Metalloproteinase Expression in PMA-stimulated THP-1 Cells

EFFECTS OF PEROxisome PROLIFERATOR-activated RECEPTOR-γ (PPARγ) AGONISTS AND 9-CIS-Retinoic Acid

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Joanna R. Worley‡‡, Mark D. Baugh††††, David A. Hughes‡, Dylan R. Edwards‡, Aileen Hogan‡, Mike J. Sampson‡, and Jelena Gavrilovic‡‡‡

From the ‡School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ, United Kingdom, the †Bertram Diabetes Research Unit, Norfolk & Norwich University Hospital NHS Trust, Norwich NR4 7UY, United Kingdom, and the ‡Institute of Food Research, Norwich Research Park, Norwich NR4 7UA, United Kingdom

The PPARγ agonists, thiazolidinediones (TZDs), have anti-inflammatory properties as well as increasing insulin sensitivity. This has widened their therapeutic scope to treat inflammatory diseases such as atherosclerosis in addition to Type 2 Diabetes. TZDs are known to reduce monocyte/macrophage expression of Matrix metalloproteinase (MMP)-9, which is implicated in atherosclerotic plaque destabilization. This study aims to identify other metalloproteinase genes of the ADAM (A Disintegrin And Metalloproteinase) and ADAMTS families that are regulated by PPARγ or RXR agonists, which are potentially important in type 2 diabetes and/or related atherosclerosis. The synthetic PPARγ agonist, GW7845, and the natural agonist 15d-PGJ2, suppressed PMA-stimulated MMP-9 in human monocyte-like cells (THP-1) only in the presence of 9-cis-retinoic acid. Quantitative Real-Time PCR showed that this reduction was regulated at the mRNA level. Expression of ADAMs 8, 9, and 17 were increased, and ADAM15 was decreased by stimulation of THP-1 with PMA, although these ADAMs were not regulated by PPARγ or RXR agonists. PMA-induced ADAM28 expression was further enhanced by the addition of 9-cis-retinoic acid. ADAMTS4, implicated in rheumatoid arthritis, was expressed in THP-1 cells, and significantly increased after 24 h of PMA stimulation. ADAMTS4 expression was suppressed by both PPARγ and RXR agonists and was undetectable when the agonists were combined. Pretreatment of THP-1 cells with the PPARγ antagonist, GW9662, suggests that PPARγ plays subtly different roles in the regulation of MMP-9, ADAMTS4 and ADAM28 gene expression. These results indicate that PPARγ and RXR agonists have complex effects on monocyte metalloproteinase expression, which may have implications for therapeutic strategies.

Peroxisome proliferator-activated receptor (PPAR)γ has been identified as the target receptor of the thiazolidinediones (TZD), a group of insulin sensitizing drugs now widely used for treating Type 2 diabetes (1). PPARγ was originally located in human liver, heart, and skeletal muscle (2). Many of the known target genes are involved in fatty acid metabolism, as PPARγ is a key component in regulating adipocyte differentiation, and controlling plasma lipid and sugar levels. When ligand-activated, PPARγ binds the PPAR response element (PPRE) as a dimer with the retinoid X receptor (RXR) (3), and affects the transcription of PPARγ target genes. RXR is also able to form homodimers, and heterodimers with other nuclear receptors such as the retinoic acid receptor (RAR) and liver X receptor (LXR). The endogenous agonist of RXR is 9-cis-retinoic acid, a natural derivative of vitamin A, which is also able to activate the RAR. Natural ligands of PPARγ include polyunsaturated fatty acids and their metabolites, in particular the lipoxigenase products of arachidonic acid and linoleic acid, 15-hydroxyeicosatetraenoic acid and 13-hydroxyoctadecadienoic acid, respectively (4), and 15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ2) (5, 6).

