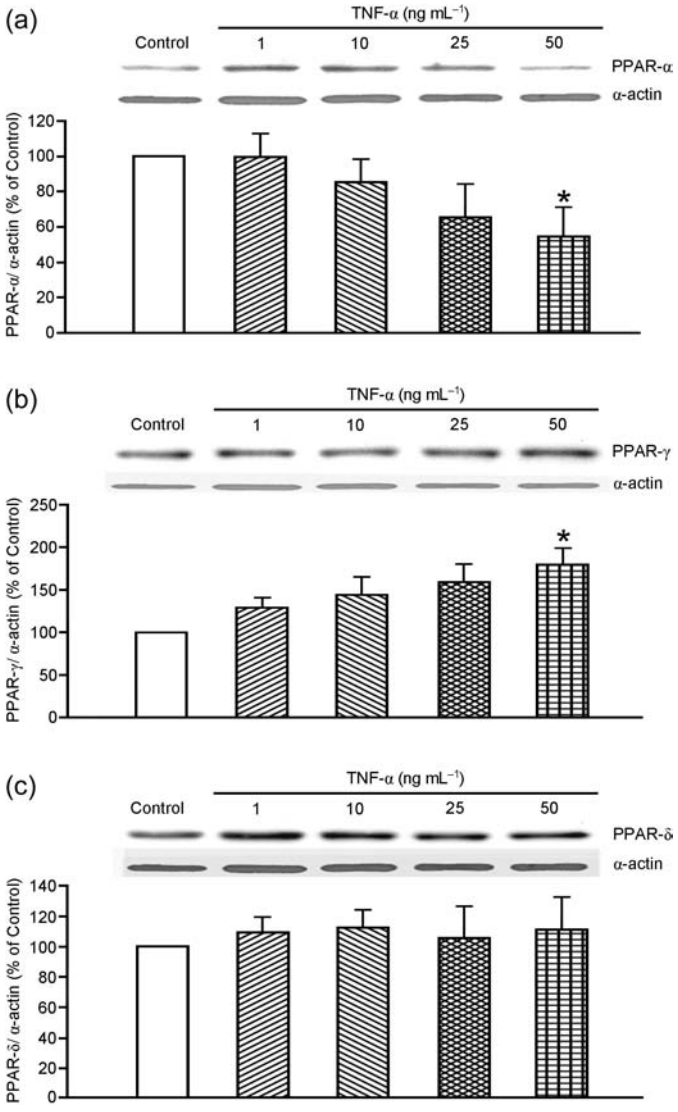

Figure 3 Effects of tumour necrosis factor-alpha (TNF-α) on peroxisome proliferator-activated receptor (PPAR) isoform protein expressions in HL-1 cardiomyocytes by Western blot analysis. (a) PPAR-α protein expression is decreased by TNF-α (50 ng mL^{-1}). Results were from five independent experiments. (b) TNF-α (50 ng mL^{-1}) increased PPAR-γ protein expressions. Results are from four independent experiments. (c) TNF-α (1, 10, 25 and 50 ng mL^{-1}) did not change PPAR-δ protein expression. Results were from four independent experiments. The protein level of PPAR isoforms were normalized with α-sarcomeric actin as internal control. *$P < 0{\cdot}05$ vs. the control group.

