Review Article

PPARγ Ligands Regulate Noncontractile and Contractile Functions of Airway Smooth Muscle: Implications for Asthma Therapy

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Received 6 February 2012; Accepted 12 June 2012

Academic Editor: Virender K. Rehan

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In asthma, the increase in airway smooth muscle (ASM) can contribute to inflammation, airway wall remodeling and airway hyperresponsiveness (AHR). Targetting peroxisome proliferator-activated receptor γ (PPARγ), a receptor upregulated in ASM in asthmatic airways, may provide a novel approach to regulate these contributions. This review summarises experimental evidence that PPARγ ligands, such as rosiglitazone (RGZ) and pioglitazone (PGZ), inhibit proliferation and inflammatory cytokine production from ASM in vitro. In addition, inhaled administration of these ligands reduces inflammatory cell infiltration and airway remodelling in mouse models of allergen-induced airways disease. PPARγ ligands can also regulate ASM contractility, with acute treatment eliciting relaxation of mouse trachea in vitro through a PPARγ-independent mechanism. Chronic treatment can protect against the loss of bronchodilator sensitivity to β2-adrenoceptor agonists and inhibit the development of AHR associated with exposure to nicotine in utero or following allergen challenge. Of particular interest, a small clinical trial has shown that oral RGZ treatment improves lung function in smokers with asthma, a group that is generally unresponsive to conventional steroid treatment. These combined findings support further investigation of the potential for PPARγ agonists to target the noncontractile and contractile functions of ASM to improve outcomes for patients with poorly controlled asthma.

1. Introduction

Asthma is a chronic inflammatory lung disease affecting over 300 million people worldwide, with 250,000 deaths per year attributed to the disease [1]. Asthma is characterized by inflammation, airway wall remodeling, and airway hyperresponsiveness (AHR), whereby airways are more sensitive to a variety of stimuli and subsequently contract too easily and too much [2].

A major feature of airway remodeling in asthma is an increase in airway smooth muscle (ASM) mass. This thickened ASM layer can act as both a source and target of inflammatory cytokines and extracellular matrix (ECM) proteins, contributing to persistent inflammation and increased airway narrowing. Proliferative, synthetic, and contractile functions of ASM can therefore play distinct roles in both the pathogenesis of asthma and perpetuation of disease symptoms (Figure 1) [3, 4].

In current asthma therapy, inhaled β2-adrenoceptor agonists are used to reverse ASM contraction while the frequency and severity of asthma attacks can be reduced by combined therapy with β2-adrenoceptor agonists and glucocorticoids (GCS). However, a significant proportion of patients have poorly controlled symptoms, with variable responses to β2-adrenoceptor agonists and persistent AHR despite optimal anti-inflammatory treatment. Cigarette smoking in asthma patients also contributes to increased severity of symptoms, with an impaired response to both inhaled and oral corticosteroids [5].

This resistance to therapy is likely to be associated with significant structural changes to the airways, including ASM accumulation, fibrosis, and increased vascularity. These changes have been mechanistically associated with disease severity and accelerated lung function decline [6] but may be difficult to reverse in established asthma. In this context
2

**Figure 1:** Potential targets for the regulation of noncontractile (proliferative and synthetic) and contractile functions of airway smooth muscle contributing to airway hyperresponsiveness.

Therefore, it is crucial to identify alternative drugs that inhibit the development of AHR, as well as the contribution of ASM to inflammation and remodeling to limit contraction or directly enhance the relaxation of the increased ASM in the airways [7, 8].

A potential novel approach to regulate ASM function in asthma is to target peroxisome proliferator-activated receptor γ (PPARγ). It has been suggested that downregulation of PPARγ signaling may be a contributing factor to the development of AHR in asthma following in utero nicotine exposure [9], while the expression of PPARγ in ASM is upregulated in the airways of asthmatic patients [10].

This paper provides a brief overview of PPARγ pharmacology and describes the contribution of ASM to inflammation, remodeling, and hyperresponsiveness in asthma. Its major focus is to outline the increasing experimental and clinical evidence that PPARγ ligands can regulate ASM function, through both PPARγ-dependent and PPARγ-independent mechanisms.

### 2. PPARγ Structure and Ligands

PPARγ is a member of the nuclear hormone receptor (NHR) family of ligand-activated transcription factors, which also includes glucocorticoid receptors (GRs) and thyroid hormone receptors. PPARγ is one of three PPAR isoforms designated PPARα (NR1C1), PPARβ (PPARδ, NR1C2), and PPARγ (NR1C3).

Like other NHR, PPARγ possesses a multidomain structure. This includes a DNA binding domain (DBD) containing two zinc finger motifs that recognise specific PPAR response elements (PPREs) sequences. These PPREs consist of hexameric direct repeats of AGGTCA recognition sequences separated by one or two random nucleotide. The DBD is linked via a hinge region to the large ligand binding domain (LBD) that occupies the C-terminal half of the receptor and an activator function (AF)-1 domain is present at the N-terminus [11–13].

