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Role of peroxisome proliferator activator receptor-gamma (PPAR-γ) in lung sepsis

Usha Q Patel[1], Grazia V Clemencet[1], Colleen B Latruffe[2], Peter A Reddy[1], Qin Chu[3], Charlene J Heyman[1], Alice E Griffin[1*]

Abstract

PPAR-gamma has been implicated in the pathology of numerous diseases including; obesity, diabetes, atherosclerosis, and cancer. PPAR-gamma agonists have been used in the treatment of hyperlipidaemia and hyperglycemia. Two isoforms of PPARG are detected in the human and in the mouse: PPAR-γ1 (found in nearly all tissues except muscle) and PPAR-γ2 (mostly found in adipose tissue and the intestine). In the present study, to directly determine the role of PPARγ in lung sepsis we used PPARγ-knockout and C57/BL6 mice model. Mice are treated with Lipopolysaccharide LPS (0.5 mg/kg, iv) for 6-hours, the plasma and tissue cytokines TNF-α, IL-1β, IL-6, IL-10, MIP-1 and KC are analyzed by ELISA. The infiltrations of the mononuclear cells in the lung tissue and degree of lung tissue injury are examined using immunofluorescent and histopathology staining respectively. In PPARγ-knockout mice, LPS induced more expression of pro-inflammatory cytokines expression, which was associated with a marked monocyte infiltration, tissue injury and elevated lung activity of myeloperoxidase compared with wild type C57/BL6 mice. Present study, suggests that PPARγ has a critical role in attenuate lung sepsis and further study need to elucidate the clinical value.

Key words: PPARγ; LPS; Proinflammatory cytokines; Lung; Immunofluorescent; PPARγ-knockout mice

*Corresponding author email: Alice.G11@yahoo.com
[1]Department of Medicine, University of Pittsburgh School of Medicine, PA, USA
[2]Department of Biochemistry, Faculty of Medicine, Johann Wolfgang Goethe-University Frankfurt, Germany
[3]Jinling Hospital, Medical School of Nanjing University, China.
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Introduction

The family of transcription factors designated peroxisome proliferator-activated receptors (PPARs) has long been studied for its role in regulation of lipid and glucose metabolism [1]. More recently, PPARs' role in immunoregulation has been recognized and is the subject of intense investigation [2]. PPARs are expressed by a variety of cells of the immune system including monocytes, macrophages, B and T lymphocytes, natural killer cells, dendritic cells, neutrophils, eosinophils, and mast cells [3].

PPARs belong to the nuclear hormone receptor superfamily that regulates a multitude of genes [4]. There are three PPARs encoded by separate genes: PPAR*α*, PPAR*β*/*δ*, and PPAR*γ* [5].

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The three PPARs differ in their structure, function, and tissue distribution [6]. PPARγ has received significant attention as a key regulator of adipocyte differentiation as well as glucose and lipid homeostasis [1]. PPARγ can be transcribed from three distinct mRNAs, γ1, γ2, and γ3, based on sites of transcription initiation and splicing [4–6]. However, there are only two protein isoforms, PPARγ1 and PPARγ2, as translation of γ1 and γ3 mRNAs results in indistinguishable proteins [6]. PPARγ1 is the predominant isoform [7]. Whereas expression of PPARγ2 and PPARγ3 mRNAs is restricted [6], PPARγ1 mRNA is expressed fairly ubiquitously [2].

A variety of ligands, natural and synthetic, are capable of stimulating PPARγ activity. Natural PPARγ ligands include saturated and unsaturated fatty acids, eicosanoid derivatives such as 15-deoxy-Δ- prostaglandin J_2 (15d-PGJ_2), and nitrated fatty acids such as nitrated linoleic and oleic acids [8]. Synthetic PPARγ agonists are represented by thiazolidinediones (TZDs) such as pioglitazone, rosiglitazone, troglitazone, and ciglitazone.

In addition, some nonsteroidal anti-inflammatory drugs such as indomethacin, fenoprofen, and ibuprofen can activate PPARγ, although their binding affinity is lower than that of TZDs. In the absence of these agonists, PPARγ remains inactive, bound to a series of corepressors. Upon ligand activation, these corepressors are displaced, allowing PPARγ to heterodimerize with retinoid X receptors and initiate transcriptional control by binding to specific peroxisome proliferator response elements in the promoter regions of target genes [9].

PPARγ agonists may have other activities, though. For example, pioglitazone has been shown to alter mitochondrial function in a PPARγ-independent manner [10] and nitrated fatty acids are electrophiles that alkylate and may inactivate target proteins [3]. These off-target effects may be either helpful or harmful, depending on the context. Additionally, PPARγ agonists are known to upregulate the receptor's expression, which may render the effects of repeated dosing greater than would be anticipated from single-dose results [6].

Recognition that PPARγ is expressed by a variety of immune cells stimulated interest in its immunoregulatory function, especially its anti-inflammatory role [2]. Involvement of PPARγ in several leukocyte functions supports its prominent role in immunoregulation [7]. Protein and mRNA expression and activity of PPARγ are altered during many inflammatory conditions, and such alterations appear to be a significant factor in the pathogenesis of some diseases [12, 13].

We are surrounded by a variety of microbial species and are constantly interacting with them throughout our lives. Some of these microorganisms are commensal or even beneficial, while others are pathogens that can cause significant morbidity and mortality. The innate immune system, characterized by secretion of proinflammatory cytokines and antimicrobial molecules and recruitment of phagocytes, is a major mediator of resistance to infection by pathogenic bacteria.

Compromised or dysregulated immunity can allow development of major illnesses requiring therapeutic intervention, yet in some cases inflammatory responses themselves can become life-threatening. Although many infectious diseases can be controlled by antibacterial drugs,

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antimicrobial resistance poses a significant threat to our healthcare system worldwide, compromising therapy, complicating treatment, increasing mortality, and resulting in substantial financial costs [14]. Drugs with novel mechanisms of action are therefore urgently needed. Recent advances in our understanding of PPARγ's role in immunity, infection, and inflammation, as discussed below, offer the opportunity for intervention with a novel approach to bacterial infections. Appropriate antimicrobial therapy will continue to be the standard of care, but adjunctive use of PPARγ agonists or antagonists may reduce the required antibiotic dosages and improve outcomes.

PPARγ's anti-inflammatory function is also prominent in chronic granulomatous disease (CGD), an inherited disorder in which phagocytes' defective ability to kill certain infectious pathogens results in chronic and recurrent infections and inflammation. In a mouse model of CGD, macrophages demonstrate reduced PPARγ expression and activation and impaired efferocytosis of apoptotic neutrophils during zymosan-induced acute inflammation [15]. Monocytes from human CGD patients similarly show defective efferocytosis [16]. Furthermore, neutrophils and monocytes/macrophages from these CGD mice as well as monocytes from human CGD patients exhibit defects in PPARγ-dependent production of mitochondrial reactive oxygen species (ROS) that contribute to bacterial killing [17]. These defects can be largely restored by PPARγ activation with pioglitazone, given prophylactically or during preexisting inflammation [18], providing further evidence for the antibacterial effect of PPARγ activation. Importantly, the authors showed that pioglitazone is capable of enhancing CGD phagocytes' ability to clear pathogens such as *S. aureus* and *Burkholderia cepacia*, restoring host defense against these pathogens [4]. Furthermore, the PPARγ agonist pioglitazone produced marked clinical improvement in a 5-month-old boy with CGD and multiple severe infections [19]. Significantly improved ROS production was associated with reductions in pathogen burden and improvements in overall clinical condition that allowed curative hematopoietic stem cell transplantation. Although this reflects an unusual setting, these direct clinical results support the ability of PPARγ agonists to upregulate pathogen killing and clearance.

