Prostaglandin-J$_2$ upregulates expression of matrix metalloproteinase-1 independently of activation of peroxisome proliferator-activated receptor-γ[★][✪]

Alicja Józkowicz[1,2][✉], Ihor Huk[2], Anneliese Nigisch[3], Jarosław Cisowski[4], Guenter Weigel[3] and Józef Dulak[4]

[1]*Department of Molecular Genetics, Faculty of Biotechnology, Jagiellonian University, Kraków, Poland*; [2]*Department of Vascular Surgery, and* [3]*Department of Cardiothoracic Surgery, University of Vienna, Austria*; [4]*Department of Cell Biochemistry, Faculty of Biotechnology, Jagiellonian University, Kraków, Poland*

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-inducible nuclear receptor that functions as a transcription factor involved in lipid metabolism, inflammatory response and angiogenesis. The most potent endogenous PPARγ activator is 15-deoxy-$\Delta^{12,14}$prostaglandin-J$_2$ (15d-PGJ$_2$), whereas synthetic ligands include the oral antidiabetic drugs thiazolidinediones (TZDs). Activation of PPARγ was reported to decrease the synthesis of matrix metalloproteinases (MMPs) in vascular smooth muscle cells and macrophages. We aimed to investigate the effect of PPARγ ligands on

Abbreviations: AP-1, activating protein-1; COX-2, cyclooxygenase-2; Cu,ZnSOD, copper-zinc superoxide dismutase; DP-R, prostaglandin-D$_2$ receptor; EGF, epidermal growth factor; FCS, fetal calf serum; FP-R, prostaglandin-F$_{2\alpha}$ receptor; GSH, glutathione; HMEC-1, human microvascular endothelial cells; LDH, lactate dehydrogenase; MMPs, matrix metalloproteinases; MnSOD, manganese superoxide dismutase; NAC, *N*-acetyl-cysteine; NFκB, nuclear factor κB; PGD$_2$, prostaglandin-D$_2$; PGE$_2$, prostaglandin-E$_2$; PGF$_{2\alpha}$, prostaglandin-F$_{2\alpha}$; 15d-PGJ$_2$, 15-deoxy-$\Delta^{12,14}$prostaglandin-J$_2$; PPARγ, peroxisome proliferator-activated receptor-γ; PPRE, PPAR response element; uPA, urokinase plasminogen activator; TZD, thiazolidinedione; VSMC, vascular smooth muscle cells.

expression of MMP-1 and urokinase plasminogen activator (uPA) in human microvascular endothelial cells (HMEC-1). We found that treatment of HMEC-1 with 15d-PGJ$_2$ increased the synthesis of MMP-1 protein up to 168% comparing to untreated cells. TZDs (ciglitazone and troglitazone), more potent activators of PPARγ in HMEC-1, did not influence MMP-1 production, arguing against the involvement of PPARγ in this process. Importantly, the stimulatory effect of 15d-PGJ$_2$ was reversed by the antioxidant N-acetyl-cysteine (NAC), suggesting a contribution of oxidative stress. We demonstrated also that 15d-PGJ$_2$ did not change the activity of MMP-1 promoter, but increased the stability of MMP-1 mRNA. In contrast, 15d-PGJ$_2$ very potently inhibited the synthesis of uPA. This effect was in part mimicked by ciglitazone and troglitazone implying an involvement of PPARγ. Accordingly, NAC did not modify the inhibitory effect of 15d-PGJ$_2$ on uPA expression. In conclusion, we postulate that 15d-PGJ$_2$ may differently regulate the synthesis of proteases involved in angiogenesis: it upregulates MMP-1 expression in HMEC-1 through induction of oxidative stress, and inhibits uPA synthesis partly by activation of PPARγ.

Matrix metalloproteinases (MMPs) are enzymes degrading almost all extracellular matrix components. They operate during fetal development, angiogenesis, or wound healing and are responsible for excessive breakdown of connective tissue in inflammatory diseases. MMPs are secreted as inactive proenzymes (zymogens), with activation occurring in the extracellular compartment (Shapiro, 1998).

One of the MMPs is MMP-1 (also known as collagenase-1), a principal proteinase capable of degrading native fibrillar collagens. MMP-1 is produced by many cell types, including endothelium. It is implicated in a wide variety of pathological processes where collagen degradation occurs, as rheumatoid arthritis, periodontal disease, tumor invasion, corneal ulceration, inflammatory bowel disease, aneurysm, and restenosis (Vincenti *et al.,* 1996). MMP-1 contributes also to the destruction of extracellular matrix at the shoulder regions of atherosclerotic plaques that leads to plaque destabilization and triggers clinical cardiovascular diseases (Bond *et al.,* 2001).

Peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-activated transcription factor of the nuclear receptor superfamily, initially identified in the adipose tissue where its activation is associated with adipocyte differentiation (Mangelsdorf & Evans, 1995). PPARγ expression has been detected also in the vessel wall, both in vascular smooth muscle cells (VSMC) and endothelium (Marx *et al.,* 1998a; Xin *et al.,* 1999; Józkowicz *et al.,* 2000;

Józkowicz *et al.,* 2001). Upon ligand binding, PPARγ forms a heterodimer with retinoid X receptor-α (RXRα) and binds the PPAR response element (PPRE) in promoters of target genes, thus directly regulating their transcription (Michalik & Wahli, 1999).

PPARγ has been intensively studied after its role had been determined in the therapeutic action of TZDs, insulin-sensitizing compounds approved for treatment of insulin-resistance in type II diabetes (Lehmann *et al.,* 1995; Sood *et al.,* 2000). Activation of PPARγ reduces inflammatory response in the vessel and plays a protective role in atherosclerosis (Patel *et al.,* 1998; Ricote *et al.,* 1998; Pasceri *et al.,* 2000). PPARγ ligands are also beneficial in protection against restenosis, acting through inhibition of MMP-9 expression and VSMC migration (Marx *et al.,* 1998a, Shinohara *et al.,* 1998; Yoshimoto *et al.,* 1999).

Down-regulation of MMPs could contribute to anti-angiogenic activity of PPARγ ligands. It has been demonstrated that both the endogenous PPARγ ligand 15-deoxy-$\Delta^{12,14}$prostaglandin-J$_2$ (15d-PGJ$_2$) and troglitazone diminish migration of endothelial cells and their differentiation into tube-like structures (Xin *et al.,* 1999; Józkowicz *et al.,* 2002). One of the mechanisms suggested was down-regulation of the synthesis of urokinase plasminogen activator (uPA), a serine protease required for degradation of extracellular matrix and for progression of capillary outgrowth (Xin *et al.,* 1999).