PPARγ has now been detected in other cell types including monocytes and macrophages, and the range of target genes has grown wider. PPARγ may have a role in inflammation and the immune response, in particular the inflammatory diseases rheumatoid arthritis (7) and atherosclerosis, as PPARγ has been detected in lesion foam cells in vivo (8), (9), and the rupture sensitive macrophage-rich shoulder region of the plaque (10). PPARγ is also implicated in the down-regulation of inflammatory cytokines, tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6, in monocytes and macrophages (11). Markers of macrophage activation, such as inducible nitric-oxide synthase and matrix metalloproteinase (MMP) −9 are also suppressed by PPARγ in monocytes (10, 12, 13), and human vascular smooth muscle cells (SMC) (14). These effects of PPARγ activation can all be considered anti-inflammatory. PPARγ activation has also been shown to have the possible pro-inflammatory effect of increasing levels of the type 2 scavenger receptor CD36 (4), which could promote differentiation to macrophage foam cell formation in atherosclerotic plaques (8). However a role for PPARγ in monocyte differentiation has been contested (15), and the PPARγ antagonist GW9662 was unable to reverse 15d-PGJ2 and all-trans-retinoic acid-induced CD36 expression in THP-1 monocytes (16).

During all stages of atherogenesis, monocytes and macrophages secrete proteolytic enzymes, which may influence plaque composition or stability (reviewed by Galis and Khatri, Ref. 17). One such protease, MMP-9 is already known to be metalloproteinase; TNF, tumor necrosis factor; Me2SO, dimethyl sulfoxide.
regulated by PPAR-γ (10, 12). The ADAMs (a disintegrin and metalloproteinase), are also members of the metalloproteinase family, but little is known about their regulation, or role in atherosclerosis, even though some are expressed in the vasculature (reviewed by Herren, Ref. 18). Many of the specific functions of ADAMs are unknown, although they are involved in cell-cell and cell-matrix interactions, and signaling (reviewed by Primakoff and Myles, Ref. 19 and Blobel, Ref. 20). The ADAMTS proteinases are closely related to the ADAMs. They are secreted, and have at least one thrombospondin-type 1 domain, which is thought to associate with proteoglycans.

Several known functions of the ADAMs could be implicated in atherosclerosis, and the vascular complications of Type 2 diabetes. Type 2 diabetes has been associated with increased circulating levels of TNF-α and its receptor, possibly shed by ADAM17 (21). Increased levels of TNF-α is thought to mediate insulin resistance (reviewed by Moller, Ref. 22). ADAM17 is also known as TNF-α-converting enzyme (TACE) as it cleaves soluble TNF-α (23, 24), and p75 TNF receptor (25) from their membrane anchored forms. Thus, insulin resistance is reduced in TNF-α-null obese mice (26), and in diabetic mice treated with a general metalloproteinase inhibitor (27).

In the initial stages of developing atheroma, monocyte recruitment involves adhesion to endothelial cells via selectins, integrins, and cellular adhesion molecules. L-selectin (28) has been shown to be shed by ADAM17 (25), and ADAM8 (29). A proteolytically processed form of ADAM8 also shows adhesion properties to ADAM8-expressing cells (30). Human ADAM28 (or MDC-L) was first identified on lymphoid cells, and also exists in a soluble form (31). The disintegrin domain of ADAM28 is able to bind integrin αβ1, which implies that it may play a role in cell to cell adhesion, or targeting or sequestering its protease activity (32). The receptor for αβ1, vascular cell adhesion molecule-1 (VCAM-1) is expressed on endothelial cells, and is also thought to be a target of MMP-mediated proteolysis (33).

Within the subendothelial space, apolipoprotein B lipoproteins can be trapped by interactions with highly sulfated glycosaminoglycan chains of proteoglycans (reviewed by Williams, Ref. 34). It is of relevance that in addition to degrading aggrecan in articular cartilage, ADAMTS4 (reviewed by Tang, Ref. 35) (also known as aggrecanase-1) is able to degrade the proteoglycan versican (36). The proteoglycan composition of SMC secreted extracellular matrix (ECM), including increased versican expression (37) and reviewed by Camejo et al. Ref. 38), is altered by elevated non-esterified fatty acids, an indicator of Type 2 diabetes. In non-human primates, versican has been localized with macrophages in intermediate and advanced lesions (39), and is increased in human atherosclerotic lesions (40). The expression of versican is increased in proliferating and migratory SMC (41), the phenotype associated with developing atheromas.