Infections by a variety of bacteria can result in sepsis, in which blood-borne toxins lead to an exaggerated and dysregulated inflammatory response that frequently results in tissue injury [20]. In severe sepsis, potentially lethal septic shock and multiple organ failure become strong possibilities [9]. PPARγ signaling has shown a protective effect in multiple models of sepsis. In the mouse model of lipopolysaccharide- (LPS-) induced sepsis involving pulmonary inflammation and injury, endothelial cell PPARγ (ePPARγ) deficiency intensifies the tissue injury with increased pulmonary edema and capillary permeability, elevated ROS and cytokine/chemokine production, infiltration of neutrophils to the lungs, and expression of inflammation-associated adhesion molecules such as ICAM-1 and PECAM-1. This exacerbation of inflammatory responses in ePPARγ-deficient mice is due to enhanced toll-like receptor-4 (TLR4) expression in the lung tissues and upregulation of TLR4 downstream signaling including the NF-κB pathway [6]. TLR4 signaling has been shown to play a key role

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in modulating inflammation/sepsis [6]. In addition to the effects of PPARγ agonists reported by others, Reddy et al. observed that physiologically relevant concentrations of 10-nitro-oleic acid reduce LPS-induced transcription of many inflammatory markers and inhibit neutrophil transmigration *in vitro* [3].

The protective effect of PPARγ activation is also demonstrated in mouse and rat models of polymicrobial sepsis using cecal ligation and puncture (CLP) [7]. Zingarelli et al. found that rats subjected to CLP exhibit reduced PPARγ expression in the lungs and thoracic aortas, increased circulating neutrophils accompanied by reduction in lymphocytes, and increased accumulation of neutrophils in multiple vital organs. Elevated levels of mediators of sepsis-associated vascular dysfunction and hypotension were also detected. These cellular and molecular changes were shown to reflect upregulation of the proinflammatory transcription factors NF-κB and AP-1. 15d-PGJ_2 and ciglitazone prolong the animals' survival, reversing the sepsis-associated proinflammatory events and improving arterial blood pressure [4]. Likewise, in mice experiencing polymicrobial sepsis, pioglitazone reduces bacterial burden at the site of infection (the peritoneum) and in the blood and alleviates edema and capillary congestion at target tissues such as the lungs by reducing neutrophil infiltration and cytokine accumulation. Survival rate of septic mice consequently improves. The authors found that PPARγ activation exerts its protective effect against bacterial sepsis via an IL-10-dependent reduction in expression of MyD88, a critical downstream component of the TLR pathway [21].

Materials and Methods

Mice

Adult male mice, weighing 25±2 g (supplied by The Field Surgery Institute, Kunming Medical University, Kunming, China), were housed in 12-h light-dark conditions with free access to water and standard laboratory chow. The animal procedures were performed in strict accordance with National Institutes of Health guidelines, which were approved by the Ethics Committee of Department of Medicine, University of Pittsburgh School of Medicine, PA, USA

Reagents

A TRIzol kit was obtained from Gibco-BRL (Carlsbad, CA, USA) and a reverse transcription kit was obtained from University of Pittsburgh School of Medicine. A polymerase chain reaction (PCR) amplification reagent kit and the DNA ladder marker. β-actin was obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and tumour necrosis factor-α (TNF-α), interleukin (IL)-6, -1β and -10 enzyme-linked immunosorbent assay (ELISA) kits were obtained from Pierce Biotechnology Inc. (Rockford, IL, USA). Salidroside (99% purity) was acquired from the National Institute for the Control of University of Pittsburgh School of Medicine.

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Model and groups

Using a random number table, 77 mice were randomly divided into four groups: Normal control, sham-operation (sham), sepsis model (model) and salidroside treatment (treatment) groups (n=20 per group). The sepsis model was induced by cecal ligation and puncture (CLP). Briefly, the animals were deprived of food, but water was permitted for 6 h prior to surgery. Under light ether anaesthesia, a laparotomy was performed through a midline abdominal incision. The cecum was punctured twice at different sites with an 18-gauge needle and gently compressed until faeces were extruded. The bowel was then returned to the abdomen and the abdominal incision was closed in two layers. Animals in the model and treatment groups were treated with 1.25 ml normal saline per 25 g body weight subcutaneously at the completion of surgery to replace the extracellular fluid sequestered during peritonitis [22]

Animals in the sham group underwent sham surgery, in which the cecum was neither ligated nor punctured. Starting at 8 h prior to surgery, animals in the treatment group were injected with 8 mg/kg salidroside every 8 h, while the animals in the normal control, sham and model groups were administered the same volume of normal saline.

Subsequently, the animals in each group were anaesthetised with ether 24 h post-surgery, and the right internal carotid artery was isolated. Blood was extracted (1 ml), centrifuged to collect the supernatant, dispensed into two sterile tubes, sealed with sealing glue and placed in freezer at −20°C for examination. All the animals were sacrificed 24 h following surgery via anesthesia and tissue samples were collected for further tests.

Extraction of RNA

For the isolation of lung tissue RNA, the mice were humanely sacrificed and under aseptic conditions, the lung tissue was removed and immediately frozen in liquid nitrogen. Prior to RNA extraction, the lung samples were homogenised in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) using a Mixer Mill 301. The total RNA was extracted according to the manufacturer's instructions. The RNA samples were electrophoresed in agarose gel and visualised with ethidium bromide for quality control.

Real-time quantitative PCR

Total cellular RNA was extracted by using the RNeasy RNA extraction kit (Qiagen, Valencia, CA, USA) and Trizol mRNA extraction kit (Thermo Fisher Scientific) according to manufacturer instructions. cDNA was synthesized from 1 μg or 500 ng total RNA by using the SuperScript first-strand synthesis system (Thermo Fisher Scientific), as previously described [22].

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Western blotting

Protein extraction, electrophoresis, and gel transfer were performed as previously described [23]. Anti-human PON-2 was purchased from Abcam (Cambridge, MA, USA). Anti-PPARγ was purchase from Cell Signaling Technology (Danvers, MA, USA) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), which was used as housekeeping gene, was purchased from Santa Cruz Biotechnology. Cell lysates were prepared by using cell lysis buffer (Cell Signaling Technology). ZO-1, claudins-1 and -4, and occludin Abs used for immunoblotting were purchased from Thermo Fisher Scientific.

Histopathological examination

Lung tissue was fixed in 10% formalin for 24 h, which was followed by dehydration. The lung tissue was embedded in paraffin wax, sectioned into 5-μm-thick slices and stained with haematoxylin and eosin. Microphotography of the lung sections was captured with a light microscope (Olympus, Tokyo, Japan). The severity of the ALI was scored in a blind manner, as previously described [24], according to the categorical degree scoring (zero, minimal or no damage; four, severe damage) of alveolar congestion, hemorrhaging, cell infiltration into the airspace or vessel wall and thickness of the alveolar wall. The mean score of five random areas per section per animal was used for data analysis.

Statistical analysis

Quantitative data are presented as the mean ± standard error of the mean (SEM) of at least three independent experiments. The histological injury scoring data were analysed by analysis of variance (ANOVA) followed by the Kruskal-Wallis nonparametric test for comparison, which was then presented as a box-and-whisker plot. The remaining data were analysed by ANOVA and then with the Newman-Keuls test for comparison. For comparisons among the groups, the unpaired Student's t-test was used (GraphPad Prism, GraphPad Software Inc., San Diego, CA, USA), in which $P<0.05$ was considered to indicate a statistically significant difference.