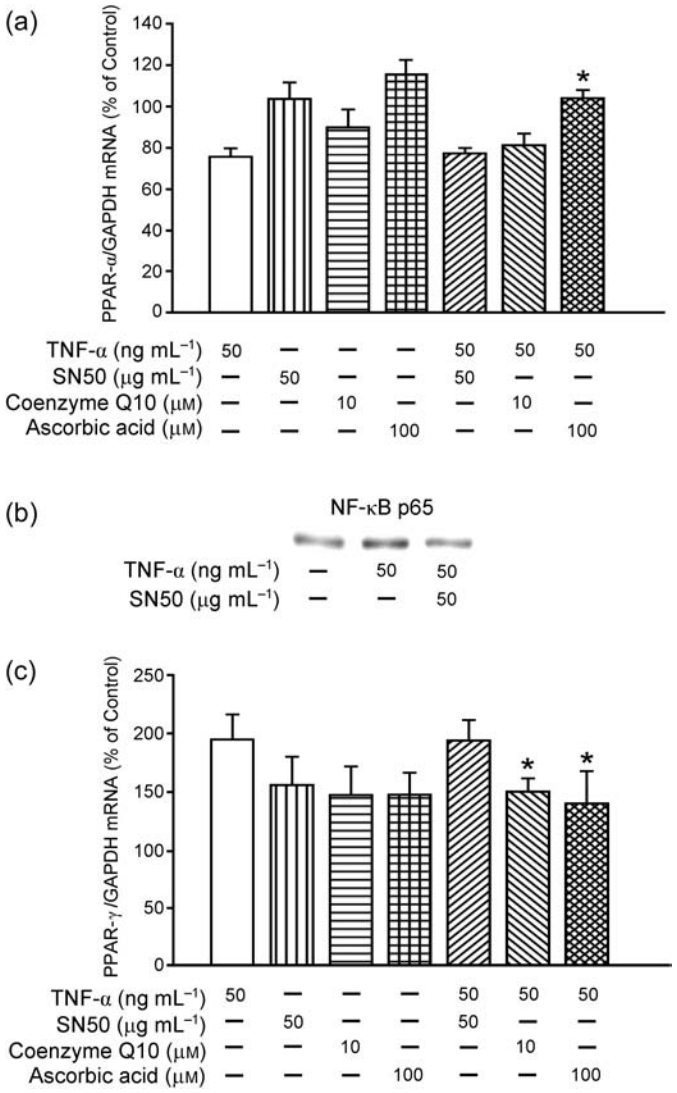

Figure 4 Effects of SN-50, ascorbic acid and coenzyme Q_{10} on tumour necrosis factor-alpha (TNF-α)-mediated peroxisome proliferator-activated receptor (PPAR) expressions in cardiomyocytes. (a) TNF-α-mediated PPAR-α mRNA down-regulation in HL-1 cell was not changed with SN50 pretreatment but was prevented by ascorbic acid. (b) TNF-α-induced nuclear p65 translocation was prevented by SN50 pretreatment, which suggests that SN50 efficiently inhibits NF-κB. The protein expression of NF-κB p65 was analysed by immunoblotting with anti-NF-κB p65 antibody from nuclear extracts. (c) Both ascorbic acid and coenzyme Q_{10} pretreatment of cardiomyocytes significantly prevented TNF-α-mediated up-regulation of PPAR-γ mRNA. Results are from four independent experiments. *$P < 0{\cdot}05$ vs. the TNF-α-treated group.

