

The PPAR γ ligand, rosiglitazone, reduces airways hyperresponsiveness in a murine model of allergen-induced inflammation

J.E. Ward*, D.J. Fernandes, C.C. Taylor, J.V. Bonacci, L. Quan, A.G. Stewart

Department of Pharmacology, University of Melbourne, Vic., 3010, Australia

Received 14 February 2005; revised 14 February 2005; accepted 22 February 2005

Abstract

There is considerable interest in the role of peroxisome proliferator activated receptors (PPARs) as ligand-activated transcription factors in the airways. This study examines the effects of a potent synthetic PPAR γ ligand, rosiglitazone (RG), in a murine model of allergen-induced inflammation, to explore its potential regulation of airways inflammation, structure and function. C57BL/6 mice were sensitised with ovalbumin (OVA, 50 μ g i.p., days 0, 12) and challenged with aerosolized OVA (1% w v⁻¹, 30 min day⁻¹) for 7 days (days 20–26). Mice were treated with RG (5 mg kg⁻¹ i.p.) or vehicle during the challenge period. The OVA challenge induced increases in leukocyte number and MMP-2 activity in bronchoalveolar lavage fluid and in goblet cell number in lung tissue obtained on Day 27. RG failed to inhibit inflammatory cell infiltration, MMP-2 activity or goblet cell hyperplasia. Respiratory resistance in response to methacholine (MCh i.v.) was greater in OVA-challenged mice than saline-challenged mice and this airways hyperresponsiveness (AHR) was reduced by RG. However, RG did not affect MCh-induced contraction in isolated guinea-pig tracheal rings, nor did it influence the airway obstruction induced by MCh in saline-challenged mice, so a direct effect on airway obstruction is unlikely. These data suggest that RG modulates AHR in this model, by a mechanism that is also potentially independent of an anti-inflammatory action.

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Keywords: Peroxisome proliferator-activated receptor γ ; Rosiglitazone; Airways hyperresponsiveness; Asthma

1. Introduction

The peroxisome proliferator-activated receptor γ (PPAR γ) is a widely expressed ligand-activated nuclear receptor, originally characterised as a regulator of adipocyte

differentiation. PPAR γ is activated by a structurally diverse range of compounds, including putative endogenous ligands such as the PGD₂ metabolite, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂ (15d-PGJ₂) [1]. Synthetic ligands include the thiazolidinedione class of anti-diabetic agents, such as the high affinity ligand rosiglitazone (RG) [2]. After ligand binding, PPAR γ forms a heterodimer with the retinoid X receptor and undergoes a conformational change that mediates exchange of corepressor and coactivator proteins to enable transcriptional activation or repression of target genes [3]. PPAR γ can also negatively regulate gene expression in a ligand-dependent manner by antagonizing the activities of other signal-dependent transcription factors, such as nuclear factor- κ B (NF- κ B) [4].

It has recently been reported that PPAR γ expression is increased in asthmatic airways. Bronchial biopsies of untreated asthmatics have considerably more PPAR γ staining than non-asthmatic tissue, particularly in mucosal eosinophils and macrophages, airway smooth muscle (ASM), and epithelial cells [5]. These cell types play a significant role in asthma pathology as sources of and/or responders to mediators implicated in the inflammation

Abbreviations 15d-PGJ₂, 15-deoxy- $\Delta^{12,14}$ -prostaglandin J₂; ABPAS, alcian blue, periodic acid-Schiff; AHR, airways hyperresponsiveness; ANOVA, analysis of variance; ASM, airway smooth muscle; AWR, airway wall remodelling; BAL, bronchoalveolar lavage; bFGF, basic fibroblast growth factor; C_{dyn}, dynamic lung compliance; CG, ciglitazone; DMSO, dimethyl sulphoxide; G-CSF, granulocyte-colony stimulating factor; GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; MCh, methacholine; MMP, matrix metalloproteinase; NF- κ B, nuclear factor- κ B; OVA, Ovalbumin; PBS, phosphate buffered saline; Penh, enhanced pause; PPAR, peroxisome proliferator activated receptor; RANTES, regulate upon activation, normal T cell expressed and secreted; RG, rosiglitazone; R_R, respiratory resistance; SEM, standard error of the mean.

* Corresponding author. Tel.: 61 3 8344 5622; fax: 61 3 8344 0241.