ADAM15 has been located in cultured human SMC, endothelial cells and atherosclerotic blood vessels, although not in normal vessels (42), where it may play a role in adhesion and migration. The soluble form of heparin-binding epidermal growth factor-like growth factor (HB-EGF) has been shown to be shed by ADAM9 (43), and is a potent mitogen for SMC (reviewed by Raab and Klagesbrunn, Ref. 44). Interestingly, the mitogenic effect of soluble HB-EGF has been shown to be increased when SMC are cultured in high glucose (45), which could have implications in diabetes-related atherosclerosis.

The anti-inflammatory properties of PPARγ and IRR agonists have lead to their possible use in the treatment of atherosclerosis (46) and arthritis (7). Combined RXX and PPARγ agonists inhibit cell growth (47), and the highly potent PPARγ agonist GW7845 reduced rat mammary carcinogenesis (48), so their therapeutic actions against cancer are also under investigation (49).

PPARγ and RXX ligands have already been shown to have a broad effect on cell physiology, and it is important to understand the full effects that these drugs will have when being used therapeutically. We investigated the effects of the synthetic PPARγ agonist GW7845, and the natural agonist 15d-PGJ2 alone, or in combination with the RXX agonist 9-cis-retinoic acid, on the MMP-9 protein and mRNA levels of porcine athero-stimulated THP-1 cells. We then looked at the effect of these agents on the transcription of other metalloproteinases: the ADAMs and ADAMTS4, and how the PPARγ antagonist GW9662 (50) affects these changes.

MATERIALS AND METHODS

Cell Culture—THP-1 cells were maintained in RPMI 1640 (Invitrogen) supplemented with 5 mM l-glutamine, 100 units/ml penicillin and streptomycin, and 10% fetal calf serum, at 37 °C and 5% CO2. For experiments, cells were cultured in the same media, except 0.2% fetal calf serum was used. GW7845 and 15d-PGJ2 (Calbiochem) were solubilized in Me2SO, and 9-cis-retinoic acid (Sigma) was dissolved in ethanol. Cells were incubated alone, with the agonists or with the vehicle controls at various concentrations for 1 h prior to the addition of 5 ng/ml PMA. In some experiments cells were pretreated with the antagonist GW9662 (5 µM) for 30 min before the agonists were added.

Gelatin Zymography—Media samples were prepared 24 h after addition of PMA to cells. MMP-9 gelatinolytic activity was assessed by gelatin zymography. Samples were prepared in non-reducing sample buffer (0.625 mM Tris-HCl, 10% glycerol, 2% SDS, 2% bromophenol blue), and run through 8% SDS-PAGE gels containing 0.5 mg/ml gelatin type A from porcine skin (Sigma). Gels were washed in 2.5% Triton X-100 solution, before incubation in 100 mM Tris-HCl pH 7.9, 30 mM CaCl2, and 0.02% sodium azide for 1.5 h at 37 °C. Gels were stained with 0.25% (w/v) Coomassie Brilliant Blue, 50% (v/v) methanol, and 20% (v/v) acetic acid and destained with 30% (v/v) methanol and 1% (v/v) acetic acid. Bands of enzyme activity appear as clear bands on a blue background.

RNA Isolation and Taqman Analysis—After PMA addition, cells were incubated for 6, 9, or 24 h before media was removed and the cells washed with phosphate-buffered saline. Total RNA was isolated using the SV Total RNA isolation System (Promega) according to the manufacturer’s instructions. RNA (1 µg) was reverse-transcribed in a 20-µl reaction using SuperScript™ II reverse transcriptase (Invitrogen) according to the manufacturers instructions. Samples were analyzed by Taqman real-time PCR (PerkinElmer) as described previously (51) using the probes and primers shown in Table I.