PPARγ possesses an unusually large T-shaped ligand-binding pocket that enables interaction with a structurally diverse library of ligands [13]. The most widely studied synthetic agonists are the thiazolidinedione class of drugs, rosiglitazone (RGZ, BRL 49653), pioglitazone (PGZ), troglitazone (TGZ), and ciglitazone (CGZ). RGZ binds the receptor with high affinity (Kd 43 nM), whereas PGZ and CGZ are less potent [14]. Alternative nonglitzone agonists include GW262570 [15] and novel triterpenoid compounds derived from oleanic acid such as 2-cyano-3,12-dioxooolean-1,9-dien-28-oic acid (CDDO) [16]. Despite binding affinities in the nM range, most biological effects of these synthetic PPARγ agonists have been observed at μM concentrations.

Potential natural ligands for PPARγ also show marked structural diversity and include prostaglandin D2 (PGD2) and its metabolites PGJ2 and 15-deoxyΔ12,14-prostaglandin-J2 (15dPGJ2) [17]. 15dPGJ2 in particular has been widely used for comparisons with glitazones in experimental settings [18]. However, these agonists, and other putative PPARγ ligands such as the oxidised lipids 9- and 13-hydroxyoctadecadienoic acid (HODE) and 12- and 15-hydroxyicosatetraenoic acid (HETE) [19], have multiple additional sites of action, suggesting that demonstrating their PPARγ-dependent actions is likely to be particularly challenging.

### 3. Mechanisms of Action of PPARγ and Its Ligands

#### 3.1. PPARγ Activation.

Cytosolic PPARγ exists as a monomer, with both the LBD and AF-1 domain regulating interactions with coactivators and corepressors that control activation of the receptor [11–13]. PPARγ does not form homodimers, but can associate with multiple partners to form heterodimers. Its most preferential binding partner is the retinoid X receptor (RXR), with 9-cis retinoic acid acting as its natural ligand [20]. Translocation of the ligand-activated PPARγ-RXR complex to the nucleus and binding to PPRE in the promoter region of target genes can result in either the upregulation or inhibition of gene expression. Multiple PPARγ-responsive genes involved in diverse cellular processes including adipogenesis, insulin sensitivity, and inflammation have been identified [21, 22].

Alternatively, PPARγ can cause the transrepression of transcriptional factors such as NfκB, CAAT/enhancer binding protein (C/EBP), signal transducers and activators of transcription (STAT), or activator protein- (AP-) 1. This transrepression may occur either by direct binding to the transcription factors, by sequestration of shared coactivators of these factors or by Small Ubiquitin-like Modifier (SUMO)ylation of PPARγ and subsequent PPARγ binding to a corepressor complex [23]. These actions also have the potential to inhibit inflammatory responses in the lung.

Given the marked differences between the reported PPARγ binding affinities of the glitazones and the concentrations required to elicit their cellular effects, multiple approaches are required to support claims for PPARγ-dependency. These include confirming PPARγ expression in cells of interest and the use of pharmacological antagonists,
with GW9662 being the most commonly used. GW9662 irreversibly inhibits PPARγ by covalently binding to Cys238 in the ligand binding pocket and prevents heterodimerisation with RXR as well as interactions with coactivator and corepressor molecules [24]. GW9662 has been used to confirm the PPARγ-dependence of known PPARγ ligands both in vitro [18] and in vivo [25]. Additional molecular techniques such as the use of dominant-negative constructs or adenoviral PPARγ (AdPPARγ) have been employed to implicate PPARγ in the regulation of cellular responses both in vitro and in vivo [26, 27].

3.2. PPARγ-Independent Mechanisms. The mechanistic complexity underlying responses to PPARγ ligands occurring via PPARγ activation is further complicated by evidence of PPARγ-independent pathways. This may involve PPARγ ligands binding directly to alternative receptors, regulating transcription factor activity, or altering signalling through enzymes or ion channels to mediate their cellular responses.

Glitazones have been shown to activate free fatty acid receptors (FFA1, also known as GPR40) causing phosphorylation of the ERK1/2 mitogen-activated protein (MAP) kinases [28]. RGZ and CGZ can also bind directly to GR independently of PPARγ, evidenced by their stimulation of GR nuclear translocation in a PPARγ-deficient cell line [29], and defining a potential alternative anti-inflammatory mechanism for these ligands.

In addition, 15dPGJ2 has been demonstrated to directly inhibit the activity of the enzyme IkB kinase, thereby reducing the phosphorylation of IkB and its subsequent dissociation from the proinflammatory transcription factor NFκB [30, 31]. Actions of PPARγ ligands may also be mediated by increasing PGE2 levels, subsequent to inhibition of its metabolism via 15-hydroxyprostaglandin dehydrogenase [32].