E-mail address: janeew@unimelb.edu.au (J.E. Ward).

and airway wall remodelling (AWR) that occurs during the development of airways hyperresponsiveness (AHR). The stimulus for increased PPAR γ levels in these airway cells is unclear, as is the functional role, if any, of this increase. Glucocorticoid-treated asthmatics had lower levels of PPAR γ expression than untreated asthmatics [5], suggesting that increased PPAR γ expression may be a product of the inflammatory pathways sensitive to steroid therapy. Inflammatory cytokines such as interleukin-4 (IL-4) and the eicosanoid, 15-Hydroxyeicosatetraenoic acid, potentially stimulate PPAR γ expression [6,7]. Given that levels of both of these mediators are elevated in asthmatic airways [8–10], they may contribute to the elevation in PPAR γ levels. The current hypothesis is that increased PPAR γ expression in response to inflammatory cytokines might provide a negative feedback mechanism, whereby endogenous PPAR γ ligands could activate these receptors to limit the cellular inflammatory response in the airways. However, PPAR γ upregulation does not appear to be a generalised response to inflammation, as PPAR γ expression is markedly reduced in a model of ulcerative colitis [11].

Recent *in vitro* studies suggest that both natural and synthetic PPAR γ ligands regulate inflammation. Both pro- and anti-inflammatory properties have been ascribed to the putative endogenous ligand 15d-PGJ₂. 15d-PGJ₂ has been shown to induce IL-8 gene expression in human T cells, macrophages and endothelial cells [12–14]. This potentially pro-inflammatory induction was attributed to a mitogen-activated protein kinase and/or NF- κ B-dependent signalling pathway [12,13], and was not related to the level of PPAR γ activation [14]. However, after inducing increased PPAR γ expression in an epithelial cell line with IL-4, 15d-PGJ₂ had no effect on cytokine-induced IL-8 secretion [7]. There are also contradictory reports on the effect of 15d-PGJ₂ in human cultured ASM cells with both increased [15] and decreased IL-1 stimulated GM-CSF levels having been reported [16].

Thiazolidinediones, such as RG and ciglitazone (CG), have been shown to inhibit release of a range of inflammatory mediators including RANTES, IL-8 and GM-CSF from monocytes [17], T cells [18,19], mast cells [20], ASM [16], and lung epithelial cells [7,21]. CG also reduced G-CSF levels, suggesting a different profile of inflammatory mediator inhibition in smooth muscle cells for CG compared with corticosteroids, which inhibited GM-CSF but not G-CSF [16]. In addition to regulating release of cytokines and chemokines promoting infiltration of inflammatory cells, thiazolidinediones have been shown to reduce IL-5-induced eosinophil survival and chemotaxis [22,23].

These studies provide considerable support for a negative immunomodulatory role for synthetic PPAR γ ligands in the regulation of airway function. In addition to regulation of cytokine release, thiazolidinediones have been shown to inhibit mitogen-induced proliferation of human cultured ASM cells [16,24]. It is of interest that the degree of inhibition of serum-induced ASM proliferation was greater

for CG than dexamethasone [16]. The mechanism of the antiproliferative effect of RG appears to involve inhibition of cell-cycle progression in late G1 phase, without affecting cyclin D1 levels [24]. This differs from the mechanism previously described for steroids such as dexamethasone [25].

The mechanism of the anti-inflammatory and anti-migratory effects *in vitro* of the synthetic ligands has not been fully characterised, and there is considerable interest in exploring whether these phenomena are restricted to cells in culture. The current evidence and their activity profile suggest that CG and RG may regulate inflammation and AWR via mechanisms that differ from steroids. This has provided the impetus for *in vivo* studies to examine the potential of these compounds to provide further clinical benefit than that available from current treatment for asthma.

Murine models of allergen-induced inflammation have been developed which display some of the features of asthma, namely inflammation, AWR and AHR. In Balb/c mice sensitised and challenged with OVA, the PPAR γ ligands CG and GI25670 inhibited the OVA-induced increase in inflammatory cells and levels of pro-inflammatory mediators in the bronchoalveolar lavage (BAL) fluid and lung [22,26,27]. In a separate study, RG inhibited the migration of antigen-loaded dendritic cells in the mediastinal lymph nodes and increased IL-10 production [28].

It has been suggested that activation of PPAR γ may also regulate airway wall remodelling since CG decreased basement membrane thickening and collagen deposition in mouse airways in response to antigen sensitization and challenge [29]. This suggests that PPAR γ ligands may influence matrix deposition in these models, perhaps through regulation of the activity of matrix metalloproteinases (MMPs) or their inhibitors, as has been described in cultured airway epithelial cells [30]. Alternatively, collagen synthesis may be directly affected, as has been shown in cultured hepatic stellate cells [31].

