



Impacts of peroxisome proliferator-activated receptor- γ activation on cigarette smoke-induced exacerbated response to bacteria

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ABSTRACT Chronic obstructive pulmonary disease (COPD) is characterised by a state of chronic pulmonary inflammation punctuated by microbial exacerbations. Despite advances in treatment options, COPD remains difficult to manage. In this study, we investigated the potential of peroxisome proliferator-activated receptor (PPAR) γ activation as a new therapy against cigarette smoke-induced inflammation and its associated bacterial exacerbation.

C57BL/6 mice were exposed to room air or cigarette smoke for either 4 days or 4 weeks and treated either prophylactically or therapeutically with rosiglitazone. The impact of rosiglitazone on cigarette smoke-induced exacerbated response to the bacterial pathogen nontypeable *Haemophilus influenzae* (NTHi) was studied using the therapeutic treatment protocol.

We found that rosiglitazone was able to reduce cigarette smoke-induced neutrophilia both when administered prophylactically or therapeutically. Therapeutic intervention with rosiglitazone was also effective in preventing cigarette smoke-induced neutrophilia exacerbation following NTHi infection. Moreover, the anti-inflammatory effects of rosiglitazone did not lead to an increase in the pulmonary bacterial burden, unlike dexamethasone.

Altogether, our data suggest that pharmacological activation of PPAR γ may be an effective therapeutic approach to improve COPD management, as it is able to reduce cigarette smoke-induced inflammation and decrease the magnitude of bacterial exacerbations, without compromising the ability of the immune system to control the infection.



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Introduction

Chronic obstructive pulmonary disease (COPD) is a chronic lung disorder characterised by irreversible airway flow obstruction [1]. COPD is punctuated by acute exacerbations, which are associated with high mortality and exert a large toll on healthcare systems [2–5]. The management of COPD exacerbations is complex, as current pharmacological therapies, such as corticosteroids, appear to have relatively little therapeutic impact on COPD [6]. Moreover, anti-inflammatory therapies have to be used with vigilance, since COPD patients are prone to pulmonary bacterial infections [7]. For this reason, current protocols to manage COPD exacerbations include antibiotics [8, 9]. Therefore, more targeted anti-inflammatory therapies are required to improve COPD management.

Peroxisome proliferator-activated receptor (PPAR) γ is a nuclear receptor involved in lipid metabolism [10]. Deletion of PPAR γ in macrophages leads to pulmonary inflammation suggesting that its expression by pulmonary macrophages is essential in the maintenance of proper lung homeostasis [11]. Moreover, PPAR γ agonists have been shown to have anti-inflammatory properties in animal models of acute lung inflammation [12–14]. Therefore, we investigated whether PPAR γ activation reduces cigarette smoke-induced inflammation and attenuates the exacerbated response to bacterial infection associated with cigarette smoke exposure.

Using pre-clinical models, we found that PPAR γ activation by rosiglitazone was able to reduce cigarette smoke-induced inflammation in both prophylactic and therapeutic settings. Unlike dexamethasone, rosiglitazone decreased the magnitude of the inflammatory response to nontypeable *Haemophilus influenzae* (NTHi) in cigarette smoke-exposed mice without impairing bacterial clearance. Altogether, PPAR γ activation may be a new target to decrease pulmonary inflammation in COPD patients and help manage the frequency and the magnitude of bacterial exacerbations.

Methods

Animals

Female 6–8 week-old C57BL/6 mice were purchased from Charles River Laboratories (Montreal, Canada). Mice were housed under specific pathogen-free conditions with *ad libitum* access to food and water and subjected to a light–dark cycle of 12 h. McMaster University Animal Research Ethics Board (Hamilton, Canada) approved all experiments.

Cigarette smoke exposure and therapy protocols

C57BL/6 mice were exposed to room air or cigarette smoke for either 4 days or 4 weeks as previously described [15, 16]. Briefly, mice were exposed twice daily for 1 h, 5 days a week to the mainstream smoke of 12 cigarettes using a whole-body exposure system (SIU48; Promech Inc., Vintrie, Sweden). Total particulate matter ranged from 700 to 900 $\mu\text{g}\cdot\text{L}^{-1}$. We previously showed that cotinine and carboxyhemoglobin levels are similar to those observed in human smokers following exposure [15].

Rosiglitazone (Sigma Aldrich, St Louis, MO, USA) was administered intraperitoneally in 125 μL of corn oil at 20 $\text{mg}\cdot\text{kg}^{-1}$. Sham mice received corn oil only. An outline of the experimental protocol for both the prophylactic and therapeutic interventions is provided in figure 1a. Dexamethasone 21-phosphate disodium salt (Sigma Aldrich) was administered *i.p.* in 125 μL of PBS at 80 $\text{mg}\cdot\text{kg}^{-1}$. All *i.p.* injections were performed daily 1 h before the first cigarette smoke exposure.

Lung processing

Lungs were removed from the thoracic cavity and the trachea cannulated with a polyethylene tube. Right lobes were tied off, dissected and either frozen in liquid nitrogen or kept for bacterial burden assessment. Bronchoalveolar lavage (BAL) was performed by instilling the lungs sequentially with 250 μL and 200 μL of ice cold PBS. The lavaged lung (left lung) was then inflated with 10% formalin at a pressure of 30 mL H_2O and kept for histological assessment. Total cell concentration was assessed using a haemocytometer. Cells were then pelleted (550 G, 4°C) and the BAL fluid (BALF) kept at -80°C for cytokine assessment. Cells were re-suspended in PBS. Cytospins were prepared and stained with Hema 3 (Biochemical Sciences Inc., Swedesboro, NJ, USA) for differential cell counts; 300 cells were counted per cytospin.

