Inhibition of MMP-9 expression by PPARγ activators in human bronchial epithelial cells

M Hetzel, D Walcher, M Grüb, H Bach, V Hombach, N Marx

AIRWAY BIOLOGY

Background: The release of matrix degrading enzymes such as matrix metalloproteinase 9 (MMP-9) from bronchial epithelial cells is critically involved in airway wall remodelling in chronic inflammatory processes of the respiratory system. MMP-9 expression is induced by inflammatory mediators such as tumour necrosis factor (TNF)-α, but to date nothing is known about the mechanisms of inhibition of MMP-9 expression in these cells.

Methods: A study was undertaken to examine whether activators of the nuclear transcription factor peroxisome proliferator activated receptor gamma (PPARγ) might modulate MMP-9 expression in two different bronchial epithelial cell lines.

Results: PPARγ was expressed and was functionally active in NL20 and BEAS cells. Activation of PPARγ by rosiglitazone or pioglitazone significantly reduced TNF-α and PMA induced MMP-9 gelatinolytic activity in a concentration dependent manner in both cell lines, but did not alter the expression of tissue inhibitor of MMPs type 1 (TIMP-1), the local inhibitor of MMP-9. Northern blot analysis revealed a decrease in MMP-9 mRNA expression following treatment with PPARγ which resulted from the inhibition of NF-κB activation in these cells, as determined by transient transfection assays and electromobility shift assays.

Conclusion: Activation of PPARγ in human bronchial epithelial cells limits the expression of matrix degrading MMP-9. This might have therapeutic applications in chronic inflammatory processes of the respiratory system.

Chronic inflammatory processes in the respiratory system such as bronchial asthma are characterised by airway wall remodelling with degradation and synthesis of interstitial matrix proteins and migration of bronchial epithelial cells.1 2 Degradation of collagen type IV is a critical step in the inflammatory disorganisation of the airway wall, and is mainly determined by the balance between matrix degrading matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of MMPs (TIMPs). Among them, gelatinase A (MMP-2) and gelatinase B (MMP-9), as well as TIMP-1, released from bronchial epithelial cells seem to be crucially involved in the pathogenesis of bronchial asthma.3 MMP-9 is increased in bronchoalveolar lavage fluid4 and bronchial tissue in patients with bronchial asthma,5 while the expression of TIMP-1 is increased to a lesser extent, thus shifting the balance towards matrix degradation in the airway wall. Various proinflammatory mediators such as tumour necrosis factor (TNF) and platelet activating factor (PAF) released from inflammatory cells in the airway system are potent inducers of MMP-9 but not MMP-2 expression.6 Recent work has shown that TNF-α induced MMP-9 expression in bronchial epithelial cells is regulated by activation of the proinflammatory transcription factor NF-κB.7 Since matrix degradation by MMPs is considered to be a major contributor to airway remodelling in bronchial asthma, the inhibition of MMP expression might preserve the histological airway structure in chronic inflammatory processes. To date, however, little is known about such counterbalancing mechanisms.

Peroxisome proliferator activated receptor gamma (PPARγ) is a nuclear transcription factor originally described as a major regulator in glucose homeostasis and adipogenesis,8 but recent work has shown that PPARγ activation might exhibit anti-inflammatory properties in different chronic inflammatory processes.9 10 PPARγ belongs to the group of nuclear hormone receptors consisting of a ligand and DNA binding domain which, upon activation by their respective ligands, bind to specific PPAR response elements (PPREs) in the promoter of their target genes, thus regulating their expression.11 PPARγ can be activated by naturally occurring ligands such as the prostaglandin D₂ derivative 15-deoxy-D₁₂,14PGJ₂,12 as well as by a group of new antidiabetic agents—thiazolidinediones (TZDs or glitazones) such as rosiglitazone or pioglitazone.13 PPARγ induces the expression of genes such as lipoprotein lipase and Glut4 and has been shown to limit the expression of proinflammatory mediators such as cytokines and chemokines. Some of these effects were mediated by an interaction with proinflammatory transcription factors like AP-1 or NF-κB.13 14 15 In addition, PPARγ has been shown to inhibit the expression and activity of MMP-9 in monocyte/macrophages and vascular smooth muscle cells through an as yet undefined mechanism.16 17 Previous work has demonstrated PPARγ expression in bronchial epithelial cells in situ and suggested an antiproliferative effect in these cells.15 However, to date nothing is known about the role of PPARγ in the regulation of MMP expression in bronchial epithelial cells.