Some of these studies have used measurements of enhanced pause (Penh) during unrestrained plethysmography to show that administration of PPAR γ ligands attenuated the OVA-induced hyperresponsiveness to MCh [22,29]. However, it may be premature to conclude that synthetic PPAR γ ligands reduce AHR on the basis of these studies. Many distinguished pulmonary physiologists consider the use of Penh without appropriate confirmation using pulmonary mechanics, as having the potential to litter the literature with misleading conclusions [32]. Confirmation of these findings requires measurement of the effects of a PPAR γ ligand on AHR using invasive measurements of respiratory resistance and compliance. In addition, it is of interest to determine the direct effects of PPAR γ ligands on ASM tone as these have not previously been examined *in vitro*.

We report here the effects of RG in a model of allergen-induced inflammation in C57BL/6 mice. We determined

whether the inhibitory effects of this synthetic PPAR γ ligand on airways inflammation, remodelling and reactivity could be demonstrated in this strain, since C57BL/6 mice have augmented eosinophilia and attenuated AHR compared to Balb/c mice following allergen challenge [33]. In this model, changes in BAL fluid cell number and MMP-2 activity were used as markers of inflammation, while measurement of goblet cell hyperplasia was used as an indicator of AWR. Invasive measurements of the obstructive response to MCh were used to assess AHR. Organ bath studies were also performed using isolated guinea pig tracheal rings to assess any direct effects of RG on the constrictor response to MCh *in vitro*.

2. Methods

2.1. *In vivo murine model*

Female C57BL/6 mice (6–8 weeks, 20–25 g) were obtained from ARC, Perth, Australia. Experiments were performed with approval from the Animal Experimentation Ethics Committee of the University of Melbourne, following guidelines from the National Health and Medical Research Council. Mice were sensitised with an intraperitoneal (i.p.) injection of 50 μ g ovalbumin (OVA)(Grade V; Sigma) mixed with Al(OH) $_3$ (10 mg ml $^{-1}$) in 200 μ l PBS on days 0 and 12. On days 19–26 mice received an i.p. injection of either vehicle (boiled peanut oil, Sigma, USA) or 5 mg kg $^{-1}$ rosiglitazone (RG) (GlaxoSmithKline, UK). Two hours post treatment on days 20–26, mice were challenged with either a nebulised solution of OVA (1% v v $^{-1}$ in PBS) or PBS alone for 30 min. On day 27, mice were anaesthetised for measurements of airway reactivity, then killed with an injection of pentobarbital sodium (600 mg kg $^{-1}$ i.p.) prior to collection of bronchoalveolar lavage fluid and lung tissue.

2.2. *Bronchoalveolar lavage (BAL) fluid collection*

The trachea was cannulated and the lungs lavaged with 400 μ l PBS (with 0.25% BSA). The lungs were gently massaged before the fluid was withdrawn and stored on ice. This procedure was repeated three more times with 300 μ l solution.

2.2.1. *Total and differential cell counts*

To determine total cell count in the BAL fluid, cells were stained with ethidium bromide and acridine orange and counted with a haemocytometer. Differential cell counts were carried out after cytoSpin (Shandon Scientific, Chesire, UK) and staining with Diff-Quik (Lab Aids, NSW). Leukocytes were identified by standard morphology with at least 200 cells being counted under $\times 400$ magnification.

2.2.2. *Assay of MMP activity*

Bronchoalveolar lavage fluid was assayed for proteolytic MMP activity by gelatin zymography. Conditioned media from human cultured airway smooth muscle was used a positive control for MMP-2 activity. Samples (40 μ l) were mixed with 20 μ l Laemmli 2 $\times$ sample buffer (0.5 M Tris–HCl, 20% glycerol, 10% SDS, 1 mg ml $^{-1}$ bromophenol blue, pH 6.8), then 40 μ l of the mixture was separated in 12% acrylamide gels containing 1 mg ml $^{-1}$ bovine skin gelatin (Type B, 75 Bloom). Once proteins were separated, gel slabs were washed for 15 min in 2.5% v v $^{-1}$ Triton-X. Gel slabs were then incubated overnight at 37 °C on a rocking platform in substrate buffer (50 mM Tris, 5 mM CaCl $_2$, 1 μ M ZnCl $_2$, 0.5% v v $^{-1}$ triton-X, 0.01% sodium azide). Following overnight incubation, gels were washed for 10 min in water, and then stained with coomassie blue for 1 h. Gels were then destained for 4–6 h by repetitive washing, and images were captured and densitometry performed using Kodak 1D software (Eastman Kodak Company, NY, USA).