Both 15dPGJ2 and CDDO have been shown to induce heme-oxi-dase by PPARγ-independent, glutathione-dependent mechanism, although this antioxidant action was restricted to PPARγ ligands possessing an electrophilic centre [33]. Additional evidence of nongenomic, rapid regulation of enzyme activity by PPARγ ligands includes activation of mitogen-associated protein kinase (MAPK), phosphoinositide-3-kinase (PI3K), and adenosine monophosphate-activated protein kinase (AMPK) pathways [34–36] with implications for regulation of cell proliferation and inflammatory cytokine production.

Some of these nongenomic effects may be mediated through altered calcium signalling. TGZ and PGZ have been shown to mobilize calcium from intracellular stores [37, 38]. Although RGZ rapidly inhibited the activity of sarco/endoplasmic reticulum Ca2+ ATPase (SERCA)-2b [39], chronic treatment with PGZ has been shown to increase SERCA activity via a PPARγ-dependent mechanism [40], suggesting that regulation of calcium homeostasis by PPARγ ligands is likely to depend on temporal and cellular contexts.

These diverse PPARγ-dependent and PPARγ-independent actions define numerous mechanisms whereby PPARγ ligands could regulate the altered proliferative, secretory and contractile functions of ASM contributing to asthma.

4. PPARγ Is Increased in Airway Smooth Muscle in Asthma

Although PPARγ was originally characterised as a regulator of adipocyte differentiation, this receptor is also widely expressed in the lung, in both inflammatory and structural cells implicated in asthma pathophysiology [41].

Regulation of PPARγ expression can occur in response to in vitro exposure to inflammatory cytokines, with acute upregulation occurring in response to interleukin-4 (IL-4) in airway epithelial cells and macrophages [42, 43] during macrophage differentiation and activation [44, 45] or following antigen exposure in sensitised mast cells [46].

PPARγ may provide a target to overcome chronic inflammation and increased airway reactivity in vivo. Higher levels of PPARγ were evident in total lung extracts from mouse models of established allergen-induced inflammation [27, 47] and could be localised to ASM and epithelium, mast cells, and some inflammatory cells [25]. In contrast, perinatal exposure to nicotine appears to decrease PPARγ expression and signaling, with increased alveolar interstitial fibroblast-to-myofibroblast differentiation contributing to the development of an asthma-like phenotype in newborn rats [48, 49].

In human airway biopsies, expression levels of PPARγ in ASM, epithelium, and mucosal eosinophils and macrophages were elevated in asthmatic patients compared with controls [10]. In the same study, asthmatics treated with GCS had lower levels of PPARγ expression compared with untreated asthmatics. Although ASM from asthmatics had lower PPARγ levels compared to healthy controls in vitro, this was reversed in the presence of a mitogenic stimulus [50]. These results suggest that increased PPARγ expression observed in situ may be a product of the inflammatory and mitogenic pathways and may also be sensitive to steroid therapy.

These combined findings suggest that PPARγ expression is increased in response to acute or chronic inflammation in multiple cell types including ASM, and that PPARγ could be targeted to limit inflammation, airway remodeling, and increased ASM contraction in asthma.

5. In Vitro Regulation of ASM Function by PPARγ Ligands

Because of the capacity of ASM to perpetuate airway inflammation, orchestrate airway wall remodelling, and modulate airway tone, it has been suggested that targeting ASM is critical for effective asthma treatment [4, 7, 8]. Accumulating in vitro evidence now supports the efficacy of PPARγ ligands in the regulation of ASM cytokine production, proliferation, and contraction, while their direct effects on the potential contribution of ASM to fibrosis and angiogenesis have yet to be confirmed.

