

ORIGINAL ARTICLE

PPAR γ Agonist PGZ Attenuates OVA-Induced Airway Inflammation and Airway Remodeling *via* RGS4 Signaling in Mouse Model

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Abstract— Peroxisome proliferator-activated receptor- γ (PPAR γ) agonist pioglitazone (PGZ) exhibits potential protective effects in asthma. Recently, regulator of G protein 4 (RGS4) has been reported to be associated with immunological and inflammatory responses. However, no evidence has shown the influence of PPAR γ on RGS4 expression in airway disorders. In this study, BALB/c mice received ovalbumin (OVA) sensitization followed by OVA intranasal challenge for 90 days to establish a chronic asthma mouse model. Accompanied with OVA challenge, the mice received administration of PPAR γ agonist PGZ (10 mg/kg) intragastrically or RGS4 inhibitor CCG 63802 (0.5 mg/kg) intratracheally. Invasive pulmonary function tests were performed 24 h after last challenge. Serum, bronchoalveolar lavage fluid (BALF), and lung tissues were collected for further analyses after the mice were sacrificed. We found that PPAR γ agonist PGZ administration significantly attenuated the pathophysiological features of OVA-induced asthma and increased the expression of RGS4. In addition, the attenuating effect of PGZ on airway inflammation, hyperresponsiveness (AHR), and remodeling was partially abrogated by administration of RGS4 inhibitor CCG 63802. We also found that the downregulation of RGS4 by CCG 63802 also significantly increased inflammatory cell accumulation and AHR, and increased levels of IL-4, IL-13, eotaxin, IFN- γ , and IL-17A in BALF, and total and OV-specific IgE in serum. Furthermore, the inhibitory effects of PGZ on the activations of ERK and Akt/mTOR signaling, and MMPs were apparently reversed by CCG 63802 administration. In conclusion, the protective effect of PGZ on OVA-induced airway inflammation and remodeling might be partly regulated by RGS4 expression through ERK and Akt/mTOR signaling.

KEY WORDS: asthma; PPAR γ ; RGS4; airway inflammation; pioglitazone; CCG 63802.

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INTRODUCTION

Asthma is a chronic airway inflammatory disease which affects all ages of the population worldwide [1]. It is come to light that most of inflammatory mediators and cytokines play their function through activating inflammatory cells including neutrophils, macrophages, eosinophils, T lymphocytes, and mast cells during airway allergic inflammation and airway remodeling processing [2].

Allergic inflammation in asthma is associated with increasing levels of T helper 2 (Th2) cell cytokines, such as interleukin (IL)-4, IL-5, and IL-13. The production of IL-4 stimulates the secretion of immunoglobulin E (IgE) by B cells, which directly activates eosinophils and mast cells to provoke inflammation [3]. Furthermore, repeated occurrence of asthma would result in airway remodeling characterized by sub-epithelium fibrosis, collagen deposit, excessive airway smooth muscle cell proliferation, and accompany with irreversible airflow obstruction [4].

The peroxisome proliferator-activated receptors (PPAR α , PPAR β/δ , and PPAR γ) are located at nucleus with distinct patterns and widely expressed in diverse cell types. They belong to the nuclear hormone receptor superfamily that regulate gene transcription and expression upon ligand binding, which play pivotal role in anti-inflammatory, immunomodulatory, and metabolic responses [5]. Among of them, PPAR γ expression has been detected in various cell types and ligand-activated PPAR γ interacts with other nuclear hormone receptors through binding to the promoter region of its target genes [6]. Accumulating evidence has reported that PPAR γ activation may exert protective effects in many animal models [7], and anti-diabetic agents named as thiazolidinediones (TZDs), including rosiglitazone, troglitazone, and pioglitazone (PGZ), are widely used as the synthetic agonists of PPAR γ for these investigations [8]. Furthermore, the applications of these agonists in clinic have obtained favorable therapeutic effects on the treatment of a large range of human disorders including asthma, chronic obstructive pulmonary disease, cancers, pulmonary fibrosis, and multiple sclerosis [9].

G protein-coupled receptors (GPCRs) comprise almost 1% of the human genome [10] and can be activated by various extracellular signals, like hormones, neurotransmitters, chemokines, and cytokines, which in turn result in various physiological and biochemical responses [11]. Regulators of G protein signaling (RGS proteins) are a newly found identified group of proteins that negatively regulate G protein function, which have been shown to hydrolyze GTP into GDP $\beta\gamma$ and G α subunits and suppress the activation of G protein. The intrinsic GTPase activity of G α subunit promotes G α re-association with G $\beta\gamma$ to form an inactive heterotrimer, which terminates G protein function indirectly [12]. At least 25 kinds of RGS proteins have been found in mammalian cells and subdivided into subfamilies according to their characteristic domains. It is worth mentioning that members of B/R4 subfamily, RGS2-5, were especially abundant in the lung [13].