Results

Salidroside upregulates the expression levels of PPAR-γ and Iκβ and blocks NF-κβ p65 expression in lung tissue

To assess the potential role of salidroside in CLP-induced ALI, the mRNA and protein expression levels of PPAR-γ, NF-κβ p65 and Iκβ in the lung tissue of the lipopolysaccharide (LPS)-induced ALI mice were determined using qPCR and western blot analysis at 24 h after the CLP challenge. As shown in Figs. 1–4, the expression levels of PPAR-γ and Iκβ were markedly reduced and the NF-κβ p65 expression levels were markedly enhanced in the model group compared with those in the normal control and sham groups. However, following the administration of salidroside, the expression levels of PPAR-γ and Iκβ were markedly

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upregulated and NF-κβ p65 expression levels were significantly decreased. In combination, these observations indicated that salidroside may be involved in the increased expression levels of PPAR-γ and Iκβ and the reduced NF-κβ p65 expression levels in CLP-induced ALI. A negative correlation was shown to exist between PPAR-γ and NF-κβ p65 mRNA expression levels, as well as between PPAR-γ and NF-κβ p65 protein (r=−0.452, P<0.05; r=0.613, P<0.05).

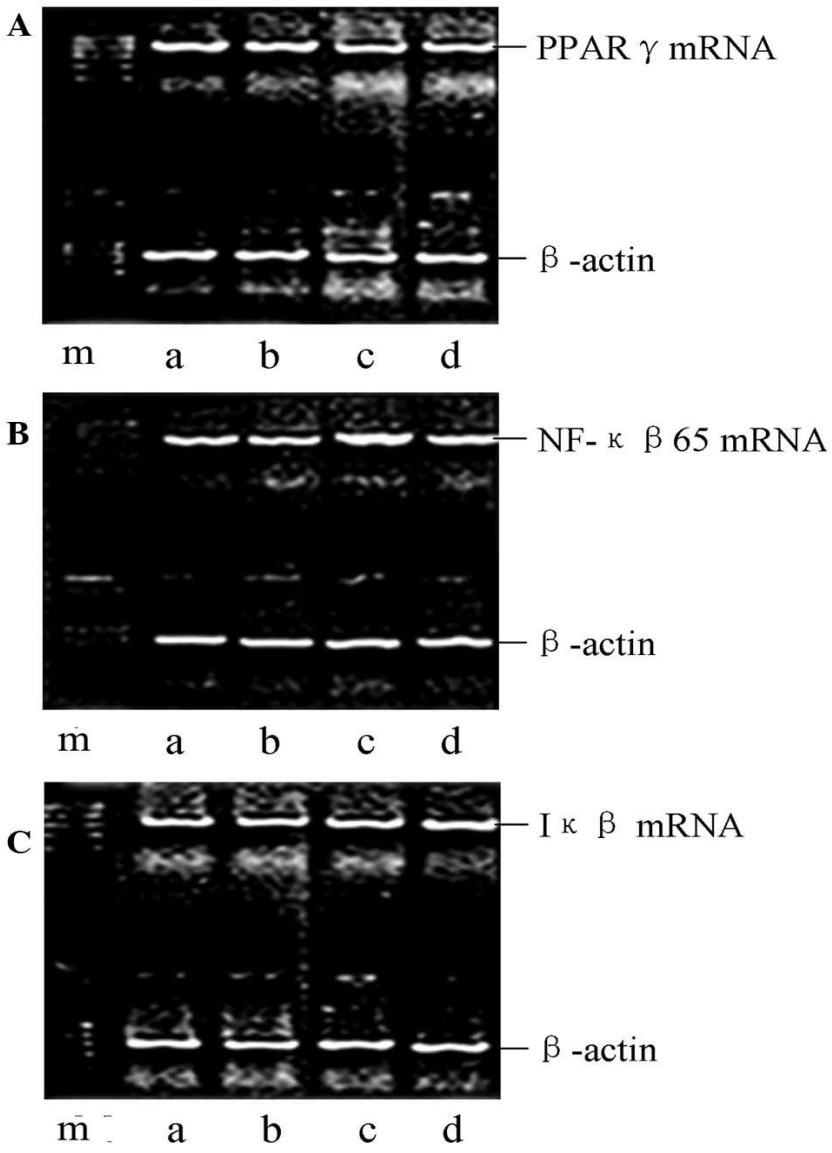

Figure 1.

Effect of salidroside on the mRNA expression levels of PPAR-γ, Iκβ and NF-κβ p65 in the lung tissue of CLP-ALI mice, as determined by qPCR. Representative gels show the mRNA expression levels of (A) PPAR-γ, (B) NF-κβ p65 and (C) Iκβ in the four groups of mice: m, marker; a, normal control; b, sham surgery; c, model; and d, treatment groups. PPAR-γ, peroxisome proliferator-activated receptor γ; NF-κβ, nuclear factor-κβ; CLP, cecal ligation and puncture; ALI, acute lung injury; qPCR, quantitative polymerase chain reaction; Iκβ, inhibitor-Iκβ.

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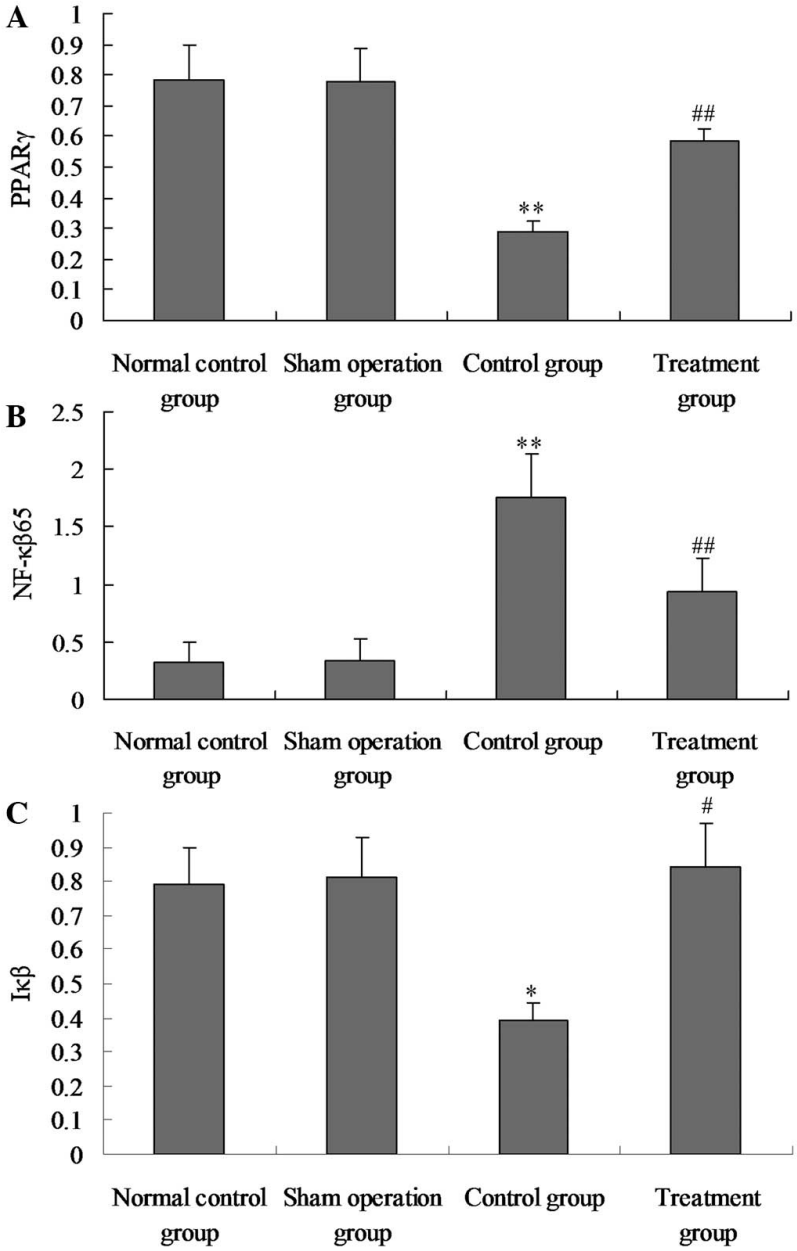

Figure 2.