5.1. Regulation of ASM Inflammatory Cytokine Production. In response to inflammatory stimuli, ASM can secrete various cytokines and chemokines contributing to the pathophysiology of asthma [51–53]. These mediators include factors such as granulocyte/macrophage-colony-stimulating factor (GM-CSF) [54], granulocyte-colony stimulating factor (G-CSF)
PGE2 levels to provide negative feedback to inhibit cytokine proinflammatory effects. PPAR γ also inhibited TNF-α and RANTES, and IL-4-induced release of eotaxin, while 4 PPAR Research migration also playing a potential role. To assess the potential remodeling in asthma is the increased ASM layer associated 5.2. Regulation of ASM Proliferation. A key feature in airway remodeling in asthma is the increased ASM layer associated with increases in both size (hypertrophy) and number (hyperplasia, migration) of myocytes [65] with ASM cell migration also playing a potential role. To assess the potential efficacy of antiremodeling agents, ASM proliferation can be induced in vitro in response to the cocktail of mitogens present in serum, and to specific stimuli such as thrombin or fibroblast growth factor 2 (FGF2), known to be increased in the asthmatic airway [66, 67]. PPARγ ligands have now been shown to inhibit proliferation of human ASM in culture. The increase in [3H]-thymidine incorporation in response to serum was completely abolished by both CGZ and 15dPGJ2 [55], while RGZ and 15dPGJ2 significantly attenuated both FGF2 and thrombin-stimulated increase in ASM cell numbers [18], demonstrating that the antiproliferative effects are mitogen-independent. Unlike GCS, inhibition of proliferation was not associated with reduced cyclin D1 levels [18, 68]. Responses were mediated by both PPARγ-dependent and PPARγ-independent mechanisms, as the PPARγ antagonist GW9662 inhibited the antiproliferative effects of RGZ but not 15dPGJ2 [18], with cell cycle analysis suggesting that neither mediated ASM apoptosis [18]. Although CGZ and 15dPGJ2 had previously been reported to cause nuclear condensation, a characteristic morphological change associated with apoptosis [55], this single finding was not consistent with the known resistance of ASM to apoptosis [69]. Cultured ASM derived from asthmatic patients has been shown to proliferate faster than cells from nonasthmatic patients [70]. Since GCS can only inhibit the in vitro proliferation of ASM from subjects without asthma [68, 71], alternative therapeutic approaches are required to target this steroid-resistant mitogenic response. In a recent study, the effects of CGZ were assessed in cells from nonasthmatic and asthmatic patients cellular proliferation in response to serum by measuring bromodeoxyuridine uptake [50]. Further studies are required to explain why CGZ failed to inhibit serum-induced proliferation in either group [50], since this finding contradicts the previously reported antiproliferative effects of both CGZ [55] and RGZ [18]. CGZ did upregulate PPARγ expression in ASM cells derived from both asthmatic and nonasthmatic subjects, and in ASM from asthmatics in the presence of serum [50], however the functional significance of these changes and their potential impact on ASM in remodeled airways remain to be determined.

5.3. Regulation of Extracellular Matrix Production and Turnover. Airway remodeling in asthma is also characterized by alterations in the amount and composition of ECM proteins, including increases in collagen I and fibronectin deposition [72]. Subepithelial fibrosis is associated with increased transforming growth factor β (TGFβ), with this profibrotic cytokine present at relatively higher levels in BAL fluid from asthmatic subjects compared to healthy subjects [73]. Although fibroblasts are considered the major resident cells contributing to the increased collagen deposition in the asthmatic airway, ASM is also known to produce ECM proteins and to regulate their turnover by secreting matrix modifying enzymes.

In this context, it is important to consider that the ECM exists not only as a structural scaffold in the airways but as a partner in bidirectional interactions with ASM, influencing proliferation and cytokine release as well as contractility [74]. Since in vitro secretion of collagen and fibronectin from ASM...
derived from asthmatic patients is increased by GCS, and TGFβ-induced ECM protein synthesis is unaffected by GCS, this aspect of remodeling appears to be resistant to steroids [75] and alternative strategies to minimize the impact of the altered ECM on ASM function need to be identified.

Confirmation of the ability of PPARγ ligands to inhibit TGFβ-induced collagen synthesis from ASM would suggest that these agents have the capacity oppose proinflammatory changes associated with increased ASM-ECM interactions. To date, the effects of PPARy ligands have only been assessed in human lung fibroblasts which express PPARγ, and respond to TGFβ treatment by differentiating into myofibroblasts expressing a smooth muscle actin (aSMA), and increasing their synthesis of fibrillar collagen I [26]. Both differentiation and collagen I secretion were abrogated by treatment with RGZ, CGZ, or 15dPGJ2. These antifibrotic effects of the PPARγ ligands were shown to be at least partially mediated by PPARγ receptor activation as inhibition was attenuated by transfection of TGFβ-treated fibroblasts with a dominant negative PPARγ receptor [26]. Similar antifibrotic properties have also been described for RGZ and CGZ in the regulation of epithelial-mesenchymal transition in alveolar epithelial cells [76].

An alternative way to regulate ASM-ECM interactions would be by regulating the activity of matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs). Activation of PPARγ by RGZ or PGZ in human bronchial epithelial cells reduced TNFα-induced MMP-9 gelatinolytic activity via inhibition of NF-κB, but did not alter the expression of its endogenous inhibitor TIMP-1 [77]. These results suggest that limiting the expression of MMP-9 by PPARγ activation might have therapeutic potential in the treatment of chronic inflammatory diseases of the respiratory system. However, the effects of PPARγ ligands on ASM-derived MMPs and TIMPs in the asthma context have yet to be directly assessed.

5.4. Regulation of Angiogenesis. Significant increases in the number and size of blood vessels supplying the remodeled airway wall are seen in asthma [6, 78]. This expanded vascular compartment is likely to contribute to asthma symptoms through tissue swelling and amplification of inflammatory cell trafficking [79]. ASM has the potential to promote angiogenesis as cultured ASM has been shown to constitutively release factors such as VEGF, which can be further increased in response to inflammatory mediators such as IL-1β, TNFα, and TGFβ [80]. Of note, these proangiogenic responses have recently been shown to be further elevated in ASM from asthmatics [81].