In early stages of angiogenesis, the proteolytic activity of MMP-1 in endothelial cells is necessary. MMP-1 deficiency leads to decreased migration of aged microvascular endothelial cells and impaired neovascularization in diabetic patients (Partridge *et al.,* 2000, Reed *et al.,* 2000, Taniyama *et al.,* 2001). Data concerning the role of PPARγ in regulation of MMP-1 expression in endothelium are, however, lacking. Therefore we decided to determine the effect of 15d-PGJ$_2$, troglitazone and ciglitazone on expression of MMP-1 in human microvascular endothelial cells. Additionally, we compared the basic mechanism underlying the expression of MMP-1 and uPA in cells treated with ligands of PPARγ.

MATERIALS AND METHODS

Reagents. 15-Deoxy-$\Delta^{12,14}$prostaglandin-J$_2$ (15d-PGJ$_2$), troglitazone and ciglitazone were obtained from Biomol. NAC, AH6809, L-glutamine, EGF, actinomycin-D, and hydrocortisone were purchased from Sigma; fetal calf serum (FCS) was procured from Promo-Cell. CytoTox-96 Non-Radioactive Cytotoxicity Assay, SV Total RNA Extraction Kit, Reverse Transcription System, PCR Core System, control pSVβgal plasmid, Luciferase Assay Reagents were obtained from Promega; Maxiprep QIAfilter EndoFree Plasmid Isolation Kit and SuperFect Transfection Reagent were purchased from Qiagen. ELISA kit for human proMMP-1 was obtained from R&D Systems, and ELISA kit for human uPA was procured from Fresenius Kabi. All others reagents were purchased from Gibco.

Cell culture and incubation experiments. Human microvascular endothelial cells (HMEC-1) were purchased from the Center for Disease Control and Prevention (Atlanta, GA, U.S.A.) and cultured in DMEM F-12 medium containing 10% FCS, L-glutamine (2 mM), EGF (10 ng/ml), hydrocortisone (1 μg/ml), penicillin (100 U/ml), and streptomycin (10 μg/ml). Cells were placed into 24-well plates and grown to full confluence. Then, fresh medium was introduced and supplemented with 15d-PGJ$_2$, ciglitazone, or troglitazone (3–10 μM). After 24 h, media were collected for determining MMP-1 or uPA protein concentrations and LDH activities. To investigate MMP-1 mRNA stability, some cells were treated with 15d-PGJ$_2$ (10 μM) in the presence of actinomycin-D (1 μg/ml) for 4, 8, and 12 h.

RT-PCR. Total RNA was isolated from the cells by acid guanidinium thiocyanate-phenol-chloroform extraction (Chomczynski & Sacchi, 1987). Reverse transcription was carried out for 1 h at 42°C using AMV reverse transcriptase and random primers, according to vendor's instruction. Then PCR with Taq DNA polymerase and primers recognizing human MMP-1 (5′-CCT TGC ACT GAG AAA GAA GA-3′ and 5′-ACT TGC CTC CCA TCA TTC TT-3′) was performed for 35 cycles using the following protocol: 95°C — 40 s, 58°C — 40 s, and 72°C — 50 s. PCR products were analyzed by electrophoresis in 2% agarose gels. The product length for the MMP-1 was 183 bp. The primers, designed using human mRNA sequence (Gene Bank accession number: NM 002421), did not amplify genomic MMP-1 DNA, as checked by negative results of PCR reaction when not preceded by reverse transcription.

Transient transfection with reporter plasmids. Plasmid pGL3-PPRE containing three copies of PPAR-responsive element (PPRE) regulating luciferase gene expression was kindly donated by Dr. Lluis Fajas (France). Construct pGL2-MMP1, containing luciferase cDNA driven by full-length human MMP-1 promoter (+49 to −1773) was kindly provided by Dr. Tsutomu Ogura (Japan). Plasmids were amplified in HB-101 *Escherichia coli* bacteria and isolated on maxiprep columns. The quality of the DNA was assessed by spectrophotometry and by electrophoresis in 1% agarose gel.

HMEC-1 grown to 80% confluence were transfected in 24-well plates using 0.5 μg of

plasmid DNA and 2.5 μl of SuperFect Reagent per well, according to vendor's protocol. After transfection, cells were exposed to 10 μM 15d-PGJ$_2$ or 10 μM troglitazone for 24 h. Then, they were collected and cell lysates were assayed for luciferase activity according to manufacturer's instruction.

Measurement of MMP-1 and uPA protein concentrations. Concentrations of MMP-1 and uPA proteins in culture media were quantified using sandwich ELISA, following manufacturer's instructions.

Cell viability assay. Cell viability was assessed colorimetrically by measuring in culture media the activity of lactate dehydrogenase (LDH), a stable cytosolic enzyme released upon cell lysis.

Statistical analysis. All experiments were performed in duplicates or triplicates and were repeated 2–7 times. Data are presented as mean ± S.D. Statistical evaluation was done with Student's *t*-test. Differences were accepted as statistically significant at $P < 0.05$.

RESULTS

PPARγ is an active transcription factor in HMEC-1

In earlier study we demonstrated that PPARγ1 mRNA is expressed in HMEC-1 (Józkowicz *et al.*, 2001). Here we measured the ability of three PPARγ ligands to activate PPAR response element (PPRE). In cells transfected with pGL3-PPRE, 10 μM concentrations of 15d-PGJ$_2$, ciglitazone and troglitazone strongly increased expression of the luciferase reporter gene, indicating that PPARγ is an active transcription factor in HMEC-1 (Fig. 1). The level of PPRE stimulation was, however, significantly weaker in response to 15d-PGJ$_2$ (261% ± 36) than that induced by the thiazolidinediones (394% ± 84 and 477% ± 33 for ciglitazone and troglitazone, respectively). At the doses used, the PPARγ ligands were not toxic to HMEC-1, as assayed by the LDH release test (not shown).
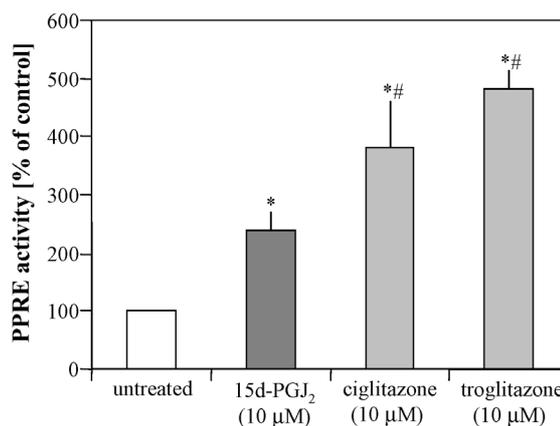
Figure 1. Effect of PPARγ ligands on activity of PPARγ in HMEC-1.