Administration of salidroside enhanced the protein expression levels of PPAR-γ and Iκβ and inhibited the protein expression of NF-κβ p65 in the lung tissue of CLP-ALI rats. The groups of mice were challenged with CLP and treated with salidroside 24 h later. PPAR-γ, NF-κβ65 and Iκβ were assayed by western blotting and a statistical summary of the densitometric analysis of (A) PPAR-γ, (B) NF-κβ p65 and (C) Iκβ protein expression levels in the four groups of mice is shown. Data are presented as the mean ± standard deviation of one experiment consisting of three replicates. The experiments were performed in triplicate. **P<0.01, vs. normal control and sham surgery groups. #P<0.05 and ##P<0.01, vs. control group. PPAR-γ, peroxisome proliferator-activated receptor γ; NF-κβ, nuclear factor-κβ; CLP, cecal ligation and puncture; ALI, acute lung injury; Iκβ, inhibitor-Iκβ.

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Salidroside increases PPAR-γ and Iκβ activation in lung tissue, and inhibits NF-κβ p65 activation in lung tissue

To study the effect of salidroside on the positive expression of PPAR-γ, NF-κβ p65 and Iκβ in CLP-induced ALI, immunohistochemical staining was performed on the lung sections. As shown in Figs. 2 and 3, in the model group, the activation of PPAR-γ and Iκβ was significantly suppressed and the activation of NF-κβ p65 was significantly increased at 24 h after the CLP challenge, as compared with the sham group. However, in the CLP-induced mice that received salidroside, the positive expression levels of PPAR-γ and Iκβ were significantly increased and the NF-κβ p65 positive expression levels were markedly inhibited compared with the model group. Therefore, salidroside may suppress the positive expression of NF-κβ p65 and promote the positive expression of PPAR-γ and Iκβ.

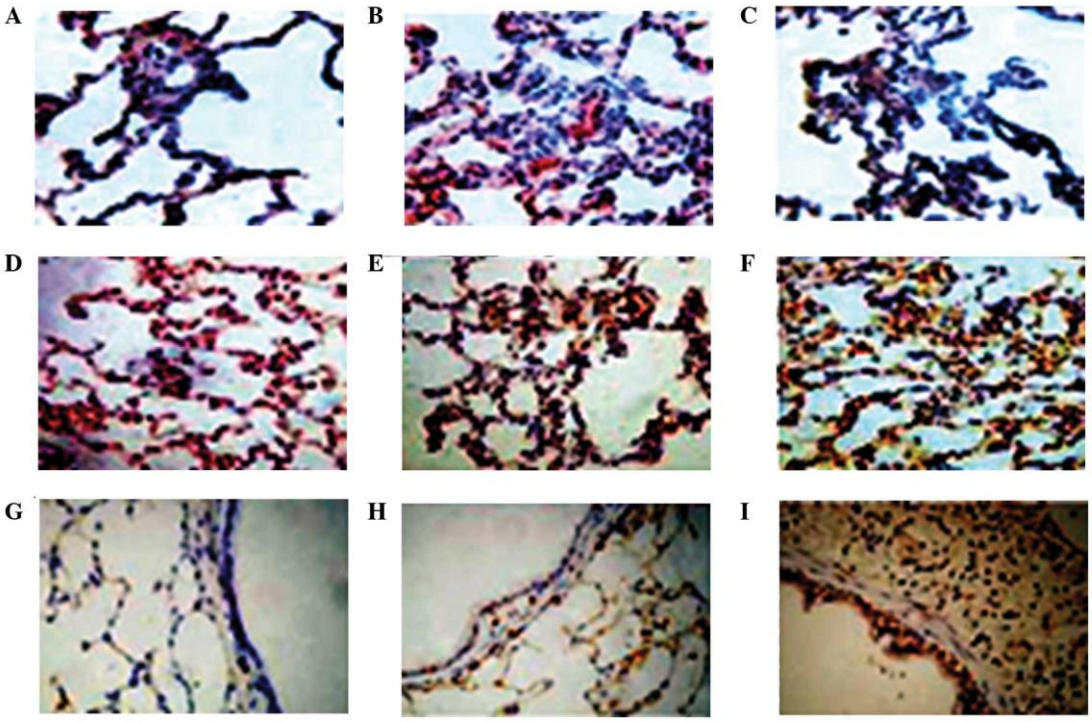

Figure 3

Effect of the administration of salidroside on the PPAR-γ, NF-κβ p65 and Iκβ positive expression levels in the lung tissue of CLP-ALI mice. The groups of rats were challenged with CLP and treated with salidroside 24 h later. Immunostaining was performed on the lung sections following antigen retrieval using Retrievagen A. Representative immunostaining images show the positive expression levels of PPAR-γ, NF-κβ and Iκβ in three groups of mice (immunofluorescence staining; magnification, ×200). Positive expression levels of (A–C) NF-κβ p65 (A, sham surgery group; B, model group; C, treatment group); (D–F) PPAR-γ (D, sham surgery group; E, model group; F, treatment group); (G–I) Iκβ (G, sham surgery group; H, model group; I, treatment group). PPAR-γ, peroxisome proliferator-activated receptor γ; NF-κβ, nuclear factor-κβ; CLP, cecal ligation and puncture; ALI, acute lung injury; Iκβ, inhibitor-Iκβ.

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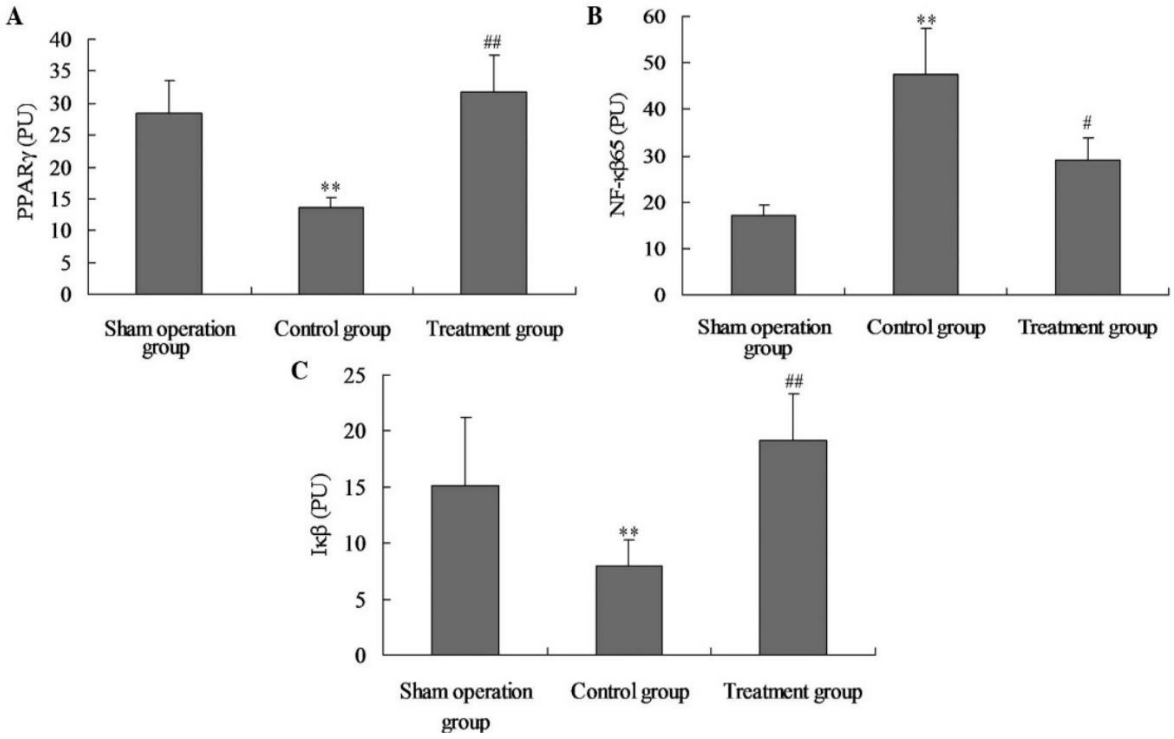

Figure 4.