Statistical Analysis—Data were analyzed by analysis of variance using SigmaStat™. Comparisons of group means were performed by Tukey test. Data are shown as mean ± S.E. A value of p < 0.05 was considered statistically significant.

RESULTS

GW7845 and 9-cis-Retinoic Acid Additively Suppress MMP-9 Activity—THP-1 cells were treated with increasing concentrations of the endogenous PPARγ agonist 15d-PGJ2 and the synthetic agonist GW7845 alone, or with increasing concentrations of the RXX agonist, 9-cis-retinoic acid for 1 h before the cells were stimulated with PMA. After 24 h, the gelatinase activity of secreted MMP-9 was assessed by gelatin zymography (Fig. 1). In the absence of PMA, MMP-9 is undetectable using the zymogram conditions described in materials and methods (data not shown). GW7845 and 15d-PGJ2 caused a dose-dependent decrease in PMA induced MMP-9 gelatinolytic activity, however, this was much more pronounced with the GW7845. THP-1 cells stimulated with PMA (Control), or PMA and Me3SO (vehicle control) and treated with 9-cis-retinoic acid also produced a slight decrease in MMP-9 activity at the highest concentration used (50 nM). Combined GW7845 and 9-cis-retinoic acid caused an additive decrease in MMP-9 expression, but the effect of 9-cis-retinoic acid on 15d-PGJ2-treated cells was much less pronounced. The cells remained viable through-
out the experiment, as determined by the MTT assay (52) (data not shown).

Differential Regulation of MMP-9, MMP-1, and TIMP-1 Gene Expression by GW7845 and 9-cis-Retinoic—To determine whether the decreases in secreted MMP-9 activity were regulated at the level of transcription, Taqman real-time PCR was used to measure the relative quantities of MMP-9 mRNA in THP-1 cells treated for 6 and 9 h (Fig. 2, top panel). PMA induced a 5- and 35-fold induction of mRNA over 6 and 9 h, respectively. Cells treated with 5 nM 9-cis-retinoic acid or 5 μM GW7845 suppressed PMA induced MMP-9 mRNA expression by 3-fold after 9 h. When added in combination expression was reduced 8-fold. Similar changes were observed after 6 h of treatment, but were less pronounced. 15d-PGJ2 (1 μM) did not suppress PMA-induced MMP-9 mRNA significantly, and was only reduced 2-fold when added in combination with 9-cis-retinoic acid.

TIMP-1 mRNA was induced less than 3-fold by PMA treatment over 9 h. Neither PPARγ agonists nor 9-cis-retinoic acid altered TIMP-1 mRNA levels significantly at these time points. MMP-1 mRNA was almost undetectable in untreated THP-1 cells, but this was induced by PMA stimulation. There was a trend for levels of MMP-1 mRNA to be reduced when the cells were treated with 9-cis-retinoic acid; however, these changes were not statistically significant.

Differential Regulation of ADAM8, -9, -15, -17, -28, and ADAMTS4 by PMA, GW7845 and 9-cis-Retinoic Acid—We also looked to see whether PPARγ agonists or RXR agonist treatment affect TIMP-1 mRNA levels significantly at these time points. MMP-1 mRNA was almost undetectable in untreated THP-1 cells, but this was induced by PMA stimulation. There was a trend for levels of MMP-1 mRNA to be reduced when the cells were treated with 9-cis-retinoic acid; however, these changes were not statistically significant.
and ADAMTS4 after 9 h. Neither of the PPARγ agonists or 9-cis-retinoic acid produced significant changes in mRNA levels of any of the ADAMs tested or ADAMTS4 after 6 or 9 h (data not shown). Interestingly, after 24 h of treatment, expression of ADAM8, -9, -15, and -17 mRNA were not altered by PMA any further (data not shown). However, at this time point ADAM28 and ADAMTS4 were induced by PMA 5- and 16-fold, respectively (Fig. 4). In addition, ADAM28 gene expression was induced >2-fold by addition of 9-cis-retinoic acid, alone or in combination with GW7845 and 15d-PGJ2. PMA-induced expression of ADAMTS4 was suppressed 5-fold by 9-cis-retinoic acid, 14-fold by GW7845, and was undetectable when added in combination. 15d-PGJ2 induced a statistically significant 1.5-fold decrease in ADAMTS4 mRNA expression, which was further suppressed (10-fold) by the addition 9-cis-retinoic acid.