Cells were transfected with reporter plasmid containing luciferase cDNA driven by PPRE and then stimulated with 15d-PGJ$_2$, ciglitazone or trogitazone. Luciferase activity in cell lysates was measured after a 24 h incubation period. Each bar represents the mean ± S.D. of three experiments performed in triplicates and expressed as a percentage of control value (control, untreated cells). *$P < 0.05$ in comparison with untreated cells (transfected with reporter plasmid and not stimulated with PPARγ ligands); #$P < 0.05$ in comparison with 15d-PGJ$_2$-treated cells.

15d-PGJ$_2$ upregulates MMP-1 expression

After 24 h incubation, confluent, resting HMEC-1 released 850 ± 48 pg/ml of MMP-1 protein as measured by ELISA. Treatment of HMEC-1 with 15d-PGJ$_2$ at the doses which activated PPARγ (3 μM and 10 μM), concentration-dependently increased the synthesis of MMP-1 up to 168% when compared to untreated cells (Fig. 2). Importantly, ciglitazone and troglitazone, which are much more potent activators of PPARγ, did not exert any effect on MMP-1 production. This suggests that 15d-PGJ$_2$ upregulates MMP-1 expression independently of the PPARγ pathway.

15d-PGJ$_2$ is also a ligand of prostaglandin-D$_2$ surface receptor (DP-R) (Hirata *et al.*, 1994). Therefore we decided to check the effect of a specific DP-R antagonist, AH6809, on 15d-PGJ$_2$-augmented synthesis of MMP-1. As
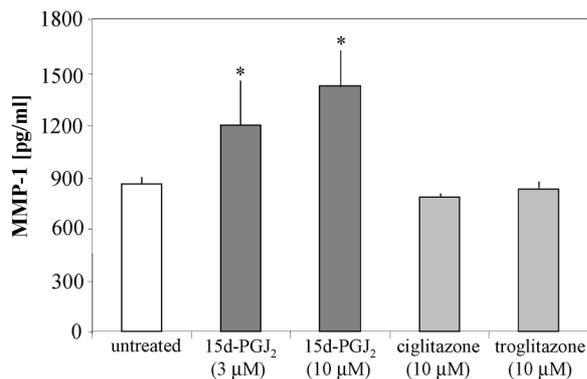
Figure 2. Effect of PPRAγ ligands on release of MMP-1 protein from HMEC-1.

Note that only 15d-PGJ$_2$ increases production of MMP-1, whereas ciglitazone and troglitazone do not show any influence. Culture media were harvested after a 24 h incubation period and concentration of MMP-1 was measured by ELISA. Each bar represents the mean ± S.D. of 3–7 experiments performed in duplicates. *P < 0.05 in comparison to untreated cells.

shown in Fig. 3, we did not observe any influence of this blocker on 15d-PGJ$_2$ activity. Additionally, using RT-PCR analysis we were not able to detect the expression of DP-R in HMEC-1 (not shown). These results convincingly indicate that DP-R is not involved in the regulation of MMP-1 synthesis by 15d-PGJ$_2$.

Finally, since it was recently suggested that 15d-PGJ$_2$ can induce oxidative stress (Kondo *et al.* 2001), we investigated the effect of the antioxidant *N*-acetyl-cysteine (NAC) on 15d-PGJ$_2$ activity. We found that a 20-minute pretreatment of HMEC-1 with 1 mM NAC significantly attenuated the stimulatory effect of 15d-PGJ$_2$ on MMP-1 synthesis (Fig. 3), which implies that the augmentation of metalloproteinase production in response to 15d-PGJ$_2$ is mediated by changes in an oxidative status of HMEC-1.

15d-PGJ$_2$ does not influence the activity of MMP-1 promoter

To investigate the effect of PPARγ ligands on MMP-1 transcription, we transfected HMEC-1 with a construct containing lucifer-
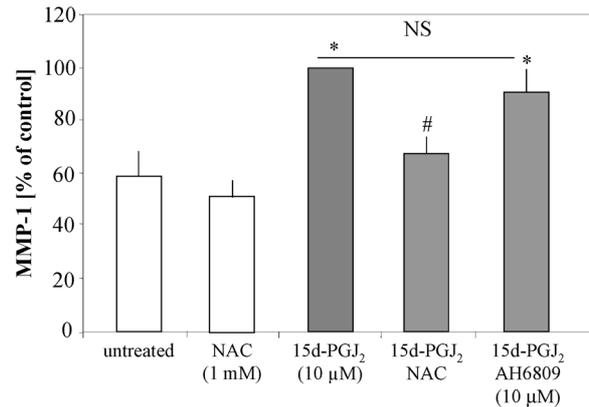
Figure 3. Effect of preincubation with NAC and AH6809 on 15d-PGJ$_2$-augmented synthesis of MMP-1 in HMEC-1.

Concentration of MMP-1 in culture medium harvested 24 h after treatment was measured by ELISA. Each bar represents the mean ± S.D. of 2–3 experiments performed in duplicates and expressed as a percentage of control value (control, cells treated with 15d-PGJ$_2$ alone). *P < 0.05 in comparison with untreated cells; #P < 0.05 in comparison with 15d-PGJ$_2$-treated cells; NS, not significant difference.

ase cDNA regulated by full-length human MMP-1 promoter (pGL2-MMP1). The efficiency of gene transfer into HMEC-1 ranged from 10% to 20% as assessed in cells transfected with a control plasmid encoding β-galactosidase (not shown). We demonstrated that the activity of MMP-1 promoter was not affected by 15d-PGJ$_2$ or troglitazone (Fig. 4A). Very similar results were obtained when pGL2-MMP1 plasmid was introduced to NIH3T3 murine fibroblast (not shown). This evidences that MMP-1 expression in response to 15d-PGJ$_2$ is not regulated at the transcriptional level.