We hypothesised that PPARγ is functionally active in human bronchial epithelial cells and that PPARγ activators might modulate the expression of MMP-9 in these cells.

METHODS

Cell culture

Normal human bronchial epithelial cells (cell line NL20) were obtained from ATCC and the human bronchial epithelial cell line BEAS-2B was provided by Berthold Fischer, Department of Internal Medicine II, University of Mainz. Both cell lines were established by transfection with the origin of replication defective SV40 large T plasmid. NL20 cells were cultured in Ham’s F12 medium with supplements according to the manufacturer’s protocol. BEAS-2B cells were cultured in
PPARγ activators inhibit MMP-9 expression

**Figure 1** Expression of functionally active PPARγ by human bronchial epithelial cells. (A) RT-PCR of PPARγ RNA in cells of the NL20 (NL) and the BEAS cell line (BE) showing a cDNA fragment of the expected size. Also shown are a DNA ladder (MW), RT-PCR product from endothelial cells as a positive control (EC), and a negative control consisting of RT-PCR reactions lacking reverse transcriptase (Co). (B) and (C) Representative transient transfection assays in (A) BEAS or (B) NL20 cells using a promoter-reporter construct containing three copies of a PPAR response element (PPRE). Transfected cells were stimulated with different concentrations of rosiglitazone for 24 hours before luciferase activity was measured in cell lysates. Data are normalised to the activity of a cotransfected β-galactosidase reporter construct. Three independent experiments yielded similar results.

RPMMi supplemented with penicillin, streptomycin, and 10% fetal calf serum. Primary human bronchial epithelial cells were obtained from BioWhittaker and cultured according to the manufacturer’s protocol.

**RT-PCR**
Total RNA from NL20 and BEAS cells was isolated for RT-PCR with amplification of PPARγ and GAPDH cDNA as described previously.17

**Figure 2** Inhibition of TNF-α induced MMP-9 gelatinolytic activity by PPARγ activators in human bronchial epithelial cells. (A) NL20 (left panel) and BEAS cells (right panel) were stimulated with TNF-α (10 ng/ml) in the absence or presence of the PPARγ activators pioglitazone and rosiglitazone. After 12 hours, cell-free supernatants were subjected to gelatin zymography. (B) Densitometric analysis of MMP-9 gelatinolytic activity in conditioned media from NL20 (left panel; n=5) or BEAS cells (right panel; n=3). The results are expressed as the percentage of TNF-α activated cells (% control). Bars represent mean (SE), *p<0.05.
Gelatin zymography and ELISA

To examine the effect of PPARγ activators on MMP-9 gelatinolytic activity, cells were treated with TNF-α (10 ng/ml) or PMA (10 ng/ml) in the absence or presence of two different PPARγ activators—rosiglitazone (GlaxoSmithKline) and pioglitazone (Takeda)—in serum-free media for 12 hours. Culture supernatants were mixed in SDS-PAGE loading buffer (lacking reducing agents), applied to 10% SDS-polyacrylamide gels containing 1 mg/ml gelatin (Bio-Rad), and separated by electrophoresis. Subsequently, SDS was removed from the gels by two washes (15 minutes) with 2.5% Triton X-100 (VWR Scientific). After the washes, gels were incubated overnight (37°C) in zymography buffer (50 mmol/l Tris, pH 7.3, 10 mmol/l CaCl2, and 0.05% Brij 35 (Sigma)) and stained with Coomassie brilliant blue (Sigma). Gelatinolytic activity was visualised as clear zones of lysis against a dark background. In some experiments cells were treated with TNF-α and PPARγ activators for 12 hours, then cultured again in regular media for 12 hours before additional stimulation with TNF-α or TNF-α and PPARγ activators for 12 hours.