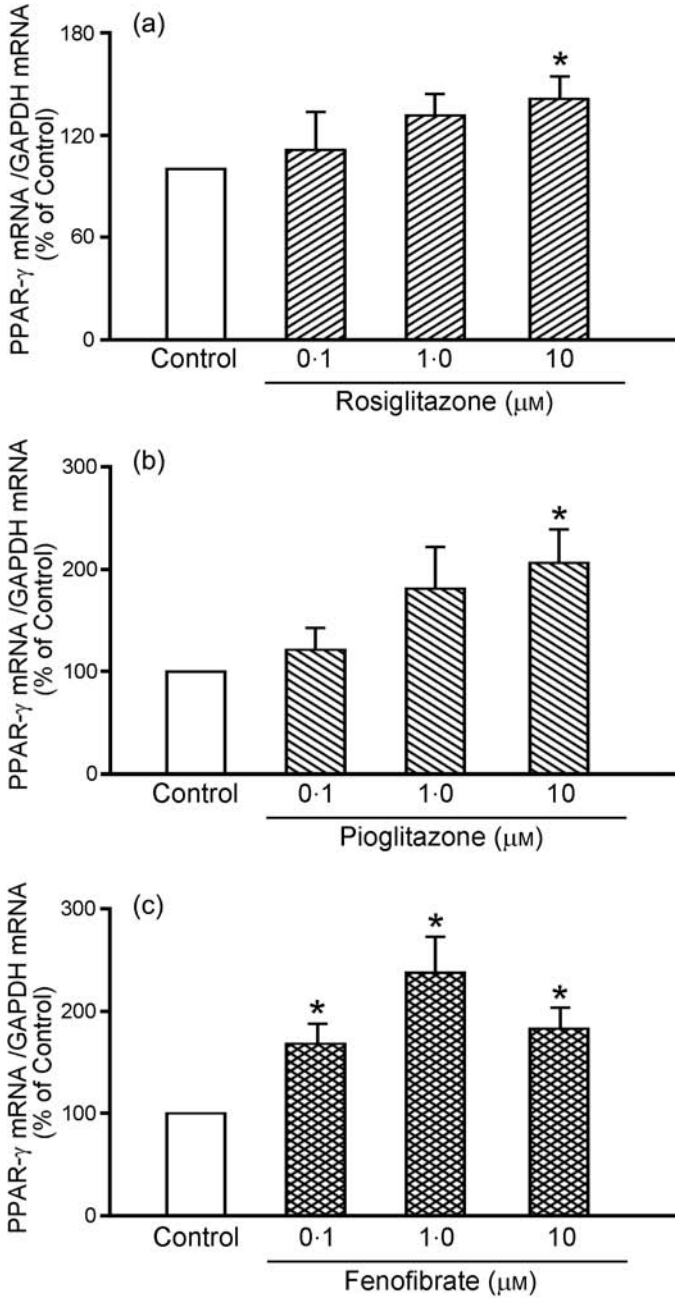

Figure 5 Effects of peroxisome proliferator-activated receptor (PPAR) ligands on the expression of PPAR-γ by real-time PCR in cardiomyocytes. (a) PPAR-γ mRNA expression was increased at 10 μM of rosiglitazone. (b) Pioglitazone (10 μM) increased PPAR-γ mRNA expression. (c) Fenofibrate (0·1, 1 and 10 μM) significantly increased PPAR-γ mRNA expression. Results are from four independent experiments. The mRNA levels were normalized with GAPDH as internal control. *$P < 0{\cdot}05$ vs. the control group.