15d-PGJ$_2$ increases stability of MMP-1 mRNA

Stability of mRNA was estimated in HMEC-1 incubated with actinomycin-D in the presence or absence of 10 μM 15d-PGJ$_2$. After the 4, 8, and 12 h incubation, RNA was isolated from the cells and RT-PCR with primers specific for MMP-1 was performed. We found that signal
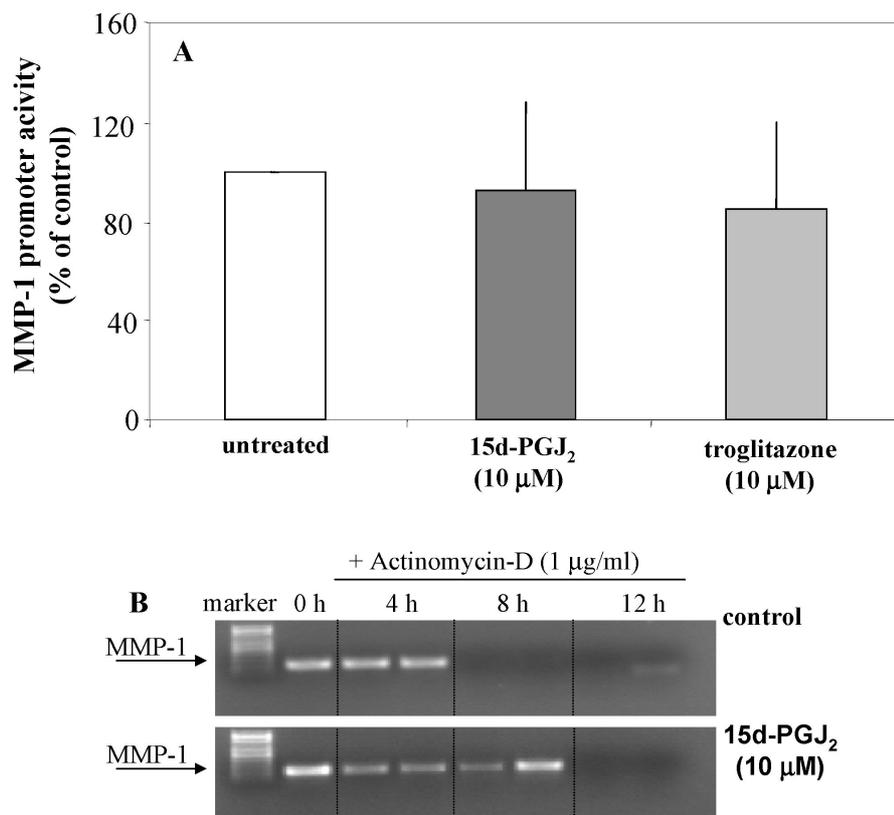
Figure 4. Effect of 15d-PGJ$_2$ on major pathways regulating the MMP-1 expression.

A. 15d-PGJ$_2$ and troglitazone did not influence the activity of MMP-1 promoter. Cells were transfected with reporter plasmid containing luciferase cDNA driven by full-length human MMP-1 promoter and then stimulated with 15d-PGJ$_2$ or troglitazone. Luciferase activity in cell lysates was measured after a 24 h incubation period. Each bar represents the mean ± S.D. of six experiments performed in duplicates and expressed as a percentage of control value (control, untreated cells). **B**. 15d-PGJ$_2$ increased the stability of MMP-1 mRNA. Cells were treated with actinomycin-D (1 μg/ml) in the presence or absence of 15d-PGJ$_2$. One of four similar RT-PCR analysis.

for MMP-1 was detectable in control cells (not stimulated with 15d-PGJ$_2$) untill 4 h after addition of actinomycin-D, but disappeared after 8 h (Fig. 4B). In contrast, in cells supplemented with actinomycin-D in the presence of 15d-PGJ$_2$, MPP-1 mRNA was present even 8 h after stimulation. This suggests that an increase in stability of mRNA may be an important mechanism involved in the upregulation of MMP-1 synthesis in response to 15d-PGJ$_2$.

PPARγ ligands down-regulate the expression of uPA

We studied also the effect of 15d-PGJ$_2$ on expression of uPA, a matrix-degrading serine protease. Treatment of HMEC-1 with 15d-PGJ$_2$ (3 μM and 10 μM) very potently and dose-dependently inhibited the release of uPA into culture media (Fig. 5). Importantly, both ciglitazone and troglitazone down-regulated uPA synthesis, which may indicate an involvement of PPARγ in this effect. Inhibition by 15d-PGJ$_2$ was, however, much stronger, suggesting that some additional, PPARγ-independent pathway(s) play a role. We also found that AH6809 did not influence 15d-PGJ$_2$ action, pointing that DP-R is not involved in uPA regulation (Fig. 5). Interestingly, pretreatment of HMEC-1 with 1 mM NAC did not significantly change the inhibitory effect of 15d-PGJ$_2$ (Fig. 5), indicating that pathways underlying the regulation of uPA expression by 15d-PGJ$_2$ are different than in the case of MMP-1.
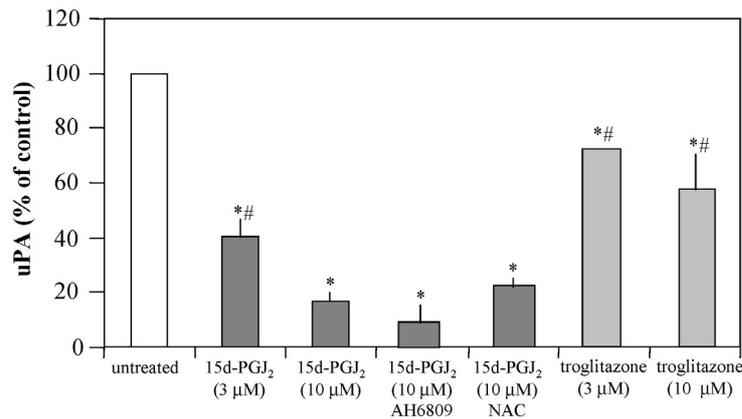
Figure 5. Effect of PPARγ ligands on release of uPA protein from HMEC-1.

Culture media were harvested after a 24 h incubation period and concentration of uPA was measured by ELISA. Each bar represents the mean ± S.D. of 2–5 experiments performed in duplicates and expressed as a percentage of control value (control, untreated cells). *$P < 0.05$ in comparison with untreated cells, #$P < 0.05$ in comparison with cells treated with 10 μM 15d-PGJ$_2$.

DISCUSSION

Ligands of PPARγ are commonly regarded as potent anti-inflammatory and anti-angiogenic agents. They are also well-known inhibitors of matrix metalloproteinases in several cell types. It was demonstrated that TZDs and 15d-PGJ$_2$ potently decreased MMP-3 and MMP-9 expression in VSMC (Marx *et al.,* 1998a; Bond *et al.,* 2001), macrophages (Marx *et al.,* 1998b; Bond *et al.,* 2001; Ricote *et al.,* 1998) and chondrocytes (Sabatini *et al.,* 2002). In addition, 15d-PGJ$_2$ significantly suppressed MMP-2 and MMP-9 synthesis in pancreatic cancer cells, which was associated with strong reduction of cancer invasiveness (Hashimoto *et al.,* 2002). Importantly, 15d-PGJ$_2$ and TZDs can inhibit also the expression of MMP-1, as shown in synovial fibroblasts (Fahmi *et al.,* 2002).