Fig. 2. PPARγ agonist and 9-cis-retinoic acid in combination affect the transcription of MMP-9 mRNA, but not TIMP-1 or MMP-1 mRNA in THP-1 cells stimulated with PMA. THP-1 cells (0.83 million/ml) were treated with 5 nM 9-cis-retinoic acid (RA), 5 μM GW88945, GW9662, or 1 μM 15d-PGJ2 alone or in combination with 5 nM RA. Cells were also left untreated, or with 0.025% Me2SO or 0.0005% ethanol (EtOH) as vehicle controls. After 1 h, 5 ng/ml PMA was added. 6 and 9 h after PMA addition, media was removed and cells lysed for RNA isolation. Relative quantities of mRNA for MMP-9, TIMP-1, and MMP-1 were assessed, after reverse transcription, by Taqman real-time PCR (see “Materials and Methods” for details). Graphs show the mean and S.E. of triplicate samples taken at 6 h (black bars) and 9 h (striped bars), *, p < 0.05 compared with PMA treated and vehicle control groups.

Fig. 3. PMA stimulation of THP-1 cells differentially regulates the mRNA expression of ADAMs. The same RNA samples as Fig. 2 were used to assess relative quantities ADAM-8, -9, -15, -17, -28, and ADAMTS-4 mRNA by Taqman real-time PCR after treatment with 5 ng/ml PMA for 0 h (black bars), 6 h (striped bars), and 9 h (black with white spots), *, p < 0.05 compared untreated controls.

Effects of PPARγ Antagonist, GW9662, on GW7845 and 9-cis-Retinoic Acid-treated PMA-stimulated MMP-9, ADAMTS4, and ADAM28 Expression—Suppression of PMA-induced MMP-9 mRNA by GW7845 or 9-cis-retinoic acid alone was not affected by pretreatment with the PPARγ antagonist GW9662. However, the additional effect of 10 nM GW7845 combined with 9-cis-retinoic acid was suppressed by the addition of GW9662. This was also reflected in the secreted MMP-9 activity assessed by gelatin zymography 24 h after PMA stimulation (Fig. 5, inset).
agonist GW7845 was also added. The effects of GW9662 were not observed when the cells were treated 1 \( \mu M \) GW7845, which suggests that PPAR\( \gamma \) is saturated by the agonist.

**DISCUSSION**

We have shown that PMA-stimulated THP-1 monocytic cells show altered MMP, ADAM, and ADAMTS4 mRNA expression, which can be differentially regulated by the addition of PPAR\( \gamma \) and RXR agonists. This has important implications for the use of PPAR\( \gamma \) and RXR agonists in the treatment of disease, especially Type 2 diabetes and atherosclerosis.