2.3. *Airway histology and measurement of goblet cells*

Lung tissue was collected immediately following BAL fluid collection, and perfused with 10% neutral buffered formalin solution via a tracheal cannula for 15 min, at a pressure of 25 cmH $_2$ O. Tissue was then placed in formalin overnight before dissection to obtain the right middle lobe and bronchus stump which were then stored in 100% ethanol. The tissue was embedded in paraffin for histological analysis, with all tissues embedded in the same orientation. Transverse sections of the bronchus were stained with haematoxylin and eosin for morphology, and a combined alcian blue, periodic acid-Schiff (ABPAS) stain (Sigma Chemical Co., St Louis, MO) was used to allow enumeration of goblet cells.

Images of stained sections were captured via a video camera (Hitachi) at low magnification and stored for image analysis (Image Pro Plus, ver. 4.1.22.1, Media Cybernetics). ABPAS-stained sections were used to enumerate goblet cells (under 400 $\times$ magnification) and all data were normalised for basement membrane length.

2.4. *Measurement of airway reactivity*

On day 27, mice were anaesthetised with a mixture of ketamine (200 μ g g $^{-1}$) and xylazine (10 μ g g $^{-1}$) (Parnell Laboratories, Australia). A tracheostomy was performed, and a catheter placed in the jugular vein to administer MCh. Mice were then placed in whole body plethysmograph and mechanically ventilated. The transpulmonary pressure was measured with a differential pressure transducer with one port attached to the interior of the plethysmograph and the other to the intratracheal cannula. The airflow rate was measured with a pneumotachograph. The airways resistance and compliance in response to intravenous MCh

(62–1000 $\mu\text{g kg}^{-1}$, given 4 min apart) was calculated on-line with a Buxco pulmonary mechanics analyser (Buxco Inc., Connecticut, USA). At the conclusion of the experimental protocol, the animals were killed by an overdose of anaesthetic.

2.5. *In vitro organ bath studies using isolated guinea pig tracheal rings*

Following CO_2 -induced anaesthesia (80% CO_2 , 20% O_2) female guinea pigs (~ 400 g) were killed by exsanguination. Trachea was removed and cut into 8 equivalent sections ~ 0.5 cm. Tracheal rings were mounted at 2 g tension in organ baths containing Krebs solution (in mM) (NaCl 118, KCl 4.7, NaHCO_3 25, $\text{MgCl}_2 \cdot \text{H}_2\text{O}$ 0.5, $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 1.0 and D (+) glucose 11.1) bubbled with carbogen. After 1 h equilibration, a maximum contraction was obtained using acetylcholine (ACh, 1 mM). After 1 h, RG (1 μM , 3 μM , 10 μM) or vehicle (dimethyl sulphoxide [DMSO]) was added and incubated for 30 min. Cumulative concentration response curves to MCh (10^{-8} to 10^{-4} M) were generated until a maximum contraction was achieved.

2.6. Statistical analysis of results

All data are presented as mean $\pm$ standard error of the mean (SEM) for n observations. For total and differential cell counts, the data for each group were log transformed and a one-way analysis of variance (ANOVA) was used to compare the effects of drug treatment and challenge protocol. Dunnett's posthoc test was used for comparison between selected pairs of data. One-way ANOVA was also used to compare goblet cell numbers and MMP-2 activity. A two-way ANOVA with repeated measures was used to compare the effects of drug treatment and challenge protocol on the changes in resistance and compliance with MCh. Probability levels of $P < 0.05$ were considered to be of statistical significance. Statistical analyses were carried out using the GraphPad PRISMTM (Version 4) statistical package.

3. Results

3.1. Effect of rosiglitazone on OVA-induced BAL inflammation

After 7 days challenge with OVA, the total cell number in the BAL fluid was $1.18 \pm 0.28 \times 10^6$ (Table 1), compared with $0.15 \pm 0.01 \times 10^6$ cells in the saline-challenged group. RG (5 mg kg^{-1} i.p. daily) had no effect on the low numbers of inflammatory leukocytes in the saline-challenged mice and did not inhibit the OVA-induced increase in BAL cellularity ($P > 0.05$ cf vehicle/OVA). Differential cell count analysis established that OVA challenge significantly increased the numbers of eosinophils, lymphocytes, neutrophils, and monocytes in the BAL fluid, with the greatest increase observed in eosinophils (Table 1). RG treatment did not modulate these measures of airways inflammation, as it had no effect on individual cell types.