Unexpectedly, our present research demonstrates that in human microvascular endothelial cells, 15d-PGJ$_2$ significantly and dose-dependently augments the expression of MMP-1. Concomitantly, the same doses of 15d-PGJ$_2$ induce PPARγ transcriptional activity as evidenced in HMEC-1 transfected with a reporter plasmid regulated by PPRE. We postulate, however, that activation of PPARγ is not involved in the 15d-PGJ$_2$-exerted augmentation of MMP-1 synthesis, as ciglitazone and troglitazone, more potent activators of PPARγ in HMEC-1, do not influence MMP-1 production. A similar discrepancy between the effects of 15d-PGJ$_2$ and TZDs we described earlier in studies on the regulation of synthesis of interleukin-8 (IL-8) in HMEC-1. 15d-PGJ$_2$ potently and dose-dependently increased both the steady-state and LPS-induced generation of IL-8 mRNA and IL-8 protein, whereas neither basal nor LPS-elicited expression of IL-8 was influenced by ciglitazone (Józkowicz *et al.,* 2001).

A growing body of evidence has emerged that 15d-PGJ$_2$, in addition to PPARγ activation, exerts PPAR-independent effects (Vaidya *et al.,* 1999; Thieringer *et al.,* 2000). A PPARγ-independent action was suggested by Goetze and colleagues, who found that 15d-PGJ$_2$ exhibited a much stronger anti-migration activity, although its potency in activating PPARγ was lower than that of the studied TZD (Goetze *et al.,* 1999). Similarly, 15d-PGJ$_2$ was much more effective than TZDs in regulation of gene expression in activated macrophages and microglia (Petrova *et al.,* 1999) or in blocking endothelial proliferation and morphogenesis (Xin *et al.,* 1999). Finally, the supposition that 15d-PGJ$_2$ can act through mechanisms not involving PPARγ was elegantly evidenced by Chawla and colleagues, who demonstrated that in macrophages homozygous for a null PPARγ gene, 15d-PGJ$_2$ is still an effective modulator of gene expressions (Chawla *et al.,* 2001). Our results add an additional example of a 15d-PGJ$_2$-specific activity not mimicked by TZD.

Studies on activated monocytes (Ardans *et al.,* 2002) or corneal organ cultures (Ottino & Bazan, 2001) revealed that indomethacine, an inhibitor of cyclooxygenase-2 (COX-2), suppresses MMP-1 synthesis. This suggests that prostaglandins can act as inducers of MMP-1. Indeed, prostaglandin-E_2 (PGE_2) increased MMP-1 expression in monocytes (Ardans *et al.,* 2002) and fibroblasts (Liu *et al.,* 2001), while supplementation with PGE_2, prostaglandin-D_2 (PGD_2), or prostaglandin-$F_{2\alpha}$ ($PGF_{2\alpha}$) increased MMP-1 levels in the cornea (Ottino & Bazan, 2001). These effects can be, however, cell-type specific, as PGE_2 and prostacyclin (PGI_2) inhibited production of MMP-1 in human aortic smooth muscle cells (Kato *et al.,* 1993).

Interestingly, it was documented in human fibroblasts that the $PGF_{2\alpha}$-induced increase in MMP-1 production is mediated by FP-R, a surface receptor for $PGF_{2\alpha}$ (Noguchi *et al.,* 2001). Therefore, although we could not detect the expression of DP-R, a surface receptor binding PGD_2 and 15d-PGJ_2, we decided to investigate its potential involvement in the upregulation of MMP-1 in HMEC-1. To this aim we preincubated HMEC-1 with a specific antagonist of DP-R, AH6809, and then stimulated the cells with 15d-PGJ_2. We found that AH6809 did not influence 15d-PGJ_2 activity. Accordingly, the upregulation of MMP-1 in response to 15d-PGJ_2 was not mimicked by treatment of HMEC-1 with a DP-R agonist, BW245C (data not shown). This implies that DP-R is not involved in 15d-PGJ_2 action in HMEC-1.

Importantly, the stimulatory effect of 15d-PGJ_2 was reversed by NAC. This compound has been used as an antioxidant in a wide variety of experiments. It may be protective by entering cells and being hydrolysed to cysteine, which stimulates GSH synthesis. Additionally, NAC may directly scavenge several reactive oxygen/reactive nitrogen species including HOCl, ONOOH, $O_2^{-\bullet}$, $OH^\bullet$, and H_2O_2 (Halliwell & Gutterige, 2001).

Inhibitory effects of NAC on MMPs have been observed in earlier experiments. NAC was reported to reduce the expression of MMP-9 in macrophages (Galis *et al.,* 1998), VSMC (Gurjar *et al.,* 2001), human umbilical endothelial cells (HUVEC) (Cai *et al.,* 1999), cancer bladder cells (Kawakami *et al.,* 2001), and segments of atherosclerotic aorta (Galis *et al.,* 1998). It inhibited also MMP-1 production in neurons (Kamata *et al.,* 1996).

The mechanisms of inhibitory action of NAC on MMPs are not fully clarified. It seems that its capability of scavenging H_2O_2 may be of essence. Several experiments pointed out the importance of elevation of H_2O_2 in upregulation of MMP-1 production (Brenneisen *et al.,* 1997; Wenk *et al.,* 1999; Ranganathan *et al.,* 2001). One of the sources of intracellular H_2O_2 are MnSOD and Cu,ZnSOD due to dismutation of the superoxide anion to hydrogen peroxide (Halliwell & Gutterige, 2001). In fact, MnSOD activity increased steady-state levels of H_2O_2 leading to augmented synthesis of MMP-1 in dermal fibroblast (Ranganathan *et al.,* 2001). Interestingly, 15d-PGJ_2 increases expression of Cu,Zn-SOD in primary endothelial cells (Inoue *et al.,* 2001). Thus one can suppose that induction of H_2O_2 generation may be responsible for the observed upregulation of MMP-1 synthesis in HMEC-1, and scavenging of H_2O_2 by NAC may decrease this stimulatory effect.

A second possibility is the regulation of MMP-1 expression by NAC-induced changes in the cellular oxidative status. Studies on fibroblasts isolated from patients suffering from systemic sclerosis demonstrated that synthesis of MMP-1 is more strongly induced in cells with lower antioxidant capacity (Yin *et al.,* 2003). Furthermore, increased glutathione concentration reduced MMP-1 production in transformed fibroblast, although this effect was cell-type specific (Tyagi *et al.,* 1996). Recent studies indicate that 15d-PGJ_2 can influence the GSH:GSSG ratio (Kawamoto *et al.,* 2000; Kondo *et al.,* 2001; Satoh *et al.,* 2001).