NTHi infection and lung bacterial burden assessment

Strain 11P6 of NTHi used in the present study was isolated from the sputum of a patient with COPD experiencing an acute exacerbation. 12 h prior to sacrifice, $\times 10^6$ bacteria in 35 μL of PBS were administered intranasally. Details of the experimental protocol have been reported previously [17]. Sham mice received vehicle only (PBS). At the time of sacrifice, the right lung was harvested prior to lavage and homogenised in 1 mL of sterile PBS using a Polytron PT 2100 homogeniser (Kinematica, Luzern,

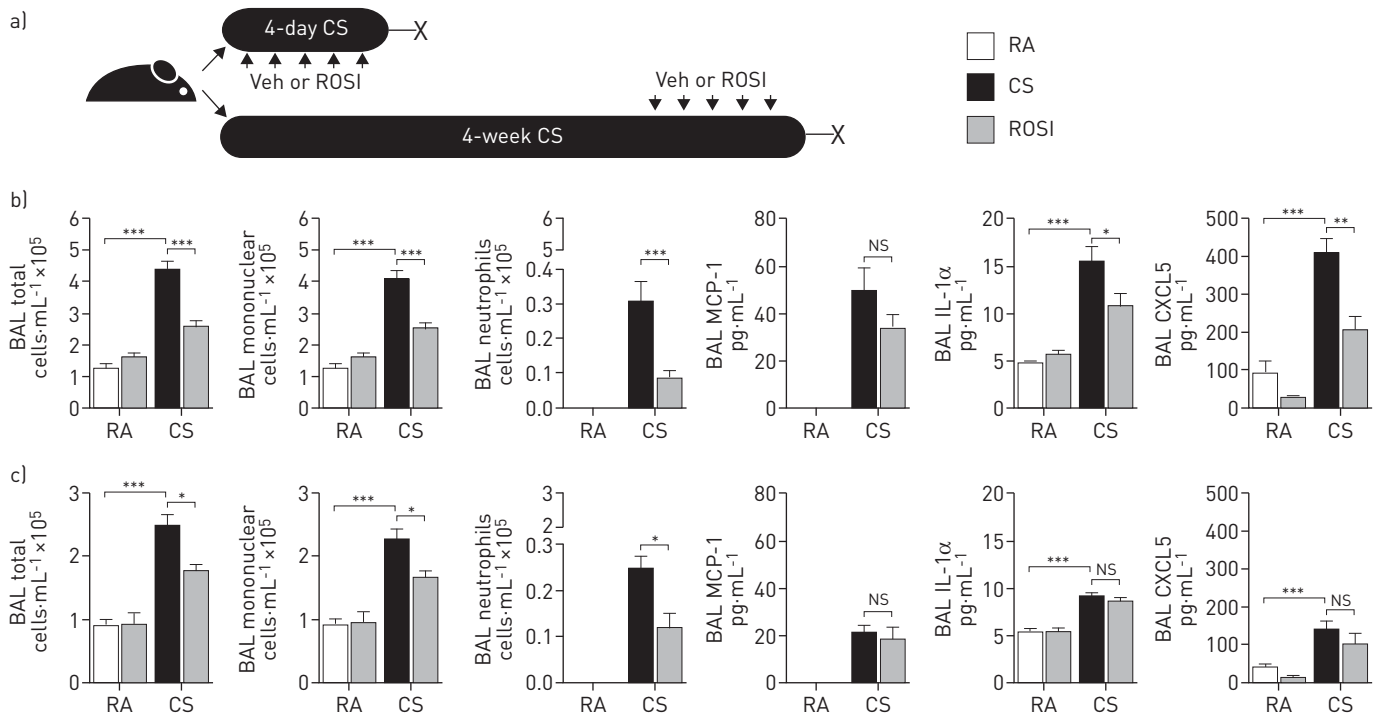


FIGURE 1 Prophylactic and therapeutic administration of rosiglitazone (ROSI) reduces cigarette smoke-induced inflammation. C57BL/6 mice were exposed to room air (RA) or cigarette smoke (CS) for 4 days or 4 weeks. ROSI was delivered prophylactically in the 4-day or therapeutically in the 4-week exposure protocol. Control animals received vehicle (Veh) only. a) Schematic of the experimental protocol. b, c) Bronchoalveolar lavage (BAL) cellular and cytokine assessment from RA- and CS-exposed mice treated either b) prophylactically or c) therapeutically with ROSI were measured by ELISA. MCP: monocyte chemotactic protein; IL: interleukin; NS: nonsignificant. Data are presented as mean \pm SEM. n=5 per group. *: p<0.05; **: p<0.01; ***: p<0.001.

Switzerland). Lung homogenates were serially diluted in PBS and plated on chocolate agar plates. Bacterial burden was expressed as colony forming units per mL of homogenate.

Ex vivo alveolar macrophage NTHi binding/phagocytosis assay

Mouse lungs were lavaged five times with 1 mL of PBS. Lavage fluids were pooled and centrifuged at 550 \times g. Pellets were resuspended in RPMI (10% fetal bovine serum, L-glutamine, β -mercaptoethanol) and alveolar macrophages isolated by adherence to a culture-treated 96-well plate (5×10^4 alveolar macrophages per well; 2 h at 37 $^{\circ}$ C, 5% CO₂). Alveolar macrophages were incubated with $\times 10^6$ freshly grown NTHi (multiplicity of infection of 20) for 2 h at 37 $^{\circ}$ C, 5% CO₂ in Hanks' balanced salt solution. Alveolar macrophages were thoroughly washed five times with PBS and then lysed in 300 μ L of sterile water for 10 min. Lysate was diluted in PBS and plated on chocolate agar. Bacterial count was used to assess to amount of bacteria that were either bound or phagocytosed by alveolar macrophages.