Studies examining the effects of PPARγ ligands on this aspect of remodelling are lacking; however, conflicting reports show that the generation of VEGF from vascular smooth muscle cells is increased by CGZ and PGJ2 [82, 83], while TGZ has been shown to inhibit VEGF-induced angiogenic signaling in endothelial cells [84]. Further investigations are required to explore the potential of PPARγ ligands to regulate the contribution of ASM to angiogenesis.

5.5. Regulation of ASM Contraction. The increased contractile response of asthmatic airways which defines AHR is likely to be due to multiple factors (recently reviewed in [4]), including the presence of higher levels of contractile mediators and reduced levels of relaxant mediators. Critically, the increased ASM bulk displays alterations in contractile protein expression that favour contraction [85, 86]. In this context, it is of interest that RGZ and other PPARγ ligands can inhibit the increase in α-smooth muscle actin and calponin associated with both epithelial-mesenchymal transition of alveolar epithelial cells [76] and alveolar interstitial fibroblast-to-myofibroblast differentiation [87].

Increased excitation/contraction coupling may also occur through disruption of calcium homeostasis [88]. Indeed, increased contraction of ASM cells from asthmatic patients has been associated with downregulation in their expression and function of SERCA2 [89]. PPARγ ligands have recently been reported to increase SERCA expression and activity in pancreatic islet cells and platelets [40, 90], with PGZ inhibiting cytokine-induced increases in intracellular calcium by facilitating its reuptake into the SR [40]. In ASM, calcium plays a key role not only in enhancing ASM contractile function, but also in promoting cell proliferation, migration and the secretion of proinflammatory cytokines and chemokines [88]. It will therefore be of particular interest to determine if acute or chronic treatment with PPARγ ligands can also restore SERCA levels and activity in ASM to inhibit the diverse proinflammatory functions that could be driven by elevated intracellular calcium.

There is now evidence that acute treatment with PPARγ ligands may exert direct effects on ASM contractility. In a single study, RGZ has been reported to cause relaxation of mouse tracheal preparations precontracted with carbachol [91]. Since this response was evident within minutes and required μM concentrations, it was likely to be occurring independently of PPARγ activation. Relaxation to RGZ in the static organ bath setting was indomethacin-sensitive and was attributed to accumulation of the dilator prostaglandin PGE2 through inhibition of its breakdown rather than an increase in PGE2 synthesis. This interpretation is consistent with the previously reported finding that RGZ can inhibit its metabolism by 15-hydroxyprostaglandin dehydrogenase [32].

Further studies are required to explore acute dilator responses to RGZ and other PPARγ ligands, to compare their efficacy with β2-adrenoceptor agonists in current clinical use for the relief of asthma symptoms and to test their actions in the disease context when ASM responsiveness is altered.

6. In Vivo Regulation of ASM Function by PPARγ Ligands

6.1. PPARγ Ligands Have Efficacy in Rodent Models of Allergic and Nicotine-Induced Airways Disease. The reported effects of PPARγ ligands on ASM functions in vitro, namely inhibition of proliferation and production of cytokines from human ASM cells as well as regulation of contractile protein expression and direct relaxation intracheal preparations, has provided an impetus for considering their effects in animal models of airways disease, using perinatal exposure to maternal nicotine or chronic ovalbumin (OVA) challenge to trigger asthma-like changes in the airways.
It is well known that cigarette smoking during pregnancy increases the incidence and severity of childhood asthma, and has been associated with increased generation of contractile myofibroblasts in the developing lung (reviewed in [9]). A recent study of newborn rats following in utero nicotine exposure has revealed that increases in airway resistance under basal conditions and reactivity to acetylcholine both in vivo and in vitro has consistently shown that RGZ or CGZ treatment attenuated the increase in total and eosinophil cell numbers in BAL fluid in OVA-treated C57Bl/6 or Balb/C mice administered i.p. [99], it was ineffective in C57Bl/6 mice administered i.p. [98]. The reason for this discrepancy is therefore more likely to be due to differences in challenge protocols and the administration methods (route, dose, and duration) of different compounds, rather than the mouse strain used.

The efficacy of PPARγ ligands in these OVA challenge models in mice has been confirmed in multiple studies, assessing the effect of chronic treatment with glitazones or other PPARγ selective agonists such as GI 262570, administered either by inhaled, oral or intraperitoneal (i.p.) routes [25, 27, 47, 92–95, 97–100] or the response to transient overexpression of PPARγ via adenoviral delivery (Table 1) [27, 47, 93]. However, since the overall changes in phenotype in these models can vary with the type of OVA used [101], the duration of the challenge protocol and the species of mice in which the model is applied [102], the reported effects of pharmacological intervention with different PPARγ ligands administered by various routes must be considered and interpreted in context. Nevertheless, these models provide compelling evidence that PPARγ ligands can regulate inflammatory cell infiltration, BAL cytokines, airway remodeling particularly ASM thickening and fibrosis, and altered reactivity to MCh.