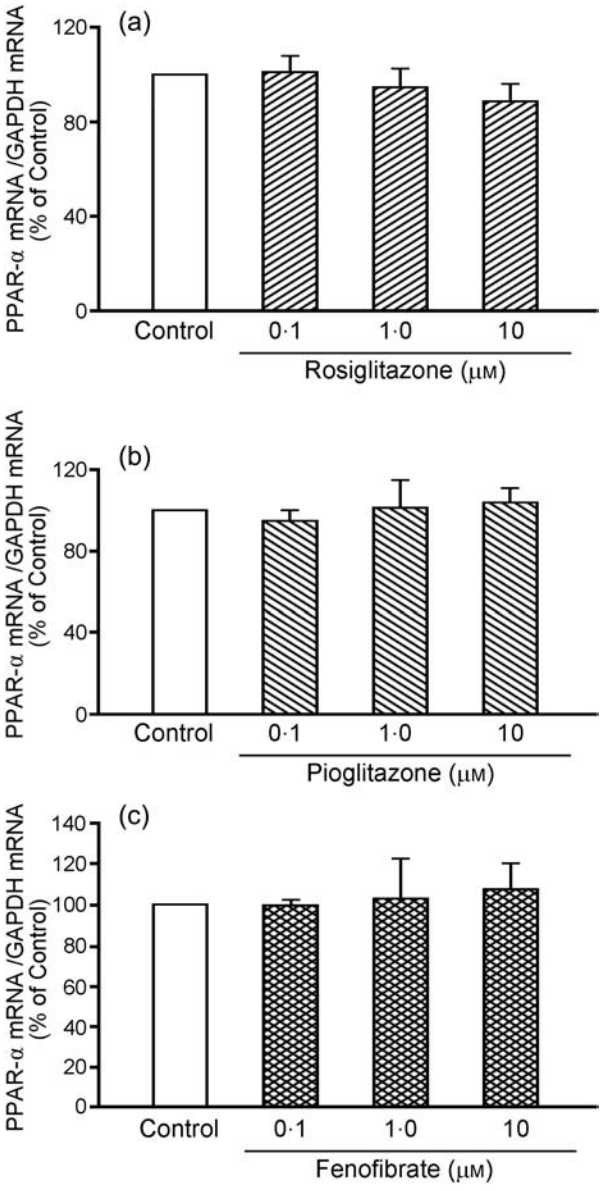

Figure 6 Effects of peroxisome proliferator-activated receptor (PPAR) ligands on the expression of PPAR-α by real-time PCR in cardiomyocytes. Rosiglitazone (0·1, 1 and 10 μM, panel a), pioglitazone (0·1, 1 and 10 μM, panel b), and fenofibrate (0·1, 1 and 10 μM, panel c) did not have significant effects on PPAR-α. mRNA levels were normalized with GAPDH. Results were from four independent experiments.

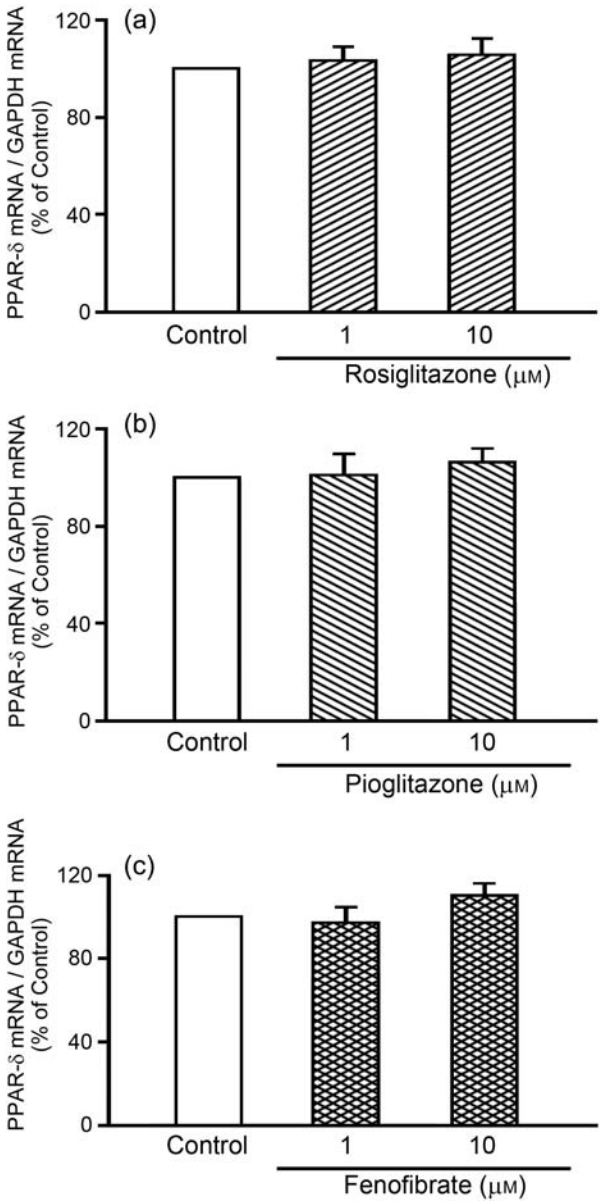

Figure 7 Effects of peroxisome proliferator-activated receptor (PPAR) ligands on PPAR-δ by real-time PCR in cardiomyocytes. Rosiglitazone (1 and 10 μM, panel a), pioglitazone (1 and 10 μM, panel b), and fenofibrate (1 and 10 μM, panel c) did not alter the expression of PPAR-δ mRNA. The mRNA levels were normalized with GAPDH as internal control. Results were from three independent experiments.

time, we demonstrated that three isoforms of PPAR are expressed in HL-1 cells. Similar to those in the ventricular myocytes [20,21], PPAR-α and PPAR-δ were expressed abundantly, but PPAR-γ was detected to be low in HL-1 atrial myocytes. Since PPAR-α plays a role in fatty acid oxidation and triglyceride synthesis, the highly expressed PPAR-α in the cardiomyocytes may contribute to the known finding about fatty acid oxidation as the principal source of heart energy [21,22].