Effect of salidroside on (A) PPAR-γ, (B) NF-κβ p65 and (C) Iκβ positive expression levels in the lung tissue of CLP-ALI mice. The groups of mice were challenged with CLP and treated with salidroside 24 h later. Immunostaining was performed on the lung sections following antigen retrieval using Retrievagen A. Using Image-Pro Plus image analysis software, the PPAR-γ, Iκβ and NF-κβ p65 positive expression levels in lung tissue were calculated. Data are presented as the mean ± standard deviation of one experiment consisting of three replicates. The experiments were performed in triplicate. *P<0.05 and **P<0.01, vs. normal control and sham surgery groups. #P<0.05 and ##P<0.01, vs. control group. PPAR-γ, peroxisome proliferator-activated receptor γ; NF-κβ, nuclear factor-κβ; CLP, cecal ligation and puncture; ALI, acute lung injury; Iκβ, inhibitor-Iκβ.

Salidroside ameliorates the histopathological changes in the lungs of CLP-ALI mice

To determine the effect of salidroside on histological lung injury, histopathological analysis was performed on the lung sections stained with hematoxylin and eosin. Histological analyses of the lungs following CLP exposure revealed a damaged alveolar structure with evident concretions and liquid draining within the bleeding inflammatory cells. In addition, the perivascular gap was widened and there was numerous alveolar stoma that were infiltrated by mononuclear inflammatory cells, including macrophages, plasma cells and neutrophils. The ALI pathology scores also increased significantly compared with that in the normal control and sham groups. The lungs of the mice in the treatment group exhibited less severe damage without significant bleeding

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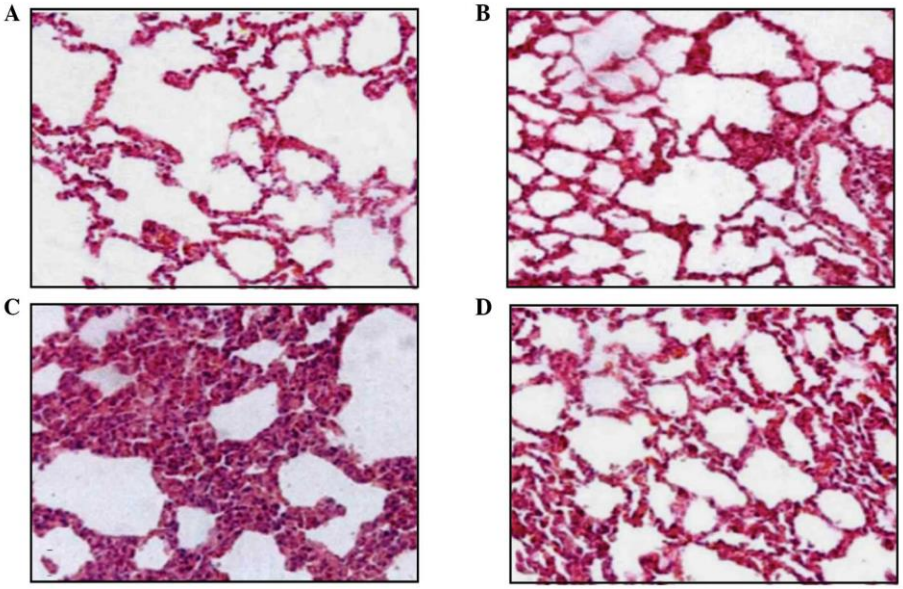

Figure 5.
Administration of salidroside ameliorated the histopathological changes of the lungs in CLP-ALI mice. Histological evaluation of the therapeutic potential of salidroside on CLP-induced lung injury in mice was analysed at 24 h after the CLP challenge (haematoxylin and eosin staining; magnification, ×200). Representative images of the haematoxylin and eosin-stained lung sections from the four experimental groups are shown: (A) Normal control; (B) sham surgery; (C) model and (D) treatment groups. CLP, cecal ligation and puncture; ALI, acute lung injury; CLP, cecal ligation and puncture.

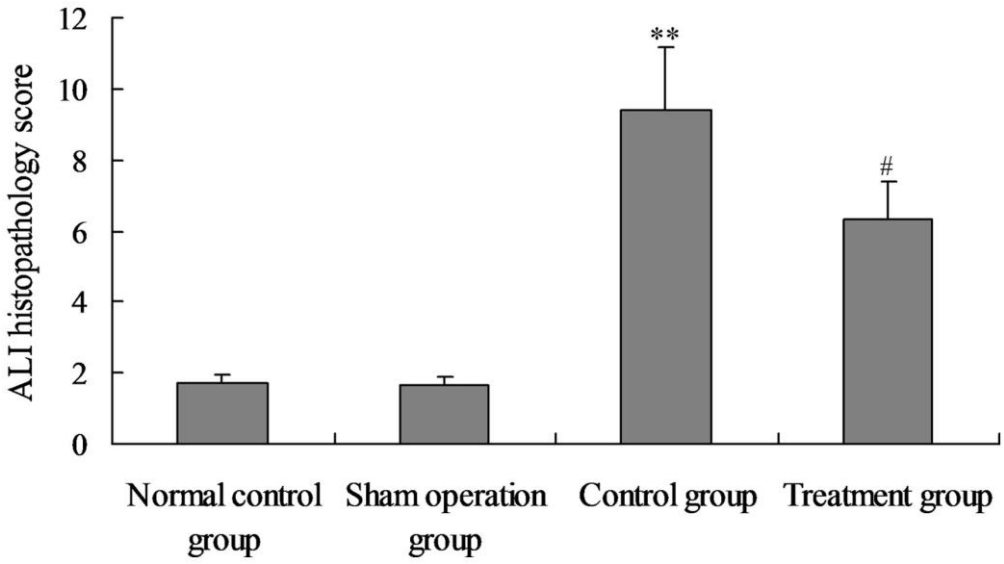

Figure 6.
Administration of salidroside decreased the ALI histopathology score of the lungs in CLP-ALI mice. Histological evaluation of the therapeutic potential of salidroside on the LPS-induced lung injury in mice was analysed at 24 h after the LPS challenge and the lung injury scores were determined. ALI pathology scores are expressed as the mean ± standard deviation. **P<0.01, vs. normal control and sham surgery groups. #P<0.05, vs. control group. ALI, acute lung injury; CLP, cecal ligation and puncture; LPS, lipopolysaccharide.

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Discussion

The positive effect of PPARγ (and/or its ligands) in bacterial infections, especially its anti-inflammatory effects via inhibition of proinflammatory molecules such as IL-6, TNF-α, IL-1β, and IL-12, has been well documented. In the *ex vivo* study by Aronoff et al., troglitazone, rosiglitazone, and 15d-PGJ_2 increased the Fcγ receptor-mediated phagocytosis of *Klebsiella pneumoniae* as well as that of IgG-opsonized nonphysiological targets by primary lung macrophages abundantly expressing PPARγ [25].