6.2. Regulation of Airway Inflammation. Assessment of airway inflammation has consistently shown that RGZ or CGZ treatment attenuated the increase in total and eosinophil cell numbers in BAL fluid in OVA-treated C57Bl/6 or Balb/C mice in a PPARγ-dependent manner (Table 1) [27, 93, 94]. Similar results were observed with GI 262570 administration to Balb/C mice, where eosinophil and lymphocytes, but not neutrophils, were reduced [92]. Although RGZ reduced eosinophilic airway inflammation when administered to Balb/C mice by oral gavage [99], it was ineffective in C57Bl/6 mice administered i.p. [98]. The reason for this discrepancy is therefore more likely to be due to differences in challenge protocols and the administration methods (route, dose, and duration) of different compounds, rather than the mouse strain used.

Regulation of cytokine production in the lung has also been assessed. OVA-induced increases in IL-4, IL-5, IL-13, eosinophil cationic protein (ECP), and eotaxin in lung tissue and BAL fluid were inhibited by administration of RGZ, PGZ, or by PPARγ overexpression [27, 93]. Similar changes were seen with nebulized CGZ, although eotaxin levels were not affected [94], while oral CGZ has been shown to reduce IL-2, IL-4, and interferon γ (IFNγ) [100]. Since cytokine release from ASM is also inhibited by PPARγ ligands in vitro, it is likely that the glitazones can reduce the contribution of ASM-derived cytokines to the levels measured in this in vivo setting.

Several potential mechanisms have been proposed to explain the anti-inflammatory effects of PPARγ ligands in these models. Regulation of NFκB has been considered since...
PPARγ activation inhibits the function of the proinflammatory transcription factor in vitro [103, 104]. Treatment of OVA-sensitised mice with RGZ, PGZ, or AdPPARγ also reduced the nuclear translocation of NFκB in response to OVA, evidenced by inhibition of increases in NFκB p65 protein in lung extracts [93], suggesting a direct action of PPARγ ligands on NFκB. Inhibition of NFκB activity by PPARγ agonists has also been associated with decreased IL-17 protein and mRNA expression. Since the effects of RGZ or PGZ could be abrogated by coadministration of rIL-17, this implicates a novel mechanism whereby PPARγ agonists regulate NFκB activity by reducing IL-17 to limit inflammation [97].

NFκB-independent mechanisms are also likely to contribute to the anti-inflammatory effects of PPARγ ligands [92]. Alternative mechanisms include PPARγ-mediated inhibition of the increase in GATA-3 expression in response to OVA [94], reducing the local Th2 response elicited by this eosinophil-derivied transcription factor. In addition, an increase in IL-10 in response to OVA, thought to occur as part of a negative feedback to inhibit inflammation, could be further increased by RGZ, PGZ, or ad PPARγ [47]. Increased IL-10 levels could explain the reported reductions in IL-4 and IL-5 as well as the inhibition of eosinophilia, since IL-10 has been shown to downregulate IL-4 and IL-5 expression by Th2 cells and reduce eosinophil survival.

In a separate study, PPARγ expression was increased in response to OVA challenge and further enhanced by the administration of the either PPARγ agonists or AdPPARγ [27]. This was associated with an upregulation of phosphatase and tensin homologue deleted on chromosome ten (PTEN) PTEN expression, correlating with decreased PI3K activity as measured by a reduction in the phosphorylation of Akt. These findings demonstrate a protective role of PPARγ in the pathogenesis of the asthma phenotype through regulation of PTEN expression [27, 93].

6.3. Regulation of Airway Remodeling. In addition to their anti-inflammatory actions in these mouse models, PPARγ ligands have also been shown to inhibit key aspects of airway remodeling, notably fibrosis, mucus production, and thickening of the ASM layer (Table 1).

Inhaled CGZ has been shown to reduce OVA-induced increases in both collagen deposition and basement membrane thickening [25]. This was associated with reduced levels of the profibrotic cytokine TGFβ [25]. Although inhaled CGZ has also been shown to decrease mucus production, based on the intensity and area of epithelial staining [25], there were no detectable effects of i.p. RGZ on goblet cell number or other aspects of airway remodeling [98], suggesting that high local concentrations may be required.

Consistent with the reported antiproliferative effects of PPARγ ligands on human ASM in vitro [18, 50, 55], intranasal administration of CGZ has been shown to reduce not only eosinophilic inflammation, but also to inhibit the thickening of the ASM layer following allergen challenge [95]. This effect appeared to be independent of regulation of TGFβ or VEGF levels, as the increased BAL levels of these potential mitogens were not reduced with CGZ treatment [95]. It would be of interest to measure endogenous factors that could contribute to ASM proliferation in this setting.