3.2. Effect of rosiglitazone on OVA-induced goblet cell hyperplasia

The bronchus was examined for changes in the number of goblet cells in the airway epithelial layer. OVA-challenged mice had four-fold higher goblet cell numbers than saline-challenged mice (Fig. 1, one way ANOVA, $P < 0.05$). RG treatment during the challenge period had no effect on goblet cell metaplasia (Fig. 1).

3.3. Effect of rosiglitazone on OVA-induced MMP-2 activity

Matrix metalloproteinase-2 (MMP-2, gelatinase A) activity in BAL fluid was increased approximately 3-fold by OVA sensitisation and challenge (Fig. 2). RG did not affect MMP-2 activity in saline- or OVA-challenged mice compared to vehicle-treated controls.

3.4. Effect of rosiglitazone on OVA-induced airways hyperresponsiveness

To examine the effect of RG on airway reactivity, we measured airways resistance and dynamic lung compliance

Table 1
The effect of rosiglitazone (RG) on BAL total cell number and differential cell count

	Total BAL cell number ($\times 10^{-3}$)	Differential cell count ($\times 10^{-3}$)			
		Neutrophils	Eosinophils	Macrophages/monocytes	Lymphocytes
Vehicle/saline	152.1 ± 12.5	0.4 ± 0.3	2.7 ± 1.4	134.4 ± 10.7	7.0 ± 1.4
RG/saline	154.3 ± 30.3	2.2 ± 1.9	20.6 ± 15.7	116.2 ± 12.8	14.0 ± 6.1
Vehicle/OVA	$1175.7 \pm 281.5^{**}$	$11.0 \pm 4.5^{**}$	$738.4 \pm 225.5^{**}$	183.7 ± 25.5	$156.0 \pm 38.6^{**}$
RG/OVA	$913.4 \pm 170.2^{**}$	$10.0 \pm 3.3^{**}$	$544.6 \pm 150.1^*$	211.2 ± 21.0	$130.1 \pm 29.3^{**}$

BAL fluid was collected 24 h after the last ovalbumin (OVA) or saline challenge and assessed for total cellularity. Differential cell counts were made on cytospin preparations from each BAL fluid sample. A minimum of 200 cells was counted for each preparation. Data are shown as mean $\pm$ SEM for $n = 14$ –16 mice per group. Statistical analysis of log-transformed data was conducted using a one-way ANOVA with posthoc Dunnett's test where appropriate. $^*P < 0.05$, $^{**}P < 0.01$ cf vehicle/saline.

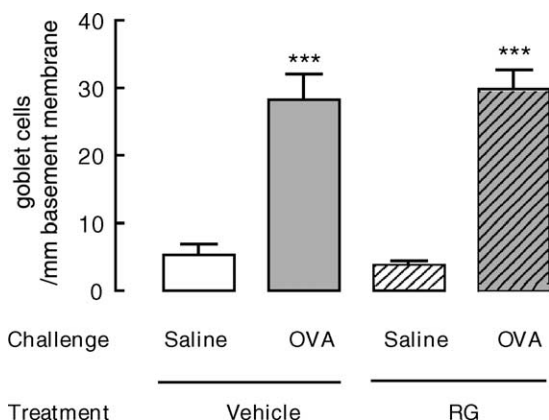


Fig. 1. The effect of rosiglitazone treatment on the increase in goblet cell number from sections of mouse bronchus following OVA challenge. The data for each group are presented as mean $\pm$ SEM of goblet cell number, normalised for basement membrane length in saline-challenged ($n=6$ for vehicle treated, $n=8$ for RG-treated) and OVA-challenged ($n=9$ for vehicle- and RG-treated) mice. *** $P<0.001$, one-way ANOVA, cf vehicle-treated, saline-challenged mice.

in anaesthetised mice using invasive whole body plethysmography.

There was no difference in the airways resistance at rest in the saline- or OVA-challenged mice with or without RG treatment. The increase in airways resistance in response to MCh ($62\text{--}1000\text{ }\mu\text{g kg}^{-1}$) was greater in OVA-challenged mice than in saline-challenged mice (* $P<0.05$ two-way ANOVA, Fig. 3). RG treatment had no effect on MCh responsiveness in saline-challenged mice, but partially reduced this measure of AHR in OVA-challenged mice.