This effect appears to be mediated through PPARγ, demonstrating the role of PPARγ in pathogen clearance during bacterial infections. This phagocytic role of PPARγ is in line with other studies showing that PPARγ activation increases expression of CD36 cell surface receptors and uptake of apoptotic neutrophils by macrophages, a process critical for resolution of inflammation [26]. Likewise, Stegenga et al. reported that the PPARγ ligand ciglitazone alleviates *Streptococcus pneumoniae*-induced lung inflammation in mice by suppressing bacterial outgrowth and proinflammatory cytokine secretion, thereby improving survival of the infected animals [27]. Interestingly, however, contrary to previous findings [28], Stegenga et al. observed no ciglitazone-induced increase in *in vitro* phagocytosis or killing ability of alveolar macrophages in response to *S. pneumoniae* infection [22]. This discrepancy may reflect differences in agonists and pathogens used as well as the cell types employed in their studies. Nevertheless, these studies highlight the role of PPARγ activation in reducing inflammation and improving pathogen clearance.

Anti-inflammatory effects of PPARγ activation are not confined to bacterial infections of the lungs.

In a study using a mouse model of central nervous system infection by *Staphylococcus aureus*, which is associated with brain abscesses in humans, ciglitazone reduced the expression of proinflammatory mediators as well as iNOS and inhibited microglia/macrophage activation. The authors of the study noted that ciglitazone's ability to suppress proinflammatory mediator secretion is only partial, a significant observation as complete absence of proinflammatory responses would result in persistence of bacteria in the brain parenchyma and therefore would be detrimental to survival of the infected animals.

Another key finding in this study was that ciglitazone is capable not only of preventing microglial activation when administered prophylactically but also of dampening the activity of microglia that have already been stimulated by on-going bacterial infections. This is clinically relevant and important because typically patients seeking treatment for brain abscess would already exhibit inflammatory central nervous system responses.

In addition to attenuation of microglial response, ciglitazone-treated animals show reduced bacterial burdens, probably due to the enhanced microglial phagocytic ability that was observed. Moreover, ciglitazone accelerates brain abscess encapsulation, as evidenced by the increased deposition and compact organization of fibronectin as well as the early emergence of α-smooth muscle actin-expressing myofibroblasts associated with development of the

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capsule, which could prevent further dissemination of the pathogens [29]. Altogether, these observations provide evidence that PPARγ activation by synthetic agonists is an attractive therapeutic intervention for brain abscesses since it is capable of achieving a balance between effective clearance of pathogen and minimal damage to the brain tissue.

CLP-induced sepsis with acute suppurative peritonitis has been demonstrated to be a typical sepsis model with Gram-negative bacteria being the predominant source of infection [11]. Gram-negative bacteria release numerous endotoxins and severe endotoxemia may activate the inflammatory cells and cause inflammatory reactions that lead to tissue and organ injury, dysfunction, and possibly mortality.

Lung tissue is one of the most vulnerable tissues to endotoxemia. LPS causes ALI, which further develops into ARDS [6]. In the present study, an endotoxemia rat model was created via the use of CLP to simulate sepsis-related lung injury in order to observe the effect of salidroside on ALI. The experimental results revealed that the rats exhibited varying degrees of lung tissue hyperaemia, haemorrhage, alveolar septal thickening, infiltration of the inflammatory cells and neutrophil accumulation, which are all pathological changes associated with ALI. These observations indicated that the model was successful.

ALI is an uncontrollable pulmonary inflammation caused by large amounts of inflammatory cells and cytokines. Under the effects of CLP, lung macrophages and neutrophils produce proinflammatory cytokines, including TNF-α and IL-1β, triggering the inflammatory reaction cascade [18].

In the present study, the plasma levels of TNF-α, IL-1β, and IL-6 significantly increased 24 h after CLP surgery. When 80 mg/kg salidroside (i.v.) was administered 24 h after the CLP-induced injury, the plasma levels of the proinflammatory cytokines and lung inflammation decreased significantly. In vitro experiments demonstrated that 80 mg/kg salidroside (i.v.) reduced the levels of TNF-α, IL-1β and IL-6 secretion by lung macrophages. IL-10 is one of the most important anti-inflammatory cytokines and salidroside administration markedly increased the IL-10 concentration in the CLP-induced ALI mice.

The administration of salidroside clearly inhibited the production of the proinflammatory cytokines, TNF-α, IL-1β and IL-6, and increased the IL-10 levels. Thus, salidroside improves the homeostasis of the cytokine network and the balance between the inflammatory and anti-inflammatory reactions associated with ALI.

PPARs are members of the nuclear receptor superfamily with three isomers existing in mammals: α, β and γ [30]. Steroid, thyroid and retinoid hormones are ligands for the receptors. PPAR-γ is highly expressed in adipose tissue and its activation plays a key role in increasing systemic insulin sensitivity. PPAR-γ agonists are clinically used in the treatment of type 2 diabetes mellitus and metabolic syndrome.

PPAR-γ has been shown to be constitutively expressed in numerous types of tissue, including lung tissue, where it has been hypothesised to play a protective role [31]. In addition, PPAR-γ expression in macrophages and lymphocytes suppresses inflammatory responses, and PPAR-

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γ agonists inhibit the production of proinflammatory cytokines and regulate the process of inflammation by activating this nuclear receptor [32]. PPAR-γ is a ligand-activated transcription factor, whose activation plays a role in controlling the inflammatory response. Several studies have demonstrated that the activation of PPAR-γ by specific ligands significantly improves survival rates in clinically relevant models of septic shock [33].

The beneficial effect of PPAR-γ activation is likely to be secondary to the inhibition of the production of several inflammatory mediators, as has been shown in vivo in septic rodents [34] and in vitro in activated macrophages and monocytes [35]. Sepsis and other inflammatory states affect the PPAR-γ expression levels and correlate with the inflammatory response. The expression levels of PPAR-γ are downregulated in the lung and vascular endothelium in rodent models of septic shock, and treatment with PPAR-γ ligands reverses the sepsis-induced reduction [36].

In adipose tissue, the expression levels of PPAR-γ decreased after the rats were challenged in vivo with endotoxins and the cytokine-induced suppression of PPAR-γ was reversed with synthetic agonists [7]. However, the mechanisms that lead to a reduction in the levels of PPAR-γ activity in the presence of sepsis remain unclear. In the present study, CLP blocked PPAR-γ expression in the lung tissue, increasing the levels of proinflammatory cytokines; however, the administration of salidroside enhanced the PPAR-γ expression levels in the lung tissue and inhibited the inflammatory response.

To further characterise the inhibitory effect of salidroside on cytokine production, the present study examined the effects of salidroside on the activation of the transcription factor NF-κβ, which regulates the expression of numerous immune and inflammatory genes and the production of cytokines. NF-κβ is essential for host defence and the inflammatory responses to microbial and viral infections [37], as it is an important transcription factor required for the expression of a number of proinflammatory cytokines [11].

In the majority of cells, NF-κβ exists in an inactive form in the cytoplasm as it is bound to inhibitory Iκβ proteins. Following CLP challenge, NF-κβ is translocated to the nucleus to drive the expression of a variety of inflammatory genes that are involved in the pathogenesis of ALI. Therefore, a blockage of NF-κβ activation and an increase in the Iκβ expression levels is expected to attenuate ALI [23].

This is supported by the results of the present study, which demonstrated that salidroside treatment following the CLP challenge inhibited NF-κβ activation, and the release of inflammatory cytokines promoted the expression of PPAR-γ. This is consistent with the theory that salidroside prevents the release of LPS-induced inflammatory cytokines via its anti-NF-κβ activity, which upregulates PPAR-γ expression levels.