Cytokine assessment

Interleukin (IL)-1 α , monocyte chemotactic protein (MCP)-1 and CXCL5 were measured by ELISA in BALF according to manufacturer's instructions (R&D Systems, Minneapolis, MN, USA).

BALF bactericidal assay

2×10^3 NTHi bacteria in 50 μ L of PBS were incubated for 1 h at 37 $^{\circ}$ C with 50 μ L of BALF from mice that were exposed to room air or cigarette smoke for 4 weeks and treated therapeutically with rosiglitazone or vehicle (fig. 1a). Samples were then diluted in PBS and plated on chocolate agar. The concentration of live bacteria was determined and compared between the groups, representing the bactericidal activity of BALF samples on NTHi.

NTHi-specific antibody measurement

BALF antibodies specific for NTHi were measured as described previously [18]. Briefly, wells of a NUNC 96-well plate (Nalge Nunc International, Penfield, NY, USA) were coated with NTHi lysate overnight at 4 $^{\circ}$ C. Wells were washed three times (0.05% Tween-20 in PBS) and blocked for 1 h at room temperature (0.05% Tween, 1% fat-free milk in PBS). Wells were incubated with diluted BALF (0.05% Tween, 0.2%

fat-free milk in PBS) for 2 h at room temperature and washed three times prior to incubation with a rabbit anti-mouse immunoglobulin (IgM+IgA+IgG) conjugated to horseradish peroxidase (1:1000; Invitrogen, Grand Island, NY, USA) for 1 h at room temperature. Wells were washed and incubated with the chromogen TMB substrate (BioFX Laboratories, Owings Mills, MD, USA). Optical density was measured at 450 nm.

Histological staining

PPAR γ protein expression was assessed by immunohistochemistry using formalin-fixed lung sections. Briefly, lung sections (3 μ m) were deparafinised in xylene and rehydrated in ethanol/water. Endogenous peroxidases were blocked in 3% H₂O₂ in methanol. Citrate buffer antigen retrieval was performed (45 min). Sections were blocked with 1% swine serum in TBS with 0.01% Tween-20. Sections were stained at 4°C overnight with a rabbit anti-mouse PPAR γ (1:50; Abcam, Cambridge, UK). Primary antibody was detected with a biotinylated, goat anti-rabbit IgG (1:100; 1 h room temperature) followed by streptavidin-horseradish peroxidase (Dako, Glostrup, Denmark). Staining was visualised using 3-amino-9-ethylcarbazole. Sections were not counterstained to avoid interference with nuclear staining.

Statistical analysis

Statistical analyses were performed using GraphPad Prism Software (La Jolla, CA, USA). Two group comparisons were made using an unpaired t-test with a significance threshold at 0.05. Experimental protocols with more than two groups were compared using a one-way ANOVA (significant threshold at 0.05) followed, if applicable, by a Bonferroni *post hoc* test.

Results

Comparable PPAR γ expression pattern in room air- and cigarette smoke-exposed mice

To investigate the impact of cigarette smoke on PPAR γ expression, we exposed C57BL/6 mice to room air or cigarette smoke for 4 weeks and assessed PPAR γ expression by immunohistochemistry. We previously reported that cigarette smoke exposure results in pulmonary inflammation characterised by increased numbers of mononuclear cells and neutrophils in BAL [15, 16]. Figure 2 shows that PPAR γ was predominantly expressed in the airways and parenchyma by alveolar macrophages and bronchial epithelial cells. The localisation was nuclear with some evidence of cytoplasmic staining. No changes in the expression pattern or staining intensity were observed between room air- and cigarette smoke-exposed mice. These findings show that PPAR γ is expressed in the lungs and suggest that cigarette smoke-induced inflammatory processes do not affect expression patterns.

PPAR γ activation attenuates cigarette smoke-induced inflammation

Next, we investigated whether PPAR γ activation using rosiglitazone attenuates cigarette smoke-induced inflammatory processes. To this end, C57BL/6 mice were exposed to room air or cigarette smoke for 4 days or 4 weeks. In the 4-day protocol, rosiglitazone was administered daily, 1 h prior to the first smoke exposure (prophylactic intervention). In the 4-week protocol, rosiglitazone was administered daily in the fourth week of smoke exposure, a time point at which cigarette smoke-induced inflammation was established. A schematic of the intervention protocol is shown in figure 1a. Prophylactic intervention with rosiglitazone attenuated the increase in BAL mononuclear cells and neutrophils by 67% and 72%, respectively, compared to cigarette smoke-exposed, vehicle-treated mice (fig. 1b). When administered therapeutically, BAL mononuclear cells and neutrophils were reduced by 45% and 52%, respectively (fig. 1c).

To investigate mechanisms of attenuated inflammation, we assessed the expression of IL-1 α and CXCL5 in the BALF of room air and cigarette smoke-exposed mice. Both inflammatory mediators have previously been implicated in cigarette smoke-induced inflammatory processes, especially neutrophil recruitment [19, 20]. We observed reduced expression of IL-1 α and CXCL5 in the prophylactic intervention protocol (fig. 1b), but not when rosiglitazone was administered therapeutically (fig. 1c). BALF levels of MCP-1, a monocyte and dendritic cell chemoattractant that is strongly induced by cigarette smoke exposure, were unchanged by either intervention protocol (fig. 1b and c). Decreased expression of inflammatory mediators probably contributed to attenuate inflammation. These data show that rosiglitazone attenuates cigarette smoke-induced inflammation when administered both prophylactically and therapeutically. Mechanistically, PPAR γ activation attenuated inflammatory mediator expression in the prophylactic, but not the therapeutic setting.