MCh caused a dose-dependent decrease in dynamic lung compliance (C_{Dyn}) in all treatment groups (Fig. 3). OVA challenge did not regulate the MCh-induced decrease in C_{Dyn} relative to saline-challenge. Similarly, RG treatment

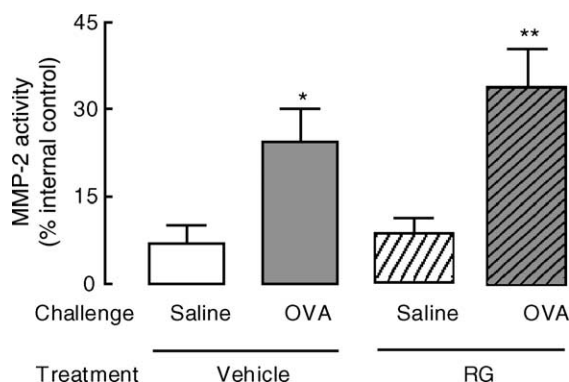


Fig. 2. The effect of rosiglitazone treatment on the increased level of MMP-2 activity in the BAL fluid following OVA challenge. The data for each group are presented as mean $\pm$ SEM of MMP-2 activity, normalised as % of positive control in saline-challenged ($n=8$ for vehicle-treated, $n=6$ for RG-treated) and OVA-challenged ($n=8$ for vehicle-treated, $n=6$ for RG-treated) mice. Conditioned media from human cultured ASM was used as a positive control for MMP-2 activity. * $P<0.05$, ** $P<0.01$ one way ANOVA, cf vehicle/saline).

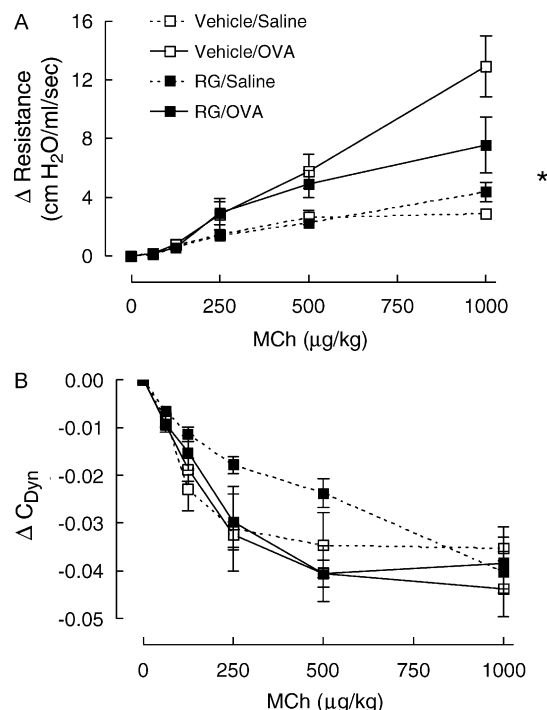


Fig. 3. Effect of rosiglitazone (RG) on OVA-induced changes in lung function. MCh-induced airways resistance (A) and dynamic lung compliance (B) were calculated in anaesthetised, mechanically-ventilated mice 24 h after the last challenge. Mean $\pm$ SEM values presented are the maximum change observed for R_R and C_{Dyn} following MCh injection in saline-challenged ($n=7$) and OVA-challenged ($n=8$) mice. A two-way ANOVA with posthoc Bonferroni's test was performed to calculate differences between treatment groups. For RG/OVA, † $P<0.05$ relative to Vehicle/OVA, * $P<0.05$ relative to vehicle/saline.

did not significantly influence C_{Dyn} relative to vehicle treatment ($P>0.05$, two-way ANOVA cf vehicle/saline)

3.5. Effect of rosiglitazone on contractile function of isolated guinea pig tracheal rings

Addition of rosiglitazone ($1\text{--}10\text{ }\mu\text{M}$) did not affect basal tension in guinea pig tracheal rings (data not shown). Rosiglitazone did not affect the sensitivity of the rings to MCh-induced contraction (Fig. 4). The pEC_{50} for the contraction was 5.16 ± 0.08 for vehicle-treated rings and 5.32 ± 0.09 for rings in the presence with $10\text{ }\mu\text{M}$ RG ($n=4$, $P>0.05$ cf control). There was no concentration-related effect of RG on the maximum response to MCh. A similar lack of effect was observed using histamine as the contractile agent (data not shown).