CLP stimulates macrophages, neutrophils and other types of immune cell to produce different mediators, including cytokines such as TNF-α and IL-6, that recruit polymorphonuclear neutrophils to the injured site and contribute to the pathogenesis of ALI and ARDS [38]. Activated neutrophils that release various types of mediators and secrete the elastase enzyme,

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whose activity is an indicator of neutrophil accumulation in tissues [12], are recognised to be a primary mechanism in the development of ALI.

In the present study, the interstitial space was shown to be filled with activated alveolar macrophages and neutrophils following the LPS challenge. These pathological changes were reversed by salidroside treatment following the challenge, indicating that salidroside may inhibit LPS-induced leukocyte rolling and transmigration into the lung tissue.

In addition, vascular leakage is a critical pathological process in sepsis [11]. Leakage permits plasma protein and leukocyte extravasation, leading to oedema and inflammatory reactions in the affected tissues [28].

Oedema causes tissue hypoxia, and leukocytes, including neutrophils, cause tissue damage through their excessive production of free radicals and proteases. Thus, vascular leakage is a promising target for the therapeutic treatment of sepsis. In the present study, CLP was found to markedly increase the albumin concentration in BALF, the W/D lung weight ratio and the water content in lung tissue.

Histological analyses of the lungs following CLP exposure revealed a damaged alveolar structure with evident concretions leaking liquid within the bleeding inflammatory cells. In addition, the perivascular gap was widened and there were numerous alveolar stoma infiltrated by mononuclear inflammatory cells. These observations indicated that CLP exacerbates the lung leakage permeability; however, the exacerbated lung leakage permeability was ameliorated by salidroside. These results provide supporting evidence that salidroside post-treatment is effective in reversing LPS-induced lung permeability and injuries.

Conclusions

In contrast to the use of antimicrobial drugs that directly target the problem's source, bacteria, treating infections with immunomodulatory agents such as PPAR*γ* ligands is more complex. The innate immune responses to invading pathogens can be divided broadly into an initial hyperinflammatory stage, termed the systemic inflammatory response syndrome, and a subsequent immunosuppressive stage called the compensatory anti-inflammatory response syndrome.

Thus, immunomodulatory drugs must achieve a fine balance between pro- and anti-inflammatory effects on the immune system, dampening excessive systemic inflammatory responses to prevent severe tissue damage and other complications without significantly affecting the essential ability of the host immune system to clear the infection.

We have here reviewed research investigating the effects of PPAR*γ* activation or inhibition during bacterial infections. These studies clearly show that PPAR*γ* is a double-edged sword, possessing both pro- and anti-inflammatory effects and exerting beneficial as well as harmful effects upon host defenses against pathogenic bacteria.

While differences in the type of pathogens, disease models, and PPAR*γ* agonists/antagonists used in the research can explain many of the variations in results reported by different research

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groups, timing, the point during the host immune response at which drugs are administered, likely plays a large part in determining which PPARγ agonist/antagonist effect predominates. For instance, blocking the anti-inflammatory cytokine IL-10, which is associated with reduced secretion of proinflammatory mediators, at an early stage of sepsis is detrimental to the host, whereas IL-10 suppression later in sepsis is linked with longer survival of the affected animals. Similarly, established sepsis may respond to immune-stimulating strategies but not to therapeutic interventions designed to suppress proinflammatory mediators secreted early during sepsis. In addition, in human patients, the effect of a PPARγ agonist or antagonist would likely differ with the immune status of each individual—the exact pathophysiologic nature of immune imbalance—as well as other factors such as age, comorbidities, and genetic background.

Patients suffering damage and symptoms due to exaggerated immune response would benefit from the anti-inflammatory effect of PPARγ ligands, while those experiencing immunoparalysis-induced symptoms would require the immune-enhancing effect of PPARγ antagonists to alleviate damage and symptoms.

Thus, it is imperative to aim for a carefully defined balance between immune stimulation and immunosuppression in each patient. Close assessment of the competence of host antimicrobial defenses and the extent of inflammation and tissue injury, including measurement of mediators of the immune response, and adjustment in the dosage and timing of PPARγ agonist/antagonist administration would be valuable for achieving the most desired outcome.

Competing interests

The authors declare that they have no competing interests.

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References

1. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med. 2003;348(16):1546–1554. [PubMed]
2. Hacham M, White RM, Argov S, Segal S, Apte RN. Interleukin-6 and interleukin-10 are expressed in organs of normal young and old mice. EUR Cytokine Netw 2004;15: 37–46. [PubMed]
3. Chandra V, Huang P, Hamuro Y, Raghuram S, Wang Y, Burris TP, Rastinejad F. Structure of the intact PPAR-γ–RXR-α nuclear receptor complex on DNA. Nature 2008; 456 (7220): 350–356. [PubMed]
4. Michalik L, Auwerx J, Berger JP, Chatterjee VK, Glass CK, Gonzalez FJ, Grimaldi PA, Kadowaki T, Lazar MA, O'Rahilly S, Palmer CN, Plutzky J, Reddy JK, Spiegelman BM, Staels B, Wahli W. International Union of Pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol. Rev 2006; 58(4): 726–41. [PubMed] [Full-Text]
5. Berger J, Patel HV, Woods J, Hayes NS, Parent SA, Clemas J, Leibowitz MD, Elbrecht A, Rachubinski RA, Capone JP, Moller DE. A PPARgamma mutant serves as a dominant negative inhibitor of PPAR signaling and is localized in the nucleus. Mol. Cell. Endocrinol 2000; 162(1–2): 57. [PubMed]
6. Abraham E, Matthay MA, Dinarello CA, Vincent JL., Cohen J, Opal SM, Glauser M, Parsons P, Fisher CJ, Repine Jr JE. . Consensus conference definitions for sepsis, septic shock, acute lung injury, and acute respiratory distress syndrome: time for a reevaluation. Crit. Care Med 2000; 28:232. [CrossRefMedline]
7. Rocchi S, Auwerx J. 1999. Peroxisome proliferator-activated receptor-γ: a versatile metabolic regulator. Ann. Med 1999; 31:342. [Medline]
8. Su CG, Wen X, Bailey ST, Jiang W, Rangwala SM, Keilbaugh SA, Flanigan A, Murthy S, Lazar MA, Wu GD. A novel therapy for colitis utilizing PPARγ ligands to inhibit the epithelial inflammatory response. J. Clin. Invest 1999; 104:383. [pubMed]
9. Hill MR, Young MD, McCurdy CM, Gimble JM. Decreased expression of murine PPARγ in adipose tissue during endotoxemia. Endocrinology 1997; 138:3073. [Abstract/FREE Full Text]
10. Atanasov AG1, Blunder M, Fakhrudin N, Liu X, Noha SM, Malainer C, Kramer MP, Cocic A, Kunert O, Schinkovitz A, Heiss EH, Schuster D, Dirsch VM, Bauer R.Polyacetylenes from Notopterygium incisum-new selective partial agonists of peroxisome proliferator-activated receptor-gamma. PLoS ONE 2013; 8 (4): e61755. [PubMed] [Full-Text]
11. Masamune A, Satoh K, Sakai Y, Yoshida M, Satoh A, Shimosegawa T. Ligands of peroxisome proliferator-activated receptor-gamma induce apoptosis in AR42J cells. Pancreas. 2002; 24(2):130-8. [PubMed]
12. Nathan C. Points of control in inflammation. Nature 2000; 420:846. [CrossRefMedline]
13. Castrillo, A., M. Mojena, S. Hortelano, L. Bosca. Peroxisome proliferator-activated receptor-γ-independent inhibition of macrophage activation by the non-thiazolidinedione agonist L-796,449: comparison with the effects of 13. 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2. J. Biol. Chem 2001; 276:34082. [Abstract/FREE Full Text]
14. Thieringer R, Fenyk-Melody JE, Le Grand CB, Shelton BA, Detmers PA, Somers EP, Carbin LD, Moller E, Wright SD, Berger J. Activation of peroxisome proliferator-activated receptor γ does not inhibit IL-6 or TNF-α responses of macrophages to lipopolysaccharide in vitro or in vivo. J. Immunol 2000; 164:1046. [Abstract/FREE Full Text]
15. Delerive P, Martin-Nizard F, Chinetti G, Trottein F, Fruchart JC, Najib J, Duriez P , Staels B. Peroxisome proliferator-activated receptor activators inhibit thrombin-induced endothelin-1 production in human vascular endothelial cells by inhibiting the activator protein-1 signaling pathway. Circ. Res 1999; 85:394. [Abstract/FREE Full Text]
16. Peters K, Unger RE, Kirkpatrick CJ. Molecular basis of endothelial dysfunction in sepsis. Cardiovasc. Res 2003; 60: 49–57. [Abstract/FREE Full Text]
17. Milam JE, Keshamouni VG, Phan SH, Hu B, Gangireddy SR, Hogaboam CM, Standiford TJ, Thannickal V J, Reddy RC. PPAR-γ agonists inhibit profibrotic phenotypes in human lung