PPAR\( \gamma \) agonists have also been shown to suppress the inflammatory cytokines TNF-\( \alpha \), IL-1\( \beta \), and IL-6 (11), and inducible nitric-oxide synthase (53) in monocytes and macrophages as well as MMP-9 (13, 10, 12, 15, 53). It was shown by transfection of transcription reporter constructs into HeLa cells (which express low levels of PPAR\( \gamma \)), that PPAR\( \gamma \) agonist repression of AP-1, NF\( \kappa \)B, and STAT minimal promoters was dependent on co-transfection of PPAR\( \gamma \). This has led to the theory that AP-1-, NF\( \kappa \)B-, or STAT-induced gene expression can be inhibited by a mechanism called transrepression, where the active PPAR\( \gamma \)-RXR heterodimer binds these transcription factors and antagonizes their transcription activation activity (53). More recently it has been reported that synthetic PPAR\( \gamma \) agonists are unable to inhibit TNF-\( \alpha \), IL-6, and IL-8 expression when administered at concentrations appropriate to their binding affinity to PPAR\( \gamma \) (12, 54, 55). Also, the potent TZD, AD-5075 was unable to lower serum levels of induced TNF-\( \alpha \) and IL-6 in LPS-challenged db/db mice (54). However, down-regulation of MMP-9 by synthetic and natural PPAR\( \gamma \) agonists has remained a consistent observation. The actual mechanism of MMP-9 down-regulation has not yet been studied directly, although it is thought to be through transrepression of AP-1 and/or NF\( \kappa \)B sites in the MMP-9 promoter (53). No PPRE has been located in the human MMP-9 promoter yet, although two possible PPRE were recently identified in 1.8 kb of the rat MMP-9 promoter (56).

Throughout this study the effects of 15d-PGJ\(_2\) were never as pronounced as the specific PPAR\( \gamma \) agonist GW7845, this could reflect its lower binding affinity to PPAR\( \gamma \) and its instability in cellular systems. Also, previous studies have shown that cellular effects of 15d-PGJ\(_2\) cannot be mimicked by synthetic PPAR\( \gamma \) agonists (54, 55, 57, 58), which suggests that 15d-PGJ\(_2\) can act through PPAR\( \gamma \)-independent pathways.

We have shown that after 9 h, MMP-9 activity and mRNA levels are reduced by both the PPAR\( \gamma \) agonist GW7845 and 9-cis-retinoic acid by >22-fold when added together. Interestingly, the combined effect of PPAR\( \gamma \) and RXR agonists on endogenous MMP-9 expression has also been investigated during the differentiation of primary monocytes, where a 2-fold reduction in MMP-9 message was observed after 1 and 4 days (15). Matrix degradation depends on a balance of active MMPs and the presence of their inhibitors. We investigated the ex-
pression of MMP-1, which has also been located in atherosclerotic plaques, and TIMP-1, a natural inhibitor of many MMPs, including MMP-1 and 9-cis-retinoic acid. Neither PPARγ agonists or 9-cis-retinoic acid affected TIMP-1 mRNA levels in this study. However, the RXR ligand AGN4204 has been shown to increase TIMP-1 protein levels in PMA stimulated THP-1 media and lysates (13). This disparity could reflect differences in the amount of PMA used to stimulated cells (100 nM compared with 8 nM in this study), or in the RXR agonists used.

In the current study, 9-cis-retinoic, but not the PPARγ agonists, had a tendency to lower MMP-1 expression in THP-1 cells, although these changes were not statistically significant. These data are in agreement with the observation that MMP-1 is suppressed by retinoic acid in a rabbit fibroblast cell line (59).

**Regulation of ADAM Family Gene Expression by PMA, PPARγ, and 9-cis-Retinoic Acid**—The transcriptional regulation of MMP genes stimulated by PMA is very complex, and is dominated by the AP-1 site present in most MMP promoters, and its interaction with other cis-acting elements (60). Our data suggest equal complexity in PMA-stimulated ADAM expression, with genes being up-regulated or down-regulated, and affected at different time points.

We have shown clearly the induction of ADAM-9, -9, and -17 and the down-regulation of ADAM15 mRNA in PMA stimulated THP-1 monocytes at 6 and 9 h. This is well established that PMA rapidly induces ADAM-mediated ectodomain cleavage of several membrane-associated growth factor receptors and cytokines (20, 61). Our data suggest that PMA additionally regulates ADAMs at the level of RNA.