PPAR γ activation reduces bacterial exacerbation of cigarette smoke-induced inflammation

Previous reports showed that PPAR γ activation attenuates inflammation induced by bacterial agents such as lipopolysaccharide [12]. For this reason, we assessed whether rosiglitazone attenuates bacterial exacerbation of cigarette smoke-induced inflammation. C57BL/6 mice were exposed to room air or

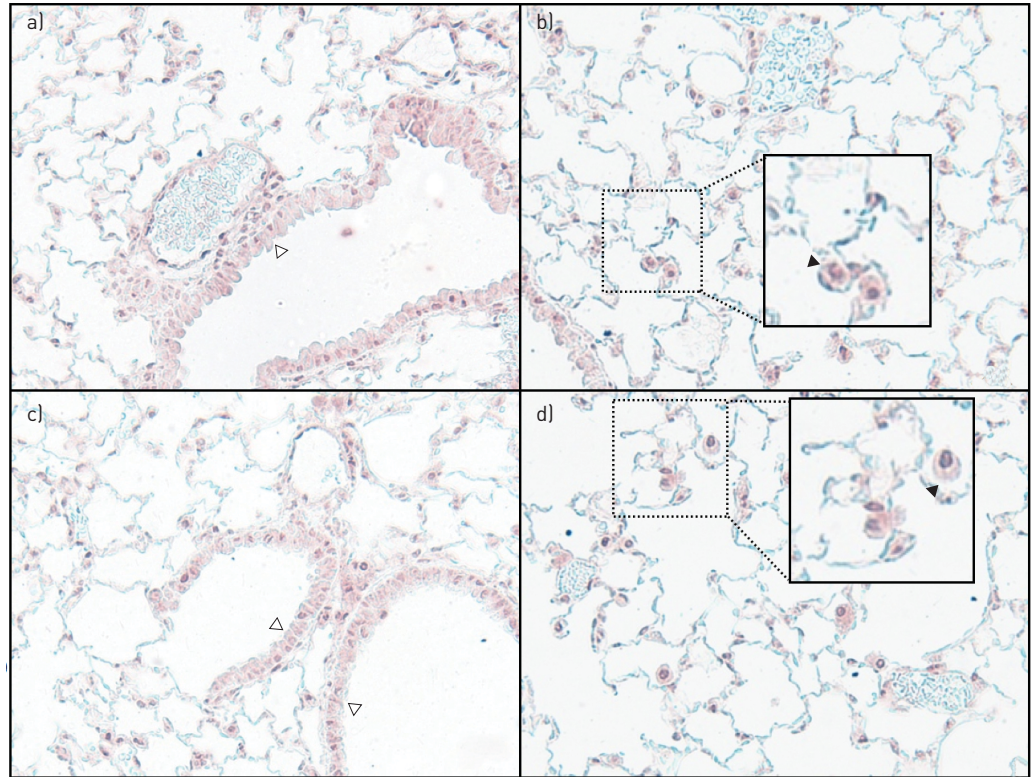


FIGURE 2 Peroxisome proliferator-activated receptor (PPAR) γ expression in room air- and cigarette smoke-exposed mice. C57BL/6 mice were exposed to a, b) room air or c, d) cigarette smoke for 4 weeks. PPAR γ expression was assessed in lung tissue sections by immunohistochemistry (brown stain). Open arrows indicate bronchial epithelial cells, closed arrows indicate alveolar macrophages. Original magnification 200 \times .

cigarette smoke for 4 weeks and infected intranasally with NTHi. Rosiglitazone was administered daily during the fourth week of cigarette smoke exposure. A schematic of the experimental protocol is shown in figure 3a. In room air-exposed mice, administration of rosiglitazone did not attenuate inflammatory processes elicited by NTHi infection (fig. 3b). Moreover, we observed similar increased levels of IL-1 α , MCP-1 and CXCL5 in the BALF of mice treated with rosiglitazone or vehicle. Of interest, rosiglitazone administration significantly attenuated BAL neutrophilia in cigarette smoke-exposed NTHi-infected mice (fig. 3c). Levels of neutrophils were comparable to room air-exposed NTHi-infected mice. While BALF levels of IL-1 α and MCP-1 remained unchanged, expression of CXCL5 was significantly decreased. Altogether, these findings suggest that therapeutic administration of rosiglitazone is effective in attenuating bacterial exacerbation of cigarette smoke-induced inflammation. This observation contrasts the lack of an anti-inflammatory effect of rosiglitazone in room air-exposed, NTHi-infected mice.

PPAR γ activation does not compromise bacterial clearance

Next, we investigated whether the anti-inflammatory properties of PPAR γ interfered with bacterial clearance. C57BL/6 mice were exposed to room air or cigarette smoke for 4 weeks and treated with rosiglitazone prior to NTHi infection as described in figure 3a. We found that room air- and cigarette smoke-exposed mice treated therapeutically with rosiglitazone had a lower lung bacterial burden compared to vehicle-treated mice following bacterial administration (fig. 3b and c). These findings show that rosiglitazone does not compromise bacterial clearance and attenuates inflammatory processes in cigarette smoke-exposed mice infected with NTHi.