There is a wealth of studies about inflammation responses modulated by PPARs. However, the effects of inflammatory cytokine on PPARs have not been fully elucidated. In this study, TNF-α was demonstrated to have different effects on the PPAR isoforms. TNF-α (50 ng mL^{-1}) increased PPAR-γ mRNA and protein expressions, but decreased PPAR-α mRNA and protein levels in the HL-1 cardiomyocytes. In addition, TNF-α has little effects on the PPAR-δ mRNA and protein expression. Similar to the previous observations, TNF-α has been demonstrated to down-regulate PPAR-α in rat liver cells, which was hypothesized to be attributed to diminution in peroxisomal β-oxidation [23]. Previous studies have also shown that cardiac hypertrophy due to ventricular pressure overload can down-regulate PPAR-α [22,24,25]. This effect is hypothesized to be a compensatory phenomenon because reversing the down-regulation of PPAR-α target genes exerts detrimental effects on cardiac performance. Therefore, PPAR-α down-regulation by TNF-α may play a role in maintaining contractile function during inflammation.

Different from the results of PPAR-α, we have found that TNF-α increased PPAR-γ mRNA and protein expression in the cardiomyocytes. This finding is similar to the previous observations in polymononuclear cells [26]. On the contrary, TNF-α-decreased PPAR-γ expression was demonstrated in other cell types such as adipocytes and hepatic cells [9,11,12,27,28]. These findings suggest that different cells may have variant responses during inflammation. A previous study has also shown that PPAR-γ was up-regulated during heart failure induced by myocardial infarction, which may be caused by the TNF-α effect [29]. The results observed in our study suggest that the increase expression of PPAR-γ might have been a cellular compensatory response to attenuate sepsis-induced cytokines.

The proinflammatory cytokine, TNF-α, plays a key role in various forms of biological processes (including induction of inflammation, proliferation and apoptosis and production of other cytokines) and immune responses. NF-κB is a transcription factor that has a vital role in the downstream signalling targets activated by TNF-α [30,31]. However, our investigation suggested that the NF-κB pathway may not play a major role in the effects of TNF-α on PPARs, since NF-κB inhibitor did not modulate this effect. On the contrary, we found that ascorbic acid may attenuate TNF-α effects on PPAR-α and PPAR-γ. It is known that TNF-α signalling is associated with enhanced generation of reactive oxygen species, which can modulate gene transcription in response to inflammatory stimulation [31]. Since ascorbic acid acts as a potent free radical scavenger protecting cells against oxidative damage, our results suggest that oxidative stress may contribute to the effects of TNF-α on PPARs [32]. Moreover, we also demonstrated that coenzyme Q_{10} can attenuate the effects of TNF-α on PPAR-γ. Coenzyme Q_{10} is an essential carrier for mitochondrial electron transport system and plays a significant role in the energy metabolism [33]. This finding further confirmed the role of oxidative stress on the regulation of PPAR-γ by TNF-α. However, coenzyme Q_{10} has insignificant effects on PPAR-α, which was different from the results in ascorbic acid. Ascorbic acid, a water-soluble antioxidant, probably has a different effect from coenzyme Q_{10}, which is a hydrophobic antioxidant.