ADAM28 and ADAMTS4 mRNA were not induced by PMA in THP-1 cells after 9 h, however, both were significantly induced after 24 h. The time scale of these observations suggests the genes are not induced directly by PMA, but by a second signaling event. A potential candidate, IL-1β, is known to induce ADAMTS4 in human and bovine chondrocytes (62, 63). IL-1β expression can be induced by PMA after 6 h in human monocytes, which is further enhanced by the addition of retinoic acid (64). In the current study, PMA induced ADAM28 mRNA expression was further enhanced by 9-cis-retinoic acid. Neither PPARγ agonist altered expression, which would suggest that 9-cis-retinoic acid is acting independently to PPARγ, possibly as a RXR homodimer, or as a heterodimer with a partner other than PPARγ. However, the effect of the PPARγ antagonist in suppressing 9-cis-retinoic acid-induced ADAM28 expression suggests that PPARγ does have a role. Modulation of ADAM28 expression in monocytes could affect their ability to bind to the endothelium, and subsequent migration into the subendothelial space. This could be due to the ability of ADAM28 to interact with integrins, such as αvβ3 (32), on the same cell or neighboring leukocytes. Migration of monocytes may also be modified by the proteolytic action of ADAM28, whose substrates are as yet unidentified, but are likely to include cell surface proteins such as intercellular adhesion molecule-1, VCAM-1, and L-selectin.

In contrast to the enhanced expression of ADAM28, PMA-induced ADAMTS4 expression was suppressed by the PPARγ agonist GW7845 and 9-cis-retinoic acid, both individually and combined. It is possible that ADAMTS4 transcription was inhibited by active PPARγ/RXR dimer antagonizing IL-1β-activated AP-1 or NF-xB. IL-1β-induced ADAMTS4 expression and activity have previously been shown to be suppressed by n-3 fatty acids in human osteoarthritic cartilage explants (62), and n-3 fatty acids can reduce the symptoms of arthritis (reviewed by Calder, Ref. 65). This supports our observation as polyunsaturated fatty acid metabolites are also ligands for PPARγ (4).

Also, PPARγ agonists are able to reduce IL-1β induced rat cartilage degradation, mediated by aggrecanase (66). In early stages of atherosclerosis, altered expression of ADAMTS4 by PPARγ agonists, such as TZD treatment, could alter the properties of the intima ECM by suppressing the ability to breakdown the proteoglycan versican, and in turn alter lipoprotein retention. Whether this affect would be beneficial needs further investigation. Versican degradation has been implicated in decreasing plaque stability in later stages atherosclerosis. It has been suggested that MMP-7 might be responsible for the breakdown versican (67), however the involvement of ADAMTS4 has not been investigated. In this case, suppression of ADAMTS4 should be beneficial in maintaining plaque stability.

The subtle differences in effects of the antagonist, GW9662 on MMP-9 and ADAMTS4 suggests that these genes are being regulated by different mechanisms. GW9662 moderately reversed suppression of MMP-9 mRNA expression only when GW7845 was combined with 9-cis-retinoic acid whereas GW9662 was able to reverse the effect of GW7845 on ADAMTS4 expression alone or in combination with 9-cis-retinoic acid. Similarly to MMP-9, GW9662 did not reverse the effect of 9-cis-retinoic acid treatment, which differs from its effect on ADAM28. Concentrations >1 μM of the PPARγ agonist GW7845 have been shown to have maximal effects on adipocyte differentiation (48). Our data also suggest that PPARγ is saturated with agonist at this concentration, as 5-fold excess of the antagonist had a much reduced effect in altering the expression of all three genes examined.

The functional consequences of the observed changes in metalloproteinase mRNA expression during PMA stimulated monocyte differentiation and PPARγ and/or RXR agonist treatment require further investigation. Future studies will uncover whether similar changes are observed at the protein level for ADAMTS4 and ADAM28. Possible functional roles of the ADAMs investigated in this study of cytokine regulation, cell to cell contact, and adhesion and migration, are all relevant to the behavior of infiltrating monocytes and the surrounding cells in atherosclerotic plaques.

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