PPAR γ activation does not restore phagocytic activity of alveolar macrophages

Cigarette smoke exposure has been shown to compromise phagocytic activity of alveolar macrophages [21, 22]. We next investigated if rosiglitazone treatment restored the phagocytic activity of alveolar macrophages *ex vivo*. C57BL/6 mice were exposed to cigarette smoke for 4 weeks and treated with rosiglitazone during the fourth week of smoke exposure. Alveolar macrophages were isolated and phagocytosis of NTHi was assessed. Figure 4a shows that rosiglitazone did not restore binding/phagocytic

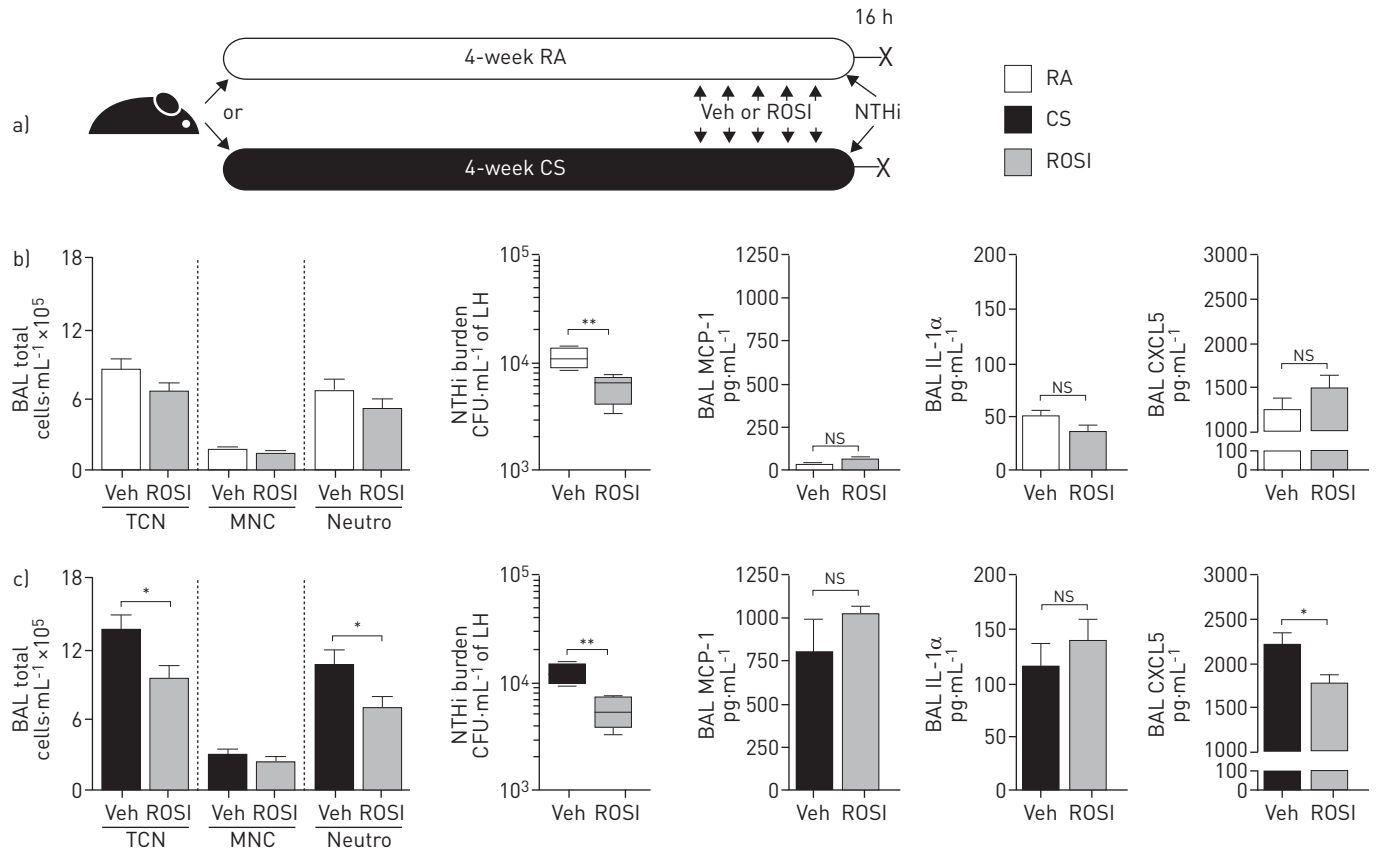


FIGURE 3 Therapeutic administration of rosiglitazone (ROSI) attenuates bacterial exacerbation of cigarette smoke (CS)-induced inflammation. C57BL/6 mice were exposed to room air (RA) or CS for 4 weeks. ROSI or vehicle (Veh) was delivered daily in the fourth week of the CS-exposure protocol. a) Schematic of the experimental protocol. b, c) Bronchoalveolar lavage (BAL) cellular and cytokine assessment from b) RA- and c) CS-exposed mice infected with nontypeable *Haemophilus influenzae* (NTHi). MCP: monocyte chemotactic protein; IL: interleukin; TCN: total cell number; MNC: mononuclear cells; Neutro: neutrophil; CFU: colony forming unit; LH: lung homogenate; NS: nonsignificant. Data are presented as mean \pm SEM. n=5 per group. *: p<0.05; **: p<0.01.

ability of alveolar macrophages. Hence, mechanisms other than improved phagocytic activity of alveolar macrophages must contribute to improved bacterial control.