Although rosiglitazone, pioglitazone and fenofibrate have several cardiovascular effects, it is not clear whether PPAR-α and PPAR-γ ligands can regulate the expressions of PPAR isoforms in the cardiomyocytes. In this study, we have found that both rosiglitazone and pioglitazone can increase PPAR-γ expression without effects on PPAR-α. These effects may contribute to the known anti-inflammatory effects of rosiglitazone and pioglitazone. Interestingly, we also demonstrated that fenofibrate can increase PPAR-γ in a biphasic dose-dependent manner. Although the mechanism remains uncertain, it might be caused by the resemblance in some of the transcription factor response elements in the promoter region of PPAR-α and PPAR-γ.

In conclusion, TNF-α may differentially regulate PPAR isoforms in cardiomyocytes through oxidative stress. Rosiglitazone, pioglitazone and fenofibrate can increase the expression of PPAR-γ, which may account for the anti-inflammation potentials of PPAR ligands.

Acknowledgements

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References

1 Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* 1990;**347**:645–50.
2 Plutzky J. Peroxisome proliferator-activated receptors as therapeutic targets in inflammation. *J Am Coll Cardiol* 2003;**42**:1764–6.
3 Staels B, Fruchart JC. Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes* 2005;**54**:2460–70.

4 Smeets PJ, Planavila A, van der Vusse GJ, van Bilsen M. Peroxisome proliferator-activated receptors and inflammation: take it to heart. *Acta Physiol (Oxf)* 2007;**191**:171–88.
5 Moraes LA, Piqueras L, Bishop-Bailey D. Peroxisome proliferator-activated receptors and inflammation. *Pharmacol Ther* 2006;**110**:371–85.
6 Jiang C, Ting AT, Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. *Nature* 1998;**391**:82–6.
7 Ricote M, Li AC, Willson TM, Kelly CJ, Glass CK. The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature* 1998;**391**:79–82.
8 Yuan Z, Liu Y, Zhang J, Kishimoto C, Wang Y, Ma A *et al*. Peroxisome proliferation-activated receptor-γ ligands ameliorate experimental autoimmune myocarditis. *Cardiovasc Res* 2003;**59**:685–94.
9 Matsumori A, Yamada T, Suzuki H, Matoba Y, Sasayama S. Increased circulating cytokines in patients with myocarditis and cardiomyopathy. *Br Heart J* 1994;**72**:561–6.
10 Neumann FJ, Ott I, Gawaz M, Richardt G, Holzapfel H, Jochum M *et al*. Cardiac release of cytokines and inflammatory responses in acute myocardial infarction. *Circulation* 1995;**92**:748–55.
11 Zhou M, Wu R, Dong W, Jacob A, Wang P. Endotoxin downregulates peroxisome proliferator-activated receptor-γ via the increase in TNF-α release. *Am J Physiol Regul Integr Comp Physiol* 2008;**294**:R84–92.
12 Sung CK, She H, Xiong S, Tsukamoto H. Tumor necrosis factor-α inhibits peroxisome proliferator-activated receptor γ activity at a posttranslational level in hepatic stellate cells. *Am J Physiol Gastrointest Liver Physiol* 2004;**286**:G722–9.
13 Lakshminarayanan V, Drab-Weiss EA, Roebuck KA. H_2O_2 and tumor necrosis factor-α induce differential binding of the redox-responsive transcription factors AP-1 and NF-κB to the interleukin-8 promoter in endothelial and epithelial cells. *J Biol Chem* 1998;**273**:32670–8.
14 Yue TL, Nerurkar SS, Bao W, Jucker BM, Sarov-Blat L, Steplewski K *et al*. *In vivo* activation of peroxisome proliferator-activated receptor-δ protects the heart from ischemia/reperfusion injury in Zucker fatty rats. *J Pharmacol Exp Ther* 2008;**325**:466–74.