Northern blot analysis

Total RNA (5 µg) was used for standard northern blotting.

Transient transfection assays

To examine whether PPARγ is functionally active in the cells used, NL20 and BEAS cells were transiently transfected with...
To assess the presence of functional endogenous PPARγ activity, we transiently transfected NL20 and BEAS cells with a PPARD-luciferase construct and stimulated cells with increasing amounts of the PPARγ activator rosiglitazone. Luciferase activity was assayed and normalised to β-galactosidase activity of a cotransfected pCMV-B GAL construct. Stimulation with rosiglitazone increased normalised luciferase activity in a concentration dependent manner in both cell lines (fig 1B). These results suggest the presence of inducible PPARD activity in these cell types.

Inhibition of TNF-α or PMA induced MMP-9 gelatinolytic activity by PPARD activators in human bronchial epithelial cells

Given the presence of functionally active PPARD in bronchial epithelial cells, we examined the effect of PPARD activators on MMP activity in these cells. Conditioned media harvested from unstimulated NL20 or BEAS cells exhibited little gelatinolytic activity as determined by substrate zymography. As expected, stimulation with TNF-α significantly induced MMP-9 activity in the supernatant but had no effect on MMP-2 (fig 2A). Incubation of cells with rosiglitazone or pioglitazone significantly reduced this increase in a concentration dependent manner with a maximal reduction to 60 (7)% with 10 μM pioglitazone or to 49 (8)% with 10 μM rosiglitazone (p<0.05 compared with TNF-α-stimulated cells, n=5) in NL20 cells, and to 60 (8)% with 10 μM pioglitazone or to 45 (9)% with 10 μM rosiglitazone (p<0.05 compared with TNF-α-stimulated cells, n=3) in BEAS cells. None of the PPARD activators significantly altered MMP-2 activity (fig 2A and B).

Sequential stimulation of cells with PPARD activators revealed that cells do not become refractory to PPARD stimulation in inhibiting TNF-α induced MMP-9 activity (fig 3D).

Effect of PPARD activators on TIMP-1 expression in human bronchial epithelial cells

Since the effects of PPARD activation on MMP expression did not differ in the two cell lines, further experiments were performed only in BEAS cells. To examine whether PPARD activators shift the balance of matrix degrading enzymes and their inhibitors towards matrix stabilisation, we measured the release of TIMP-1 (the local inhibitor of MMP-9) after stimulation with PPARD activators. As shown in fig 4, neither rosiglitazone nor pioglitazone had a significant effect on TIMP-1 protein content in the supernatants from BEAS cells.
Effect of PPARγ activators on MMP-9 mRNA expression in human bronchial epithelial cells

To investigate whether the decrease in MMP-9 activity by PPARγ activators resulted from reduced mRNA expression, northern blot analysis was performed after 6 hour stimulation. Both rosiglitazone and pioglitazone markedly reduced the TNF-α induced MMP-9 mRNA content in a concentration dependent manner, but did not affect mRNA levels of the constitutively expressed gene GAPDH (fig 5).

Effect of PPARγ activators on NF-κB activation in human bronchial epithelial cells

Previous work has shown that TNF-α induced MMP-9 expression in human bronchial epithelial cells is regulated by activation of NF-κB. To assess the effect of PPARγ activation on NF-κB, transient transfection experiments of a reporter construct containing three copies of the MHC class I NF-κB site were used. Stimulation of transfected cells with TNF-α (10 ng/ml) induced a 3.6 (0.6) fold increase in normalised luciferase activity which was reduced to 2.1 (0.2) fold by rosiglitazone and to 2.5 (0.4) fold by pioglitazone (fig 6A). To further investigate whether PPARγ activators inhibit binding of NF-κB transcription factors to the respective NF-κB DNA binding site, we performed electromobility shift assays using oligonucleotides corresponding to the MHC1 NF-κB site. As shown in fig 6B, both rosiglitazone and pioglitazone inhibited TNF-α induced binding of the NF-κB proteins to DNA.