PPAR γ activation does not increase BALF bactericidal activity or pulmonary NTHi-specific antibodies

As rosiglitazone treatment improved NTHi clearance *in vivo* but did not restore the phagocytic activity of alveolar macrophages, we next investigated whether rosiglitazone increases the levels of soluble anti-bacterial factors and/or NTHi-specific antibodies in the BALF. To this end, NTHi was incubated with BALF from mice exposed to room air or cigarette smoke for 4 weeks and treated therapeutically with rosiglitazone, as described in figure 1a. We found that neither cigarette smoke nor rosiglitazone had an effect on the bactericidal activity of the BALF (fig. 4b). Moreover, as previously reported, cigarette smoke exposure led to an increase in NTHi-specific antibodies in the BALF [18]. This increase was prevented by treatment with rosiglitazone (fig. 4c). Therefore, the increased bacterial clearance associated with rosiglitazone administration does not appear to be caused by the induction of soluble anti-bacterial factors or NTHi-specific antibodies.

Comparison between rosiglitazone and dexamethasone therapy

Clinically, corticosteroids are used to manage COPD exacerbations [6]. For this reason, we directly compared rosiglitazone and dexamethasone interventions in our model of bacterial exacerbation of cigarette smoke-induced inflammation. C57BL/6 mice were exposed to room air or cigarette smoke for 4 weeks. Dexamethasone and rosiglitazone were administered *i.p.* in the fourth week of the smoke exposure protocol as described in figure 3a. Mice were infected with NTHi and sacrificed 12 h later. Both dexamethasone and rosiglitazone significantly attenuated bacterial exacerbation of cigarette smoke-induced neutrophilic inflammation (fig. 5a). The magnitude of the effect was comparable between dexamethasone and rosiglitazone. However, while dexamethasone intervention was associated with a six-fold increase in bacterial burden compared to cigarette smoke-exposed, NTHi-infected mice, the bacterial burden

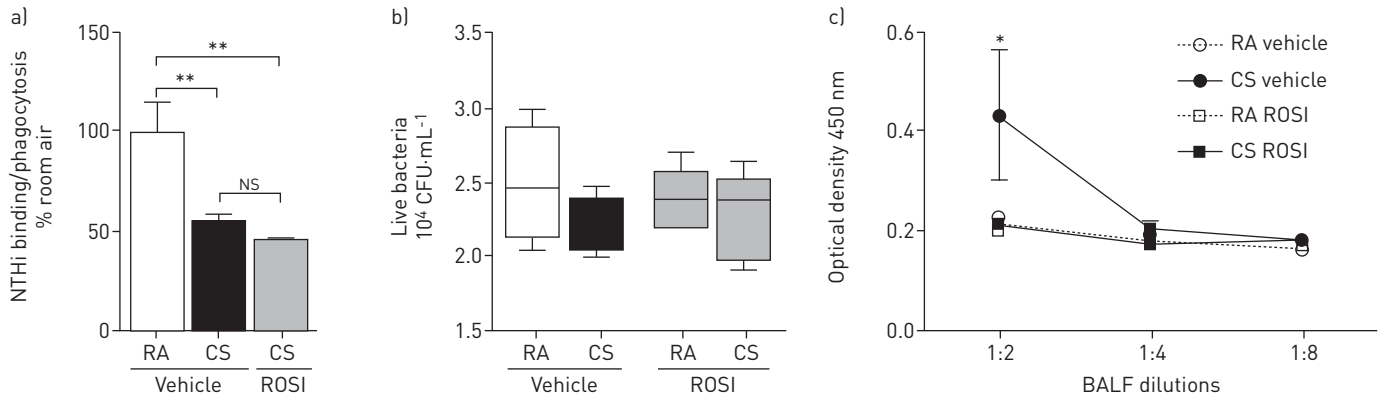


FIGURE 4 Impact of *in vivo* rosiglitazone (ROSI) treatment. a) Alveolar macrophages were isolated and subjected to *ex vivo* nontypeable *Haemophilus influenzae* (NTHi) binding/phagocytosis assay. b) Bronchoalveolar lavage fluid (BALF) bactericidal assay activity on NTHi was assessed *ex vivo*. c) Total NTHi-specific antibody levels were measured by ELISA. C57BL/6 mice were exposed to room air (RA) or cigarette smoke (CS) for 4 weeks. ROSI or vehicle was delivered daily in the fourth week of the CS-exposure protocol. Data are presented as mean±SEM. n=5 per group. NS: nonsignificant; CFU: colony forming unit. *: p<0.05; **: p<0.01.

remained controlled in rosiglitazone-treated mice (fig. 5b). Levels of MCP-1 and IL-1 α in the BALF remained unchanged in both treatment groups, while levels of CXCL5 were similarly decreased by the dexamethasone and rosiglitazone treatment. These findings suggest that rosiglitazone intervention is superior to dexamethasone, as rosiglitazone exerts anti-inflammatory effects without compromising functions essential to controlling bacteria.

Discussion

Accumulating evidence suggests that PPAR γ activation has beneficial effects in preclinical pulmonary diseases models [12–14]. In the present study, we investigated the therapeutic potential of the PPAR γ activation in models of cigarette smoke-induced inflammation, as well as bacterial exacerbation. To the best of our knowledge, we are the first to report that activation of PPAR γ is a potential target to attenuate bacterial exacerbation of cigarette smoke-induced inflammation.

