Prostaglandin-J₂ upregulates expression of matrix metalloproteinase-1 independently of activation of peroxisome proliferator-activated receptor-γ*

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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-Δ¹²,¹⁴-prostaglandin-J₂ (15d-PGJ₂), 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

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Abbreviations: AP-1, activating protein-1; COX-2, cyclooxygenase-2; Cu,ZnSOD, copper-zinc superoxide dismutase; DP-R, prostaglandin-D₂ receptor; EGF, epidermal growth factor; FCS, fetal calf serum; FP-R, prostaglandin-F₂α 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; PGD₂, prostaglandin-D₂; PGE₂, prostaglandin-E₂; PGF₂α, prostaglandin-F₂α; 15d-PGJ₂, 15-deoxy-Δ¹²,¹⁴-prostaglandin-J₂; 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-PGJ2 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-PGJ2 was reversed by the antioxidant N-acetyl-cysteine (NAC), suggesting a contribution of oxidative stress. We demonstrated also that 15d-PGJ2 did not change the activity of MMP-1 promoter, but increased the stability of MMP-1 mRNA. In contrast, 15d-PGJ2 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-PGJ2 on uPA expression. In conclusion, we postulate that 15d-PGJ2 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 protease 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-Δ12,14-prostaglandin-J2 (15d-PGJ2) 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-PGJ2, 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-DeoxyΔ12,14-prostaglandin-J2 (15d-PGJ2), 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 Promocell. 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 other 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-PGJ2, 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-PGJ2 (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**

**PPAR\(\gamma\) is an active transcription factor in HMEC-1**

In earlier study we demonstrated that PPAR\(\gamma\)1 mRNA is expressed in HMEC-1 (Józkowicz et al., 2001). Here we measured the ability of three PPAR\(\gamma\) 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 PPAR\(\gamma\) 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 PPAR\(\gamma\) ligands were not toxic to HMEC-1, as assayed by the LDH release test (not shown).

**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 PPAR\(\gamma\) (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 PPAR\(\gamma\), did not exert any effect on MMP-1 production. This suggests that 15d-PGJ\(_2\) upregulates MMP-1 expression independently of the PPAR\(\gamma\) 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
shown in Fig. 3, we did not observe any influence of this blocker on 15d-PGJ2 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-PGJ2.

Finally, since it was recently suggested that 15d-PGJ2 can induce oxidative stress (Kondo et al. 2001), we investigated the effect of the antioxidant N-acetyl-cysteine (NAC) on 15d-PGJ2 activity. We found that a 20-minute pretreatment of HMEC-1 with 1 mM NAC significantly attenuated the stimulatory effect of 15d-PGJ2 on MMP-1 synthesis (Fig. 3), which implies that the augmentation of metalloproteinase production in response to 15d-PGJ2 is mediated by changes in an oxidative status of HMEC-1.

**15d-PGJ2 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 luciferase 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-PGJ2 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-PGJ2 is not regulated at the transcriptional level.

**15d-PGJ2 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-PGJ2. 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...
for MMP-1 was detectable in control cells (not stimulated with 15d-PGJ2) until 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-PGJ2, 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-PGJ2.

**PPARγ ligands down-regulate the expression of uPA**

We studied also the effect of 15d-PGJ2 on expression of uPA, a matrix-degrading serine protease. Treatment of HMEC-1 with 15d-PGJ2 (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-PGJ2 was, however, much stronger, suggesting that some additional, PPARγ-independent pathway(s) play a role. We also found that AH6809 did not influence 15d-PGJ2 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-PGJ2 (Fig. 5), indicating that pathways underlying the regulation of uPA expression by 15d-PGJ2 are different than in the case of MMP-1.
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-PGJ2 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-PGJ2 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-PGJ2 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-PGJ2 significantly and dose-dependently augments the expression of MMP-1. Concomitantly, the same doses of 15d-PGJ2 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-PGJ2-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-PGJ2 and TZDs we described earlier in studies on the regulation of synthesis of interleukin-8 (IL-8) in HMEC-1. 15d-PGJ2 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-PGJ2, 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-PGJ2 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-PGJ2 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-PGJ2 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-PGJ2 is still an effective modulator of gene expressions (Chawla et al., 2001). Our results add an additional example of a 15d-PGJ2-specific activity not mimicked by TZD.
Studies on activated monocytes (Ardans et al., 2002) or corneal organ cultures (Ottino & Bazan, 2001) revealed that indomethacin, an inhibitor of cyclooxygenase-2 (COX-2), suppresses MMP-1 synthesis. This suggests that prostaglandins can act as inducers of MMP-1. Indeed, prostaglandin-E2 (PGE2) increased MMP-1 expression in monocytes (Ardans et al., 2002) and fibroblasts (Liu et al., 2001), while supplementation with PGE2, prostaglandin-D2 (PGD2), or prostaglandin-F2α (PGF2α) increased MMP-1 levels in the cornea (Ottino & Bazan, 2001). These effects can be, however, cell-type specific, as PGE2 and prostacyclin (PGI2) inhibited production of MMP-1 in human aortic smooth muscle cells (Kato et al., 1993).

Interestingly, it was documented in human fibroblasts that the PGF2α-induced increase in MMP-1 production is mediated by FP-R, a surface receptor for PGF2α (Noguchi et al., 2001). Therefore, although we could not detect the expression of DP-R, a surface receptor binding PGD2 and 15d-PGJ2, we decided to investigate its potential involvement in the upregulation of MMP-1 in human aortic smooth muscle cells (Kato et al., 1993).

Importantly, it was documented in human fibroblasts that the PGF2α-induced increase in MMP-1 production is mediated by FP-R, a surface receptor for PGF2α (Noguchi et al., 2001). Therefore, although we could not detect the expression of DP-R, a surface receptor binding PGD2 and 15d-PGJ2, 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-PGJ2. We found that AH6809 did not influence 15d-PGJ2 activity. Accordingly, the upregulation of MMP-1 in response to 15d-PGJ2 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-PGJ2 action in HMEC-1.

Importantly, the stimulatory effect of 15d-PGJ2 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, O2−•, OH•, and H2O2 (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 H2O2 may be of essence. Several experiments pointed out the importance of elevation of H2O2 in upregulation of MMP-1 production (Brenneisen et al., 1997; Wenk et al., 1999; Ranganathan et al., 2001). One of the sources of intracellular H2O2 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 H2O2 leading to augmented synthesis of MMP-1 in dermal fibroblast (Ranganathan et al., 2001). Interestingly, 15d-PGJ2 increases expression of Cu,Zn-SOD in primary endothelial cells (Inoue et al., 2001). Thus one can suppose that induction of H2O2 generation may be responsible for the observed upregulation of MMP-1 synthesis in HMEC-1, and scavenging of H2O2 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-PGJ2 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-PGJ2 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, H2O2 overproduction or exposure to NO (Vincenti et al., 1996; Wenk et al., 1999; Ishii et al., 2003).

15d-PGJ2 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-PGJ2 (data not shown). Regardless of this inhibitory potential, 15d-PGJ2 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-PGJ2 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-PGJ2 very potently inhibited the synthesis of uPA in HMEC-1. This observation is concordant with the earlier report showing that 15d-PGJ2 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-PGJ2 on uPA expression.

In conclusion, we postulate that in human microvascular endothelial cells 15d-PGJ2 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.

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