15 Nissen SE, Wolski K. Effect of rosiglitazone on the risk of myocardial infarction and death from cardiovascular causes. *N Engl J Med* 2007;**356**:2457–71.
16 Nissen SE, Wolski K, Topol EJ. Effect of muraglitazar on death and major adverse cardiovascular events in patients with type 2 diabetes mellitus. *JAMA* 2005;**294**:2581–6.
17 Claycomb WC, Lanson NA Jr, Stallworth BS, Egeland DB, Delcarpio JB, Bahinski A *et al*. HL-1 cells: a cardiac muscle cell line that contracts and retains phenotypic characteristics of the adult cardiomyocyte. *Proc Natl Acad Sci USA* 1998;**95**:2979–84.
18 Tsuneki H, Sekizaki N, Suzuki T, Kobayashi S, Wada T, Okamoto T *et al*. Coenzyme Q_{10} prevents high glucose-induced oxidative stress in human umbilical vein endothelial cells. *Eur J Pharmacol* 2007;**566**:1–10.
19 Duarte TL, Jones GD. Vitamin C modulation of H_2O_2-induced damage and iron homeostasis in human cells. *Free Radic Biol Med* 2007;**43**:1165–75.
20 Gilde AJ, Van Bilsen M. Peroxisome proliferator-activated receptors (PPARS): regulators of gene expression in heart and skeletal muscle. *Acta Physiol Scand* 2003;**178**:425–34.
21 Yang Q, Li Y. Roles of PPARs on regulating myocardial energy and lipid homeostasis. *J Mol Med* 2007;**85**:697–706.
22 Finck BN, Han X, Courtois M, Aimond F, Nerbonne JM, Kovacs A *et al*. A critical role for PPARα-mediated lipotoxicity in the pathogenesis of diabetic cardiomyopathy: modulation by dietary fat content. *Proc Natl Acad Sci USA* 2003;**100**:1226–31.
23 Beier K, Völkl A, Fahimi HD. TNF-α downregulates the peroxisome proliferator activated receptor-α and the mRNAs encoding peroxisomal proteins in rat liver. *FEBS Lett* 1997;**412**:385–7.
24 Finck BN, Lehman JJ, Leone TC, Welch MJ, Bennett MJ, Kovacs A *et al*. The cardiac phenotype induced by PPARα overexpression mimics that caused by diabetes mellitus. *J Clin Invest* 2002;**109**:121–30.
25 Leone TC, Weinheimer CJ, Kelly DP. A critical role for the peroxisome proliferator-activated receptor α (PPARα) in the cellular fasting response: the PPARα-null mouse as a model of fatty acid oxidation disorders. *Proc Natl Acad Sci USA* 1999;**96**:7473–8.
26 Reddy RC, Narala VR, Keshamouni VG, Milam JE, Newstead MW, Standiford TJ. Sepsis-induced inhibition of neutrophil chemotaxis is mediated by activation of peroxisome proliferator-activated receptor-γ. *Blood* 2008;**112**:4250–8.
27 Zhang B, Berger J, Hu E, Szalkowski D, White-Carrington S, Spiegelman BM *et al*. Negative regulation of peroxisome proliferator-activated receptor-γ gene expression contributes to the antiadipogenic effects of tumor necrosis factor-α. *Mol Endocrinol* 1996;**10**:1457–66.
28 Sewter C, Blows F, Considine R, Vidal-Puig A, O'Rahilly S. Differential effects of adiposity on peroxisomal proliferator-activated receptor γ1 and γ2 messenger ribonucleic acid expression in human adipocytes. *J Clin Endocrinol Metab* 2002;**87**:4203–7.
29 Daniela F, Dirk W, Alexander R, Carola S, Eva B, Jens F *et al*. Up-regulation of PPAR-γ in myocardial infarction. *Eur J Heart Fail* 2008;**10**:30–8.
30 Li H, Lin X. Positive and negative signaling components involved in TNF α-induced NF-κB activation. *Cytokine* 2008;**41**:1–8.
31 Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science* 2002;**296**:1634–5.
32 Carr A, Frei B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J* 1999;**13**:1007–24.
33 Kettawan A, Takahashi T, Kongkachuichai R, Charoenkiatkul S, Kishi T, Okamoto T. Protective effects of coenzyme Q_{10} on decreased oxidative stress resistance induced by simvastatin. *J Clin Biochem Nutr* 2007;**40**:194–202.