Thus, it cannot be excluded that modulation of the cellular redox state can be responsible for the induction of MMP-1 expression by 15d-PGJ$_2$ and for the inhibitory effect of NAC in HMEC-1.

The expression of MMP-1 is controlled mainly at the transcriptional level, and characterization of the MMP-1 gene promoter has revealed that AP-1 is an essential transcription factor for its induction (White & Brinckerhoff, 1995). Many agents have been shown to regulate the expression of MMP-1 *via* modulation of AP-1 activity. Thus, inhibition of AP-1 is responsible for reduced expression of MMP-1 under treatment with glucocorticoid hormones and retinoids, whereas augmented binding of AP-1 leads to increased MMP-1 synthesis in response to basic calcium phosphate crystals, H$_2$O$_2$ overproduction or exposure to NO (Vincenti *et al.,* 1996; Wenk *et al.,* 1999; Ishii *et al.,* 2003).

15d-PGJ$_2$ down-regulates AP-1 activity in many cell types (for a review see Józkowicz *et al.,* 2002b). Accordingly, in human synovial fibroblasts it reduces the basal and IL-1β-induced AP-1 levels and, in consequence, decreases MMP-1 expression (Fahmi *et al.,* 2002). Also in HMEC-1 we confirmed using EMSA analysis the inhibition of AP-1 binding capacity by 15d-PGJ$_2$ (data not shown). Regardless of this inhibitory potential, 15d-PGJ$_2$ did not influence the activity of MMP-1 promoter in HMEC-1, as evidenced by luciferase assay in cells transfected with pGL2-MMP1 plasmid.

Instead, we demonstrated that 15d-PGJ$_2$ augments the expression of MMP-1 through stabilization of MMP-1 mRNA. Data on the involvement of mRNA stability in the regulation of MMP-1 synthesis are very scarce. It was revealed, for example, that in rabbit synovial fibroblasts the IL-1β-induced MMP-1 expression results from a prolonged MMP-1 half-life (Vincenti *et al.,* 1994). In addition, in transformed human fibroblasts the decrease in GSH:GSSG ratio augments MMP-1 expression independently of MMP-1 promoter, thus

stabilization of mRNA can be hypothesized (Tyagi *et al.,* 1996). It fact, the numerous AU-rich elements in MMP-1 mRNA may be indicative of the importance of regulation *via* mRNA stability (Fini *et al.,* 1987).

In contrast to the augmentation of MMP-1 expression, 15d-PGJ$_2$ very potently inhibited the synthesis of uPA in HMEC-1. This observation is concordant with the earlier report showing that 15d-PGJ$_2$ reduced uPA mRNA level in HUVEC (Xin *et al.,* 1999). Importantly, we found that this effect was in part mimicked by ciglitazone and troglitazone, which suggests an involvement of PPARγ. Unlike in the case of MMP-1, treatment of HMEC-1 with NAC did not influence the inhibitory effect of 15d-PGJ$_2$ on uPA expression.

In conclusion, we postulate that in human microvascular endothelial cells 15d-PGJ$_2$ may differently regulate the synthesis of proteases involved in angiogenesis and cell migration: it upregulates MMP-1 expression through induction of oxidative stress, and inhibits the synthesis of uPA by activation of PPARγ.

REFERENCES

Ardans JA, Economou AP, Martinson JM Jr, Zhou M, Wahl LM. (2002) Oxidized low-density and high-density lipoproteins regulate the production of matrix metalloproteinase-1 and -9 by activated monocytes. *J Leukoc Biol.*; **71**: 1012–8.

Bond M, Chase AJ, Baker AH, Newby AC. (2001) Inhibition of transcription factor NFκB reduces matrix metalloproteinase-1, -3, -9 production by vascular smooth muscle cells. *Cardiovasc Res.*; **50**: 556–65.

Brenneisen P, Briviba K, Wlaschek M, Wenk J, Scharffetter-Kochanek K. (1997) Hydrogen peroxide (H$_2$O$_2$) increases the steady-mRNA levels of collagenase MMP-1 in human dermal fibroblasts. *Free Radic Biol Med.*; **22**: 515–24.

Cai T, Fassina G, Morini M, Aluigi MG, Masiello L, Fontanini G, D'Agostini F, De Flora S, Noonan DM, Albini A. (1999) *N*-Acetyl-

cysteine inhibits endothelial cell invasion and angiogenesis. *Lab Invest.*; **79**: 1151–9.

Chawla A, Barak Y, Nagy L, Liao D, Tontonoz P, Evans RM. (2001) PPARγ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat Med.*; **7**: 48–53.

Chomczynski P, Sacchi N. (1987) Single step method of RNA isolation by guanidinium thiocyanate-phenol-chlorophorm extraction. *Anal Biochem.*; **162**: 156–9.

D'Armentio J. (2002) Matrix metalloproteinase disruption of the extracellular matrix and cardiac dysfunction. *Trends Cardiovasc Med.*; **12**: 97–101.

Fahmi H, Pelletier JP, Di Basta JA, Cheung HS, Fernandes JC, Martel-Pelletier J. (2002) Peroxisome proliferator-activated receptor gamma activators inhibit MMP-1 production in human synovial fibroblasts likely by reducing the binding of the activator protein 1. *Osteoarthritis Cartilage.*; **10**: 100–8.

Fini ME, Plucinska IM, Mayer AS, Gross RH. Brinckehoff CE. (1987) A gene for rabbit synovial cell collagenase: Member of a family of metalloproteinases that degrade the connective tissue matrix. *Biochemistry.*; **26**: 6156–65.

Galis ZS, Muszynski M, Sukhova GK, Simon-Morrissey E, Unemori EN, Lark MW, Amento E, Libby P. (1994) Cytokine-stimulated human vascular smooth muscle cells synthesize a complement of enzymes required for extracellular matrix degradation. *Circ Res.*; **75**: 181–9.

Galis ZS, Asanuma K, Godin D, Meng X. (1998) *N*-Acetyl-cysteine decreases the matrix-degrading capacity of macrophage-derived foam cells. New target for antioxidant therapy? *Circulation.*; **97**: 2445–53.

Goetze S, Xi XP, Kawano H, Gotlibowski T, Fleck E, Hsueh WA, Law RE. (1999) PPARγ-ligands inhibit migration mediated by multiple chemoattractants in vascular smooth muscle cells. *J Cardiovasc Pharmacol.*; **33**: 798–806.