DISCUSSION

This study demonstrates the expression and functional activity of PPARγ in two different human bronchial epithelial cell lines and shows that PPARγ activators inhibit the expression of MMP-9 in these cells through an interaction of PPARγ with the MMP-9 inducing transcription factor NF-κB.

PPARγ expression was originally described in adipocytes and hepatocytes and recent work has demonstrated its presence in inflammatory cells, endothelial cells, and various cells in the intestine. Immunohistochemical staining of normal bronchial tissue revealed PPARγ immunoreactivity in epithelial cells, and in vitro experiments suggested the expression of this receptor in lung cancer cells and NIH A549 cells. Our study extends the knowledge about PPARγ in the respiratory system by showing PPARγ expression in two different cell lines of human normal bronchial epithelial cells. In addition, the study shows that PPARγ activators are capable of activating endogenous PPARγ in these cells by increasing the activity of a transfected PPRE-luciferase construct.

Stimulation of bronchial epithelial cells with two different PPARγ activators significantly attenuated cytokine induced gelatinolytic activity of MMP-9 but not of MMP-2, another matrix degrading enzyme which is also thought to be implicated in inflammatory airway processes. The ligands employed—rosiglitazone and pioglitazone—are most probably exhibiting their effects through the activation of PPARγ given that these agents have high binding activities for PPARγ. However, recent work has suggested that thiazolidinediones might exhibit PPARγ independent effects such as regulation of cell cycle processes in cells of the monocytic lineage. We cannot therefore exclude the possibility that similar mechanisms might be involved in the effects investigated in this study.

The action of PPARγ activators on MMP-9 expression is independent of the inducing stimulus since these agents inhibited both TNF-α and PMA induced MMP-9 release, suggesting a direct effect on the expression of this gene. Transient transfection assays and gel shift analyses suggest that PPARγ exhibits its inhibitory effects on MMP-9 expression by limiting TNF-α induced NF-κB activation. In addition, PPARγ seems to limit binding of the NF-κB proteins to the respective DNA binding site. Previous work has shown inhibition of NF-κB activation by PPARγ activators in various cell types such as endothelial cells and monocyte/macrophages. However, the underlying molecular mechanism of this interaction still needs to be elucidated.
Taken together, these data suggest a novel mechanism to counterbalance the release of matrix degrading MMP-9 from bronchial epithelial cells. PPARγ activation might therefore be useful for preserving the histological airway structure in chronic inflammatory processes, but in vivo experiments and clinical data are needed to determine the biological relevance of MMP-9 inhibition mediated by PPARγ activators.

ACKNOWLEDGEMENT
This work was supported by grants from the Deutsche Forschungsgemeinschaft (MA 2047/2-2, SFB 451) and the Landesschwerpunkt Baden-Württemberg “Die NF-κB-Signalkaskade als Ziel selektiver Interventionsmaßnahmen bei entzündlichen und malignen Krankheitsgeschehen” to Dr Nikolaus Marx.

Authors’ affiliations
M Hetzel, D Walcher, M Grüb, H Bach, V Hombach, N Marx,
Department of Internal Medicine II, University of Ulm, Germany

REFERENCES
Inhibition of MMP-9 expression by PPARγ activators in human bronchial epithelial cells

M Hetzel, D Walcher, M Grüb, H Bach, V Hombach and N Marx

Thorax 2003 58: 778-783
doi: 10.1136/thorax.58.9.778

Updated information and services can be found at:
http://thorax.bmj.com/content/58/9/778

These include:

References
This article cites 25 articles, 8 of which you can access for free at:
http://thorax.bmj.com/content/58/9/778#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Airway biology (1100)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/