6.4. Regulation of Airway Hyperresponsiveness. Studies demonstrating the inhibitory effects of PPARγ ligands on AHR are consistent with the numerous in vitro findings suggesting a role for PPARγ ligands in the regulation of ASM function in asthma (Table 1). The development of AHR to cholinergic agonists subsequent to in utero nicotine exposure or in vivo allergen exposure can be alleviated by chronic treatment with PPARγ ligands, measured either indirectly using Penh [25, 27, 94, 95] or by assessing changes in airway resistance [47, 48, 97, 98].

A recent study has reported that coadministration of RGZ prevented the changes in lung function in rat offspring induced by perinatal nicotine exposure. Inhibition of the development of AHR as measured in vivo and in isolated tracheal preparations was attributed to the ability of RGZ to decrease the lipofibroblast-to-fibroblast transdifferentiation induced by nicotine, minimizing the increased expression and function of mesenchymal markers of contractility [48].

Chronic allergen studies demonstrating PPARγ ligand efficacy suggest a PPARγ-dependent mechanism in opposing ovalbumin-induced AHR, since the inhibitory effects could be mimicked by transient overexpression of PPARγ via adenoviral delivery or prevented by co-treatment with GW9662 [25, 27, 47, 93, 94, 97, 98]. It would be reasonable to attribute this reduction in AHR to the inhibition of inflammation and airway remodeling mediated by the PPARγ ligands used. However, RGZ also reduced AHR measured by invasive plethysmography in OVA-challenged C57Bl/6 mice without detectable effects on markers of inflammation or remodeling [98]. This result suggests that it is possible that PPARγ ligands may also exert a direct effect on ASM contractile function in vivo.

In this context, it is notable that chronic treatment with PPARγ ligands may not only inhibit the development of AHR, but also protect airway dilator responses. In a guinea pig model of in vivo homologous desensitization to salbutamol, chronic treatment with RGZ mitigated AHR to carbachol, preserved salbutamol relaxant activity, and partially restored β2-adrenoceptor binding sites in tracheal tissues ex vivo [105]. The potential for PPARγ ligands to maintain dilator sensitivity and reverse β2-adrenoceptor desensitization is of particular interest since GCS can prevent cytokine-induced desensitization [106], but cannot restore sensitivity once tolerance to β2-adrenoceptor agonists has developed [107].

7. Potential Clinical Benefit of PPARγ Ligands in Asthma

Although several members of the glitazone class of drugs have been used for type 2 diabetes, PGZ is the only PPARγ agonist in current clinical use for this condition, with its potential as a treatment to reduce inflammation in rheumatoid arthritis also being assessed [108]. TGZ was the first glitazone to be marketed for diabetes, but was withdrawn because of serious hepatotoxicity in some patients [109],
while RGZ has also recently been withdrawn because of potential cardiovascular risks [110].

There is currently only limited data on the efficacy of glitazones in the treatment of respiratory diseases. Further studies characterizing the effects of PPARγ ligands on lung development as well as nicotine-induced changes in lung function are required to determine whether these agents may provide a new therapeutic approach to minimize, or even reverse, the adverse impacts of maternal smoking that contribute to the development of paediatric asthma.

An isolated report described the effects of PGZ in two case subjects with both diabetes and established asthma [111]. One patient reported reduced wheezing when taking PGZ in addition to his asthma preventer medication, with deterioration of symptoms when PGZ was discontinued. In another, concurrent treatment with the sulfonylurea glibenclamide and PGZ effectively reduced the patient’s blood glucose levels and improved pulmonary function test results, increasing both forced vital capacity and force expiratory volume in one second (FEV1).

More recently, a small single-centre trial has been conducted, assessing RGZ in a double-blind, randomised, placebo-controlled, two-period crossover study in the inhaled allergen challenge model of asthma [112]. 32 steroid naïve subjects completed the study, receiving RGZ (4 mg) and placebo twice daily for 28 days in random order. The late asthmatic reaction (LAR) change from postsaline FEV1 from 4–10 hrs after allergen on day 28 was attenuated by 15% compared to the response during placebo-treatment, suggesting an inhibitory effect of RGZ on activated eosinophil recruitment. This reduction was accompanied by trends in several other markers of efficacy and anti-inflammatory activity (e.g., IL-4, IL-6, IL-13). In light of these modest changes, the authors suggested that PPARγ agonist monotherapy is unlikely to represent a clinically useful intervention, at least in the context of relatively mild asthma.