Research Article doi: 10.18081/2333-5106/014-04/228-246

fibroblasts and bleomycin-induced pulmonary fibrosis. Am. J. Physiol. Lung Cell. Mol. Physiol 2008; 294: L891–L901. [Abstract/FREE Full Text]

18. Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptor-γ is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. J Allergy Clin Immunol 2004; 113: 882–888. [OpenUrlCrossRefMedlineWeb of Science]
19. Huang JT, Welch JS, Ricote M, Binder CJ, Willson TM, Kelly C, Witztum JL, Funk CD, Conrad D, Glass CK. Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. Nature 1999; 400: 378–382. [OpenUrlCrossRefMedline]
20. Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF Jr, Motomura K, Anania FA, Willson TM, Tsukamoto H. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000; 275: 35715–35722. [OpenUrlAbstract/FREE Full Text]
21. Poleni PE, Bianchi A, Etienne S, Koufany M, Sebillaud S, Netter P, Terlain B, Jouzeau JY. Agonists of peroxisome proliferator-activated receptors (PPAR) α, β/δ or γ reduce transforming growth factor (TGF)-β-induced proteoglycan production in chondrocytes. Osteoarthritis Cartilage 2007; 15: 493–505. [OpenUrlCrossRefMedlineWeb of Science]
22. Ray DM, Akbiyik F, Bernstein SH, Phipps RP. CD40 engagement prevents peroxisome proliferator-activated receptor-γ agonist-induced apoptosis of B lymphocytes and B lymphoma cells by an NF-κB-dependent mechanism. J Immunol 2005; 174: 4060–4069. [OpenUrlAbstract/FREE Full Text]
23. Saika S, Yamanaka O, Okada Y, Miyamoto T, Kitano A, Flanders KC, Ohnishi Y, Nakajima Y, Kao WW, Ikeda K. Effect of overexpression of PPARγ on the healing process of corneal alkali burn in mice. Am J Physiol Cell Physiol 2007; 293: C75–C86. [OpenUrlAbstract/FREE Full Text]
24. Shiau CW, Yang CC, Kulp SK, Chen KF, Chen CS, Huang JW, Chen CS. Thiazolidenediones mediate apoptosis in prostate cancer cells in part through inhibition of Bcl-xL/Bcl-2 functions independently of PPARγ. Cancer Res 2005; 65: 1561–1569. [OpenUrlAbstract/FREE Full Text]
25. Zhao C, Chen W, Yang L, Chen L, Stimpson SA, Diehl AM. PPARγ agonists prevent TGFβ1/Smad3-signaling in human hepatic stellate cells. Biochem Biophys Res Commun 2006; 350: 385–391. [OpenUrlCrossRefMedlineWeb of Science]
26. Altiok S, Xu M, Spiegelman BM. PPARγ induces cell cycle withdrawal: inhibition of E2F/DP DNA-binding activity via down-regulation of PP2A. Genes Dev 1997; 11: 1987–1998. [OpenUrlAbstract/FREE Full Text]
27. Burgess HA, Daugherty LE, Thatcher TH, Lakatos HF, Ray DM, Redonnet M, Phipps RP, Sime PJ. PPARγ agonists inhibit TGF-β induced pulmonary myofibroblast differentiation and ollagen production: implications for therapy of lung fibrosis. Am J Physiol Lung Cell Mol Physiol 2005; 288: L1146–L1153. [Abstract/FREE Full Text]
28. Fu M, Zhang J, Lin Y, Zhu X, Zhao L, Ahmad M, Ehrengruber MU, Chen YE . Early stimulation and late inhibition of peroxisome proliferator-activated receptor gamma (PPAR gamma) gene expression by transforming growth factor beta in human aortic smooth muscle cells: role of early growth-response factor-1 (Egr-1), activator protein 1 (AP1) and Smads. Biochem J 2003; 370: 1019–1025. [CrossRefMedlineWeb of Science]
29. Honda K, Marquillies P, Capron M, Dombrowicz D. Peroxisome proliferator-activated receptor gamma is expressed in airways and inhibits features of airway remodeling in a mouse asthma model. J Allergy Clin Immunol 2004; 113: 882–888. [CrossRefMedlineWeb of Science]
30. Milam JE, Keshamouni VG, Phan SH, Hu B, Gangireddy SR, Hogaboam CM, Standiford TJ, Thannickal VJ, Reddy RC. PPAR-gamma agonists inhibit profibrotic phenotypes in human lung fibroblasts and bleomycin-induced pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2008; 294: L891–L901. [Abstract/FREE Full Text]
31. Simon DM, Mariani TJ. Role of PPARs and retinoid x receptors in the regulation of lung maturation and development (Abstract). *PPAR Res* 2007; 91240. [Medline]
32. Chinetti G, Fruchart JC, and Staels B. Peroxisome proliferator-activated receptors and inflammation: from basic science to clinical applications. Int J Obes Relat Metab Disord 2003; 27 Suppl 3: S41–45. [PubMed]
33. Jiang C, Ting AT, and Seed B. PPAR-γ agonists inhibit production of monocyte inflammatory cytokines. Nature 1998; 391: 82-86. [CrossRefMedlineWeb of Science]

34. Lemberger T, Desvergne B, and Wahli W. Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology. Annu Rev Cell Dev Biol 1996; 12: 335-363. [CrossRefMedlineWeb of Science]
35. Nagy L, Tontonoz P, Alvarez JG, Chen H, and Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ. Cell 1998; 93: 229-240. [CrossRefMedlineWeb of Science]
36. Schoonjans K, Staels B, and Auwerx J. The peroxisome proliferator-activated receptors (PPARS) and their effects on lipid metabolism and adipocyte differentiation. Biochim Biophys Acta 1996; 1302: 93-109. [Medline]
37. Thieringer R, Fenyk-Melody JE, Le Grand CB, Shelton BA, Detmers PA, Somers EP, Carbin L, Moller DE, Wright SD, and Berger J. Activation of peroxisome proliferator-activated receptor γ does not inhibit IL-6 or TNF-α responses of macrophages to lipopolysaccharide in vitro or in vivo. J Immunol 2000; 164: 1046-1054. [Abstract/FREE Full Text]
38. Wu Z, Bucher NL, and Farmer SR. Induction of peroxisome proliferator-activated receptor γ during the conversion of 3T3 fibroblasts into adipocytes is mediated by C/EBPβ, C/EBPδ, and glucocorticoids. Mol Cell Biol 1996; 16: 4128-4136. [Abstract/FREE Full Text]

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