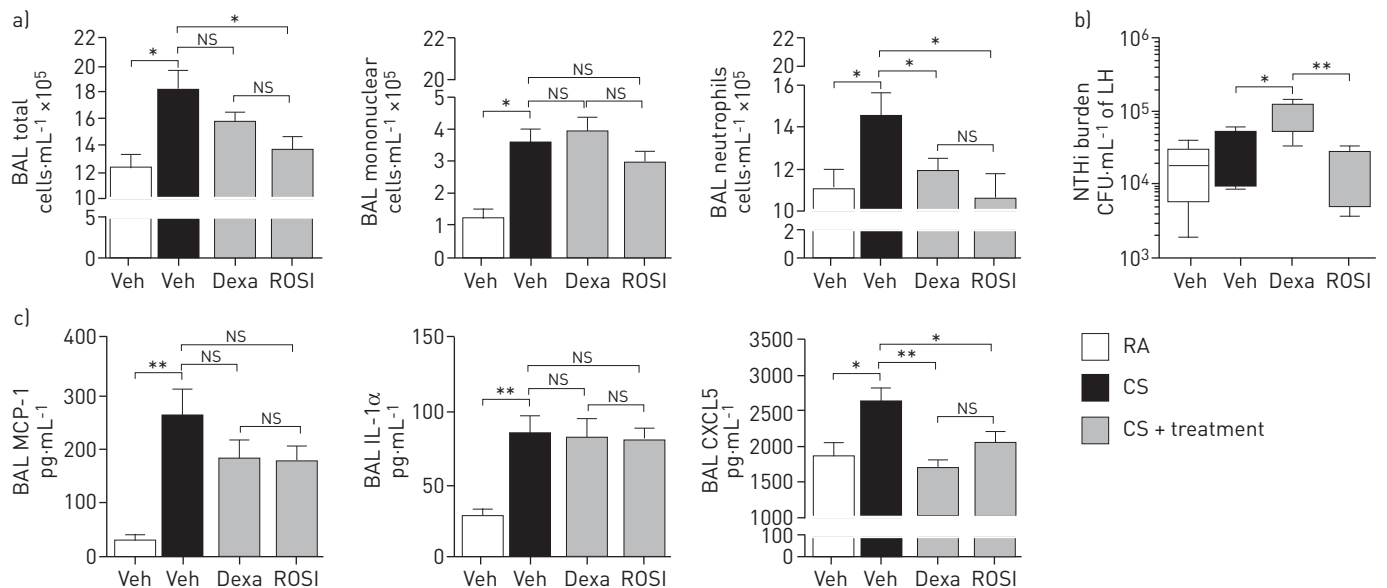


FIGURE 5 Comparison of dexamethasone (Dexa) and rosiglitazone (ROSI) treatment on bacterial exacerbation of cigarette smoke (CS)-induced inflammation. C57BL/6 mice were exposed to room air (RA) or CS for 4 weeks. ROSI, Dexa or vehicle (Veh; corn oil) were delivered daily in the fourth week of the CS-exposure protocol. a) Bronchoalveolar lavage (BAL) cellular profile, b) nontypeable *Haemophilus influenzae* (NTHi) pulmonary bacterial burden and c) BAL cytokine assessment. CFU: colony forming unit; LH: lung homogenate; MCP: monocyte chemotactic protein; IL: interleukin; NS: nonsignificant. Data are presented as mean±SEM. n=5 per group. *: p<0.05; **: p<0.01.

The experiments were carried out using a well-characterised pre-clinical model of cigarette smoke-induced inflammation in mice [16, 19]. Cigarette smoke exposure is well tolerated and elicits neutrophilic inflammation in the lungs. Bacterial exacerbation of cigarette smoke-induced inflammatory processes was modelled using a clinical isolate of NTHi, which is a Gram-negative bacterium frequently associated with COPD exacerbations [23]. Studies described here were pursued using live, replicating bacteria to investigate the consequences of PPAR γ activation on bacterial clearance. Details of the experimental protocol have been reported elsewhere [17].

In the lungs, PPAR γ is mainly expressed by alveolar macrophages and bronchial epithelial cells, which are two cell types believed to play an important role in the pathogenesis of COPD [24, 25]. Cigarette smoke exposure did not change the expression pattern of PPAR γ as assessed by immunohistochemistry. Our findings are in agreement with observations in human smokers and COPD patients [26], suggesting that the impact of PPAR γ localisation and cigarette smoke on its expression are comparable between humans and mice. This makes the pre-clinical model a valid tool to assess the therapeutic impact of PPAR γ activation on cigarette smoke-induced inflammation and the associated exacerbated inflammatory response following bacterial infection.

Prophylactic activation of PPAR γ decreased cigarette smoke-induced inflammation. These findings are in agreement with a previous report by LEA *et al.* [26] using a different cigarette smoke exposure system. This attests to the solidity and reproducibility of the observed phenomenon. However, the clinical relevance of prophylactic intervention protocols is not immediately intuitive as, clinically, most patients would initiate therapies after years of smoking. To this end, we decided to investigate whether PPAR γ activation could reduce established cigarette smoke-induced inflammation. Of note, therapeutic activation of PPAR γ reduced neutrophil levels in the BAL, providing clear evidence that PPAR γ regulates established cigarette smoke-induced inflammatory processes. SHAN *et al.* [27] recently published a study reporting that PPAR γ activation attenuated formation and reversed established emphysema in cigarette smoke-exposed mice. These observations complement our findings and suggest that activation of PPAR γ in smokers and COPD patients could have beneficial effects, as it is able to attenuate inflammatory processes, as well as improve tissue pathologies.