More positive results were obtained in another recently completed exploratory clinical trial, which compared the effects of oral RGZ (8 mg) with inhaled beclometasone in a group of forty-six smokers with asthma, a group that is generally unresponsive to conventional GCS treatment [113]. In measurements taken after two and four weeks, RGZ did not significantly reduce asthma symptoms as determined by the Asthma Control Questionnaire (ACQ) scores and only produced a borderline reduction in sputum IL-8 levels compared to beclometasone-treated patients [113]. However, the patients receiving RGZ experienced significant improvements in FEV1 and forced expiratory flow over beclometasone-treated patients, which may reflect an effect of RGZ to reduce small airway obstruction.

These promising findings support the assessment of the effectiveness of long-term treatment of RGZ in a larger treatment group. The use of substantially higher oral doses may not be associated with a positive benefit/risk profile in asthma since PPARγ agonists are associated with dose-related adverse effects such as weight gain (probably secondary to fluid retention). This suggests that a preferable alternative strategy would be to assess responses to both acute and chronic inhalation of PPARγ agonists. This route of administration would potentially minimize the reported adverse cardiovascular effects that have limited the systemic use of RGZ in diabetes [110]. In addition, it would achieve the higher local airway concentrations that may be required to exert direct effects on ASM contractile function to elicit acute bronchodilation as reported in mouse trachea, and chronic effects to regulate airway inflammation, remodeling, and the development of AHR.

8. Summary

An accumulating body of evidence supports the use of PPARγ ligands for the targeting of PPARγ receptors and other PPARγ-independent mechanisms in ASM for the treatment of inflammatory lung diseases (Figure 2) [9, 41, 114]. In vitro treatment inhibits proliferation of human ASM via PPARγ [18, 55] and also inhibits cytokine release from these cells [55, 62, 63]. Chronic in vivo treatment inhibits the development of nicotine-induced AHR in rat airways [48] as well as OVA-induced increases in ASM mass in mouse airways [95], part of a suite of actions involving inhibition of airway inflammation, remodeling and the development of AHR. PPARγ ligands may also protect dilator responses since they can preserve β2-adrenoceptor expression and function in a guinea pig model of homologous desensitization to albuterol [105]. The potential for direct bronchodilator actions is supported by the demonstration of acute PPARγ-independent relaxation in mouse trachea [91]. Although clinical trial results are limited, evidence of improved lung function in a difficult-to-treat cohort of smokers with asthma [113] supports further investigation of the potential for PPARγ agonists to target ASM proliferative, inflammatory and contractile functions and their contributions to impaired dilator responses and the consequences of AHR in asthma.

**Abbreviations**

15dPGJ₂: 15-Deoxy-D₁₂,1₄-prostaglandin-J₂
ACQ: Asthma control questionnaire
AdPPARγ: Adenoviral PPARγ
AF-1: Activator function-1
AHR: Airway hyperresponsiveness
AMPK: Adenosine monophosphate-activated protein kinase
AP-1: Activator protein-1
AR: Adrenoceptor
ASM: Airway smooth muscle
αSMA: α smooth muscle actin
BAL: Bronchoalveolar lavage
C/EBP: CAAT/enhancer binding protein
CDDO: 2-Cyano-3,12-dioxoolean-1,9-dien-28-oic acid
CGZ: Ciglitazone
DBD: DNA binding domain
ECM: Extracellular matrix
ECP: Eosinophil cationic protein
FFA: Free fatty acid
FGF2: Fibroblast growth factor
GCS: Glucocorticoid
G-CSF: Granulocyte-colony-stimulating factor
GR: Glucocorticoid receptor
HETE: Hydroxyeicosatetraenoic acid
GR: Glucocorticoid receptor
HODE: Hydroxyoctadecadienoic acid
HOG: HOG domain
IκB: Inhibitory protein kappa B
IL-1α: Interleukin-1α
i.p.: Intraperitoneal
LBD: Ligand binding domain
MAPK: Mitogen-associated protein kinase
MCh: Methacholine
MMP: Matrix metalloproteinases
NFκB: Nuclear factor kappa B
NHR: Nuclear hormone receptor
OVA: Ovalbumin
Penh: Enhanced pause
PG: Prostaglandin
PGZ: Pioglitazone
PI3K: Phosphoinositide-3-kinase
PPARγ: Peroxisome proliferator-activated receptor γ
PRRE: PPAR response element
PTEN: Phosphatase and tensin homologue deleted on chromosome ten
RANTES: Regulated on activation, normal T cells expressed and secreted
RXR: Retinoid X receptor
SERCA: Sarco/endoplasmic reticulum Ca²⁺ ATPase
STAT: Signal transducers and activators of transcription
SUMO: Small Ubiquitin-like Modifier
TGFβ: Transforming growth factor β
TGZ: Troglitazone
TNFα: Tumour necrosis factor α
VEGF: Vascular endothelial growth factor.

Acknowledgments

This work was supported by the National Health and Medical Research Council (Grant 509239) and ANZ Medical Research and Technology in Victoria Fund.

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