Gurjar MV, Deleon J, Sharma RV, Bhalla RC. (2001) Role of reactive oxygen species in IL-1β-stimulated sustained ERK activation and MMP-9 induction. *Am J Physiol Heart Circ Physiol.*; **281**: H2568–74.

Halliwell B, Gutterige JMC. (2001) *Free radicals in biology and medicine.* 3rd edn. Oxford Science Publications.

Hashimoto K, Ethridge RT, Evers BM. (2002) Peroxisome proliferator-activated receptor gamma ligand inhibits cell growth and invasion of human pancreatic cancer cells. *Int J Gastrointest Cancer.*; **32**: 7–22.

Hirata M, Kakizuka A, Aizawa M, Ushokubi F, Narumiya S. (1994) Molecular characterization of a mouse prostaglandin D receptor and functional expression of the cloned gene. *Proc Natl Acad Sci U S A.*; **91**: 11192–6.

Inoue I, Goto S, Matsunaga T, Nakajima T, Awata T, Hokari S, Komoda T, Katayama S. (2001) The ligands/activators for peroxisome proliferator-activated receptor alpha (PPARα) and PPARγ increaase Cu^{2+}, Zn^{2+}-superoxide dismutase and decrease p22phox message expression in primary endothelial cells. *Metabolism.*; **50**: 3–11.

Ishii Y, Ogura T, Tatemichi M, Fujisawa H, Otsuka F, Esumi H. (2003) Induction of matrix metalloproteinse gene transcription by nitric oxide and mechanisms of MMP-1 gene induction in human melanoma cell lines. *Int J Cancer.*; **103**: 161–8.

Józkowicz A, Dulak J, Piątkowska E, Placha W, Dembińska-Kieć A. (2000) Ligands of peroxisome proliferator-activated receptor-γ increase the generation of vascular endothelial growth factor in vascular smooth muscle cells and in macrophages. *Acta Biochim Polon.*; **47**: 1147–57.

Józkowicz A, Dulak J, Prager M, Nanobashvili J, Nigisch A, Winter B, Weigel G, Huk I. (2001) Prostaglandin-J_2 induces synthesis of interleukin-8 by endothelial cells in a PPARγ-independent manner. *Prostaglandins Other Lipid Mediat.*; **66**: 165–77.

Józkowicz A, Dulak J, Nigish A, Winter B, Weigel G, Huk I. (2002a) Ciglitazone, a ligand of peroxisome proliferator-activated receptor-γ, inhibits vascular endothelial growth factor activity. *Eur Surg.*; **34**: 127–30.

Józkowicz A, Dulak J, Nanobashvili J, Prager M, Huk I. (2002b) Role of peroxisome proliferator-activated receptor-γ ligands in the vessel wall. *Eur Surg.*; **34**: 121–6.

Kamata H, Tanaka C, Yagisawa H, Matsuda S, Gotoh Y, Nishida E, Hirata H. (1996) Suppression of nerve growth factor-induced neuronal differentiation of PC12 cells. N-acetylcysteine uncouples the signal transduction from Ras to the mitogen-activated kinase cascade. *J Biol Chem.*; **271**: 33018–25.

Kato S, Sasaguri Y, Morimatsu M. (1993) Down-regulation in the production of matrix metalloproteinase-1 by human aortic intimal smooth muscel cells. *Biochem Mol Biol Int.*; **31**: 239–48.

Kawakami S, Kageyama Y, Fuji Y, Kihara K, Oshima H. (2001) Inhibitory effect of N-acetylcysteine on invasion and MMP-9 production of T24 human bladder cancer cells. *Anticancer Res.*; **21**: 213–9.

Kawamoto Y, Nakamura Y, Naito Y, Kumagai T, Osawa T, Ohigashi H, Satoh K, Imagawa M, Uchida K. (2000) Cyclopentanone prostaglandins as potential inducers of phase II detoxification enzymes. 15-Deoxy-$\Delta^{12,14}$-prostaglandin-J$_2$-induced expression of glutathione S-transferases. *J Biol Chem.*; **275**: 11291–9.

Kondo M, Oya-Ito T, Kumagai T, Osawa T, Uchida K. (2001) Cyclopentenone prostaglandins as potential inducers of intracellular oxidative stress. *J Biol Chem.*; **276**: 12076–83.

Lehmann JM, Moore LB, Smith-Olivier TA, Wilkison WO, Willson TM, Kliewer SA. (1995) An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor-γ (PPARγ). *J Biol Chem.*; **270**: 12953–6.

Liu CM, Hong CY, Shun CT, Wang JS, Hsiao TY, Wang CC, Lin SK. (2001) Matrix metalloproteinase-1 and tissue inhibitor of metalloproteinase-1 gene expression and their differential regulation by proinflammatory cytokines and prostaglandin in nasal polyp. *Ann Otol Rhin Laryngol.*; **110**: 1129–36.

Mangelsdorf DJ, Evans RM. (1995) The RXR heterodimers and orphan receptors. *Cell.*; **83**: 841–50.

Marx N, Schoenbeck U, Lazar MA, Libby P, Plutzky J. (1998a) Peroxisome proliferator-activated receptor gamma activators inhibit gene expression and migration in human vascular smooth muscle cells. *Circ Res.*; **83**: 1097–103.

Marx N, Sukhova G, Murphy C, Libby P, Plutzky J. (1998b) Macrophages in human atheroma contain PPARγ. Differentiation-dependent peroxisomal proliferator-activated receptor (PPARγ) expression and reduction of MMP-9 activity through PPARγ activation in mononuclear phagocytes *in vitro*. *Am J Pathol.*; **153**: 17–23.

Michalik L, Wahli W. (1999) Peroxisome proliferator-activated receptors: three isotypes for a multitude of functions. *Curr Opin Biotech.*; **10**: 564–70.

Noguchi K, Tominaga Y, Matsushita K, Izumi Y, Endo H, Kondo H, Ishikawa I. (2001) Upregulation of matrix metalloproteinase-1 production by prostacyclin F$_{2\alpha}$ in human gingival fibroblasts. *J Periodontal Res.*; **36**: 334–9.

Ottino P, Bazan HE. (2001) Corneal stimulation of MMP-1, -9, and uPA by platelet-activated factor is mediated by cyclooxygenase-2 metabolites. *Curr Eye Res.*; **23**: 77–85.

Partridge CR, Hawker JR Jr, Forough R. (2000) Overexpression of a secretory form of FGF-1 promotes MMP-1 mediated endothelial cell migration. *J Cell Biochem.*; **78**: 487–99.

Pasceri V, Wu HD, Willerson JT, Yeh ETH. (2000) Modulation of vascular inflammation *in vitro* and *in vivo* by peroxisome proliferator-activated receptor-γ activators. *Circulation.*; **101**: 235–8.