4. Discussion

In vitro studies have shown that synthetic PPAR γ ligands, such as the thiazolidinediones rosiglitazone (RG) and CG, can inhibit the release of inflammatory cytokines from airway epithelial cells [7,21] and smooth muscle cells [16] and reduce smooth muscle proliferation [16,24].

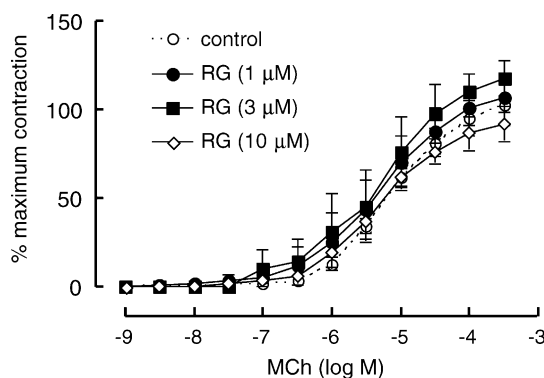


Fig. 4. Effect of rosiglitazone (RG) on contraction of isolated guinea pig tracheal rings in response to MCh. Results are expressed as mean $\pm$ SEM of the contraction for $n=4$ rings, normalised to the maximum contraction to 1 mM acetylcholine. Rings were pre-incubated with RG (1–10 μ M) or vehicle for 30 min prior to preparation of a cumulative concentration response curve to MCh.

Given the growing evidence of anti-inflammatory and anti-remodelling actions of these agents, it is not surprising that there is considerable interest in whether the promising in vitro activity profile of synthetic PPAR γ ligands would extrapolate to the in vivo setting.

The hypothesis that RG regulates inflammation, AWR and AHR was tested in the current study, using a murine model of airway inflammation. OVA was used as the allergen in C57BL/6 mice in a sensitisation and 7 day aerosol challenge protocol. In this model, OVA-treated mice showed a marked increase in BAL fluid cell number, goblet cell hyperplasia and an increased obstructive response to MCh, compared to saline-treated controls. The impact of RG on changes in these markers of inflammation, AWR and AHR after OVA exposure was assessed after administration of 5 mg kg $^{-1}$ RG, on day 19, and 2 h prior to aerosol challenge on days 20–26. This dose of RG has been shown previously to improve insulin sensitivity in mice [34] and prevent up-regulation of pro-inflammatory mediators in rats [35]. Similar doses of PPAR γ ligands have been used in recent studies in other murine models of allergen-induced inflammation. Our studies reveal that in C57BL/6 mice, treatment with RG reduces the OVA-induced increase in airway reactivity to MCh, in the absence of a significant effect on the OVA-induced increase in BAL inflammatory cells, MMP-2 activity and goblet cell number, following sensitisation and challenge with OVA. In addition, organ bath studies performed using isolated guinea pig tracheal rings showed that RG did not inhibit the constrictor response to MCh in vitro.

In our in vivo model, RG treatment had no effect on the increased number of eosinophils or other leukocytes in the BAL fluid after OVA exposure. It was proposed that RG would inhibit this increase, since numerous in vitro studies have shown that thiazolidinediones regulate release of cytokines and chemokines promoting infiltration of inflammatory cells [7,16–21], and can reduce IL-5-induced

eosinophil survival and chemotaxis [22,23]. In other acute models of lung inflammation using Balb/c mice, the PPAR γ ligands CG and GI 262570 caused a dose-dependent reduction in BAL eosinophils and lymphocytes [22,26].

Given this background, the failure to observe an effect of RG on the influx of inflammatory cells was surprising. The current finding where RG did not reduce inflammatory cell number in the BALF may reflect a relatively reduced potency of the PPAR γ ligand tested here to inhibit the augmented OVA-induced eosinophilia that has previously been described in the C57BL/6 strain [33]. In addition, the effects of RG on inflammatory cell infiltration into the lung tissue or the state of activation of these cells have not been assessed and are worthy of further investigation.

Characteristics of AWR include goblet cell metaplasia, thickening of the basement membrane and collagen deposition. The role of PPAR γ in regulating these changes is unclear. Treatment of Balb/c mice with nebulised CG has recently been associated with a reduction in all of these indicators of AWR [29]. However, our study failed to show an impact of RG on the OVA-induced increase in goblet cell number. This observation is in agreement with a lack of effect of orally administered CG on mucous production in Balb/c mice [27]. Our results may be due to route of administration, rather than a strain difference in sensitivity of this parameter to the effects of synthetic PPAR γ ligands. The degradation of interstitial matrix proteins by MMPs is a contributor to airway wall remodelling. The increase in MMP-2 (gelatinase A) proteolytic activity in the BAL with OVA treatment was not affected by RG in this study. However, it has previously been reported that RG inhibits MMP-9 (gelatinase B) but not MMP-2 expression in bronchial epithelial cell lines [30], so a role for PPAR γ ligands in regulating MMP activity in the airways cannot be excluded.