Bacterial exacerbations are a major cause of morbidity and mortality in patients with COPD [3–5]. We found that PPAR γ activation was also able to attenuate bacterial exacerbation of cigarette smoke-induced inflammatory processes. These beneficial effects were achieved therapeutically. We observed reduced numbers of neutrophils; a cell type that can cause local damage by releasing proteases and reactive oxygen species. Interestingly, the therapy had little effect on inflammatory processes in room air-exposed, NTHi-infected mice. This suggests that pharmacological activation of PPAR γ affects pathways activated by bacteria in cigarette smoke-exposed lungs, but not normal lungs. Additionally, we previously reported that PPAR γ activation attenuates viral exacerbation of cigarette smoke-induced inflammatory processes [16]. Therefore, PPAR γ -activating therapies may prove to be effective in controlling both bacterial and viral exacerbations of COPD, thus possibly reducing symptoms and the risks of hospitalisation.

A key observation of this study is that the anti-inflammatory effect of PPAR γ activation did not interfere with clearance of the bacteria from the lung. This finding becomes particularly interesting when compared to corticosteroid-based intervention. While dexamethasone administration attenuated inflammation, it was also associated with a significant increase in lung bacterial burden in NTHi-infected, cigarette smoke-exposed mice, as previously reported [17]. In support of this, mounting evidence suggests that chronic use of corticosteroids is associated with an increased risk of pneumonia [28]. Our data provide preclinical evidence that PPAR γ activation decreases cigarette smoke-induced inflammation and bacterial exacerbation without compromising bacterial clearance. Due to its anti-inflammatory properties and positive impact on bacterial clearance, PPAR γ -based therapies may help to control inflammatory processes in COPD patients, reduce the frequency of bacterial exacerbations, and improve the management of COPD exacerbation. Clinical studies, however, are required to translate our observations in animal models to the clinic.

Mechanisms that contribute to the beneficial effects of PPAR γ activation on cigarette smoke-induced inflammation and bacterial exacerbation are still not well understood. PPAR γ biology is complex and affects a multitude of cellular pathways [10]. Of interest, PPAR γ activation has been shown to dampen inflammatory processes by interfering with nuclear factor- κ B activation *in vitro* [29]. In the present study, rosiglitazone reduced IL-1 α expression when administered prophylactically, but not therapeutically. This suggests that PPAR γ activation does not mediate its effects by reducing IL-1 α production. This is surprising as IL-1 α is a critical cytokine required for cigarette smoke-induced neutrophilia [19]. It is plausible that rosiglitazone acts downstream of IL-1 α and dampens the epithelial and endothelial response, thus decreasing the release of chemokines and the recruitment of immune cells. In support of this

interpretation, levels of CXCL5, a chemokine mainly released by epithelial cells [30], were significantly attenuated by rosiglitazone in the context of bacterial exacerbation. Further investigations are required to better understand the impact of PPAR γ activation on the pulmonary response to cigarette smoke.

PPAR γ activation improved bacterial clearance, but it appears that the mechanisms do not involve the restoration of alveolar macrophage phagocytic ability. This contrasts with the positive impact of PPAR γ activation on efferocytosis capacity [26]. Moreover, it is unlikely that bacterial killing capacity of the macrophages is improved with PPAR γ agonists, as studies showed that *Mycobacterium tuberculosis* uses PPAR γ activation as a mechanism to survive within macrophages [31]. Also, improved bacterial clearance does not seem to be due to an increase in soluble anti-bacterial factors, such as defensins, as rosiglitazone did not affect the bactericidal activity of BALF. We previously reported that cigarette smoke exposure led to the accumulation of NTHi-specific antibodies in the BAL of mice. Mechanistically, these antibodies accelerated clearance of NTHi in cigarette smoke-exposed mice following bacterial inoculation [18]. Rosiglitazone attenuated the accumulation of NTHi-specific antibodies in cigarette smoke-exposed mice, ruling out the contribution of humoral immune processes to the improved bacterial control.

While we succeeded in excluding a number of potential mechanisms, how rosiglitazone improves bacterial clearance in cigarette smoke-exposed mice remains unknown. PPAR γ is known to activate lipid metabolism [10]. It has recently been shown that cigarette smoke exposure leads to the generation of modified phospholipids that inhibit phagocytosis of bacteria [22]. Therefore, PPAR γ activation may help in processing inhibitory phospholipids from the extracellular lung environment to allow binding of the bacteria by alveolar macrophages and neutrophils. Following the same line of thought, it is also possible that by reducing cigarette smoke-induced neutrophil recruitment and improving macrophage efferocytosis [26], rosiglitazone may lower the efferocytic pressure on pulmonary macrophages and allow them to more effectively clear the bacterial inoculum.

Rosiglitazone and other glitazones have been associated with an increased risk of cardiovascular events and black box warnings have been issued [32]. Since COPD is associated with cardiovascular complications [33], the use of PPAR γ agonists in these patients may need to be closely monitored. Moreover, rosiglitazone is delivered systemically in humans. It is plausible that local administration of these molecules may help reduce the impact of glitazones on the cardiovascular system.

Our study shows that therapeutic PPAR γ activation reduces cigarette smoke-induced inflammation and exacerbated inflammatory responses to pulmonary bacterial infection in a pre-clinical mouse model. A direct comparison between rosiglitazone and dexamethasone further revealed that mice treated with a PPAR γ agonist maintained control over the bacteria, while corticosteroid treatment was associated with increased bacterial burden. Our study contributes to an increasing body of literature documenting the beneficial effects of PPAR γ activation in pre-clinical models of diseases. The clinical impact of these findings is of significant interest since many PPAR γ agonists are already approved for human use, and are therefore available to COPD patients.

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