Patel SS, Thiagarajan R, Willerson JT, Yeh ETH. (1998) Inhibition of 4 integrin and ICAM-1 markedly attenuate macrophage homing to atherosclerotic plaques in apoE-deficient mice. *Circulation.*; **97**: 75–81.

Petrova TV, Akama KT, Van Eldik LJ. (1999) Cyclopentenone prostaglandins suppress activation of microglia: down-regulation of inducible nitric-oxide synthase by 15-deoxy-$\Delta^{12,14}$prostaglandin-J_2. *Proc Natl Acad Sci U S A.*; **96**: 4668–73.

Ranganathan AC, Nelson KK, Rodriguez AM, Kim KH, Tower GB, Rutter JL, Brickerhoff CE, Huang TT, Epstein CJ, Jeffrey JJ, Melendez JA. (2001) Manganese superoxide dismutase signals matrix metalloproteinase expression *via* H_2O_2-dependent ERK1/2 activation. *J Biol Chem.*; **276**: 14264–70.

Reed MJ, Corsa AC, Kudravi SA, McCormick RS, Arthur WT. (2000) A deficit in collagenase activity contributes to impaired migration of aged microvascular endothelial cells. *J Cell Biochem.*; **77**: 116–26.

Ricote M, Huang J, Fajas L, Li A, Welch J, Najib J, Witzum JL, Auwerx J, Palinski W, Glass CK. (1998) Expression of the peroxisome proliferator-activated receptor-γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density protein. *Proc Natl Acad Sci U S A.*; **95**: 7614–9.

Ricote M, Li A, Kelly C, Glass C. (1998) The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation. *Nature.*; **391**: 79–82.

Sabatini M, Bardiot A, Lesur C, Moulharat N, Thomas M, Richard I, Fradin A. (2002) Effects of agonist of peroxisome proliferator-activated receptor gamma on proteoglycan degradation and matrix metalloproteinase production in rat cartilage *in vitro*. *Osteoarthritis Cartilage.*; **10**: 673–9.

Satoh K, Hayakari M, Ookawa K, Satou M, Aizawa S, Tanaka M, Hatayama I, Tsuchida S, Uchida K. (2001) Lipid peroxidation end products-responded induction of a preneoplastic marker enzyme glutathione *S*-transferase P-form (GST-P) in rat liver on administration *via* the portal vein. *Mutat Res.*; **483**: 65–72.

Schall TJ, Bacon KB. (1994) Chemokines, leukocyte trafficking, and inflammation. *Curr Opin Immunol.*; **6**: 865–73.

Shapiro SD. (1998) Matrix metalloproteinase degradation of extracellular matrix: Biological consequences. *Curr Opin Cell Biol.*; **10**: 602–8.

Shinohara E, Kihara S, Ouchi N, Funahashi T, Nakamura T, Yamashita S, Kameda-Katemura K, Matsuzawa Y. (1998) Troglitazone suppresses intimal formation following balloon injury in insulin-resistant Zucker fatty rats. *Atherosclerosis.*; **136**: 275–9.

Sood V, Colleran K, Burge MR. (2000) Thiazolidinediones: a comparative review of approved uses. *Diabetes Technol Ther.*; **2**: 429–40.

Taniyama Y, Morishita R, Hiraoka K, Aoki M, Nakagami H, Yamasaki K, Matsumoto K, Nakamura T, Kaneda Y, Ogihara T. (2001) Therapeutic angiogenesis induced by human hepatocyte growth factor gene in rat diabetic hind limb ischemia model: molecular mechanisms of delayed angiogenesis in diabetes. *Circulation.*; **104**: 2344–50.

Thieringer R, Fenyk-Melody JE, Le Grand CB. (2000) Activation of peroxisome proliferator-activated receptor-γ does not inhibit IL-6 or TNFα responses of macrophages to lipopolysaccharide *in vitro* and *in vivo*. *J Immunol.*; **164**: 1046–52.

Tyagi SC, Kumar S, Borders S. (1996) Reduction-oxidation (redox) state regulation of extracellular matrix metalloproteinases and tissue inhibitors in cardiac normal and transformed fibroblast cells. *J Cell Biochem.*; **61**: 139–51.

Vaidya S, Somers EP, Wright SD, Detmers PA, Bansal VS. (1999) 15-Deoxy-$\Delta^{12,14}$-prostaglandin-J_2 inhibits the β-$_2$ integrin-dependent oxidative burst: involvement of a mechanism distinct from peroxisome proliferator activated receptor γ ligation. *J Immunol.*; **163**: 6187–92.

Vincenti MP, Coon CI, Lee O, Brickerhoff CE. (1994) Regulation of collagenase gene expression by IL-1β requires transcriptional and post-transcriptional mechanisms. *Nucleic Acids Res.*; **22**: 4818–27.

Vincenti MP, White LA, Schroen DJ, Bennow U, Brickerhoff CE. (1996) Regulating expression of the gene for matrix metalloproteinase-1 (collagenase): Mechanism that control enzyme activity, transcription, and mRNA stability. *Crit Rev Eukaryot Gene Expr.*; **6**: 391–411.

Wenk J, Brenneisen P, Wlachek M, Poswig A, Briviba K, Oberley TD, Scharffetter-Kochanek K. (1999) Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloproteinase-1. *J Biol Chem.*; **274**: 25869–76.

White LA, Brinckerhoff CE. (1995) Two activator protein-1 elements in the matrix metalloproteinase-1 promoter have different effects on transcription and bind JunD, c-Fos, and Fra-1. *Matrix Biol.*; **14**: 715–25.

Xin X, Yang S, Kowalski J, Gerritsen ME. (1999) Peroxisome proliferator-activated receptor-γ ligands are potent inhibitors of angiogenesis *in vitro* and *in vivo*. *J Biol Chem.*; **274**: 9116–21.

Yin L, Yamauchi R, Tsuji T, Krutmann J, Morita A. (2003) The expression of matrix metalloproteinase-1 mRNA induced by ultraviolet A1 (340–400 nm) is phototherapy relevant to the glutathione (GSH) content in skin fibroblasts of systemic sclerosis. *J Deramatol.*; **30**: 173–80.

Yoshimoto T, Naruse M, Shizume H, Naruse K, Tanabe A, Tanaka M, Tago K, Irie K, Muraki T, Demura H, Zardi L. (1999) Vasculo-protective effects of insulin sensitizing agent pioglitazone in neointimal thickening and hypertensive vascular hypertrophy. *Atherosclerosis.*; **145**: 333–40.