Despite the inability of RG to modulate inflammation and goblet cell hyperplasia in our animal model, the finding that RG reduces AHR is of great interest. This study is the first to demonstrate an effect of a synthetic PPAR γ ligand on airway reactivity measured by MCh-induced changes in respiratory resistance and compliance. This is important in light of recent criticism of the use of Penh measurements to draw conclusions about the effects of potential therapeutic agents on airways hyperreactivity [32]. Previous studies using nebulised CG in Balb/c mice showed that CG completely prevented the MCh-induced increase in Penh after allergen sensitisation and challenge [22,29]. Rosiglitazone reduced the increase in airways resistance after OVA challenge, without affecting the decrease in lung compliance, reflecting an effect on the airways rather than the parenchyma of the lung. The failure of RG to completely inhibit OVA-induced airways resistance may reflect the failure to use a high enough dose of RG, or alternatively there may be factors contributing to AHR that are resistant to the actions of RG.

Although not tested in this study, attempts to confirm the PPAR γ -dependence of the effects of thiazolidinediones have been made in some studies using the selective PPAR γ antagonist GW9662 [36]. The inhibitory effects of CG on eosinophil chemotaxis in vitro and on the OVA-induced airway eosinophilia in Balb/C mice were reversed by GW9662, suggestive of a PPAR γ -dependent mechanism [22]. In addition, GW9662 completely abrogated the inhibitory effect of CG on the MCh-induced increase in Penh after allergen sensitisation and challenge [22,29]. The current study using another PPAR γ ligand RG in C57BL/6 mice provides further support for the proposed role of PPAR γ in the regulation of AHR.

It is important to determine whether any direct effects of RG on the airways have contributed to the decrease in AHR observed. The only prior evidence suggestive of a potential effect of RG on muscle tone was a study in cultured endothelial cells showing that RG prevents the insulin-induced release of the vasoconstrictor endothelin [37]. The effects of RG were tested in isolated guinea pig tracheal rings, the benchmark tissue to examine the ability of drugs to modulate the obstructive response to a range of agents, including MCh and histamine. It is more commonly used than mouse tissue because the latter does not respond to histamine. Concentration-response curves were prepared in the absence and presence of up to 10 μ M RG, a concentration that has been shown to have both anti-proliferative and anti-inflammatory effects in human ASM [15,24]. No apparent modulation of basal tone, or contraction in response to MCh or histamine was observed. This observation was not completely unexpected, as the mechanism of action of the PPAR γ receptor signalling requires the intracellular hormone receptor to bind to DNA and modulate transcription of target genes. Therefore, the time-course of these genomic actions would limit the likelihood of observing any acute effects in vitro in an acute experiment. A limitation of the current study is the absence of data from isolated mice trachea, so that the possibility of a difference in a direct relaxant effect of RG in mice versus guinea pigs cannot be excluded. However, in the saline-challenged mice that had been treated with RG for 7 days, MCh reactivity was unchanged, providing further evidence to support the conclusion that neither acute nor chronic exposure of ASM to RG directly inhibits contractile responsiveness.

In conclusion, our studies reveal that in C57BL/6 mice, treatment with RG reduces the OVA-induced increase in airway reactivity to MCh, in the absence of a significant effect on the OVA-induced increase in BAL inflammatory cells, MMP-2 activity and goblet cell number, following sensitisation and challenge with OVA. Therefore, RG has been shown to modulate AHR in this murine model of allergen-induced inflammation by a mechanism that is independent of inhibition of inflammatory cell recruitment to the airway. Other recent reports of reductions in inflammation and AWR in addition to AHR following use

of synthetic PPAR γ agonists in other murine models [22,26,27,29] underline the importance of understanding the underlying mechanisms through which these effects occur. The implications of these findings for evaluation of PPAR γ as a potential therapeutic target for asthma therapy remain to be explored. However, the development of more selective PPAR γ ligands to modulate specific cellular processes which contribute to airway pathophysiology may provide additional therapeutic benefit in future treatment of asthma.

Acknowledgements

Rosiglitazone was a generous gift from GlaxoSmithKline, U.K. This study was funded in part by a grant from NHMRC (#299823, Australia)

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