Previous studies have suggested that PPAR γ agonists could relieve OVA-induced airway allergic inflammation

and airway remodeling [14], as well as upregulate RGS protein subtype expressions in diabetes and arteriosclerosis prevention [15]. Thus, we hypothesized that RGS protein subtype was involved in the protective function of PPAR γ agonists in ovalbumin (OVA)-induced asthmatic airway inflammation and airway remodeling. In present study, a murine model of asthma was established to clarify the possible role of RGS4 involved in the regulation of PPAR γ agonist, PGZ, against asthma.

MATERIALS AND METHODS

Animals and Experimental Protocol

Forty female BALB/c mice of 6 to 8 weeks of age and free of murine-specific pathogens were obtained from the Research Institute of Animal Department (Xi'an, China). The animal experimental procedures were approved by the Animal Research Ethics Committee of Xi'an Jiaotong University. All the mice were fed at the same condition of 20 °C room temperature with 30% humidity. Forty mice were randomly divided into four groups ($n = 10$ for each group): control group (Con); OVA-induced asthma group (OVA); PGZ treated OVA-induced asthma group (OVA + PGZ); PGZ and CCG 63802 co-treated OVA-induced asthma group (OVA + PGZ + CCG). Antigen sensitization was performed by intraperitoneal (i.p.) injection of 50 μ g OVA (grade V, Sigma-Aldrich, St. Louis, MO) mixed with alum (2 mg in 200 μ l of PBS) on day 0 and day 7. From day 14 on after initial sensitization, antigen challenge was given by intranasal with 50 μ g OVA alone every other day until day 90. For PGZ treatment, PGZ (Takeda Chemical Industries Ltd., Osaka, Japan) dissolved in dimethyl sulfoxide (DMSO) and diluted with 0.9% NaCl was administered by oral gavage (10 mg/kg body weight) 30 min before each challenge. A selective inhibitor of RGS4, CCG 63802 (0.05 mg/kg body weight; MedChemExpress; USA), dissolved in DMSO and diluted with 0.9% NaCl, was administered intratracheally once per week until the last OVA challenge. An overdose of [phenobarbital sodium](#) was administered intraperitoneally to sacrifice all mice. Serum samples, bronchoalveolar lavage fluid (BALF), and lung tissues were collected on sacrifice day.

Assessment of Airway Hyperresponsiveness

Hyperresponsiveness (AHR) tests were performed 24 h after last OVA challenge. Briefly, mice were anesthetized by intraperitoneal injection. Then a catheter was inserted into trachea at once and then connected to the

FlexiVent (SCIREQ, Montreal, PQ, Canada) and mechanically ventilated. Lung resistance was measured by using the forced oscillation technique. Airway responsiveness was determined by increasing doses of inhaled methacholine (MCh). Data were collected at 1-min interval and the lung function values, including lung resistance (cm H₂O/ml/s) and airway resistance (cm H₂O/ml/s), were plotted as a function of MCh doses.

BALF Cell Collection

After the catheter was inserted into trachea, 2 ml cold PBS was injected into the lung by syringe. And then the syringe was moved back and forth for two times. BALF samples were collected into EP tube and then were centrifuged at 4000 rpm. Then the supernatants were stored at – 80 °C for cytokine measurement. Cell pellet of BAL was resuspended with 200 µl PBS and stained with trypan blue. Then total cell counts were performed under common light microscope. Cell differentiation was determined after cyto-spin was made and stained with diff-quick solution (Fisher Scientific Co., Newark, DE).

Histology and IHC

After the mice were sacrificed immediately, the right lungs were harvested, fixed in 4% phosphate-buffered formalin, and then embedded in paraffin. Paraffin-embedded lung tissues were cut into 5 µm samples. H&E, alcian blue, and Masson's trichrome staining were performed on lung sections as described [16]. For immunohistochemistry, goat anti-mouse polyclonal antibodies against RGS4 (1:500), PPARγ (1:500), and α-actin (1:1000) were used for RGS4, PPARγ, and α-actin detection. ABC staining kits were used to amplify the signal (Santa Cruz, CA, USA). The inflammation score of H&E sections, the percent positive area of Masson's trichrome, and immunostaining sections were calculated among 10 random fields using Image-Pro 6.5 system (Media Cybernetics, Silver Spring, MD) and blindly evaluated by two experienced pathologists in our institution.

Quantification of Cytokines, Chemokines, and Immunoglobulins

Cytokines and chemokines in BALF supernatants were determined using commercially available ELISA kits according to manufacturer's instructions. Serum samples in duplicates were analyzed using ELISA kits for total and specific IgE detection according to manufacturer's instructions.

Western Blot

Harvested left lungs were minced and then lysed with RIPA buffer. The concentration of protein was determined by BCA Protein Assay Kit (Thermo). Proteins were separated by SDS-PAGE and then were electronically transferred to polyvinylidene difluoride membrane (Bio-Rad) and incubated with primary antibodies against phosphor (p)-ERK, p-Akt, p-mTOR, total (t)-ERK, t-Akt, t-mTOR, tissue inhibitor of metalloproteinase (TIMP), and GAPDH (Santa Cruz, CA, USA). An anti-rabbit IgG conjugated to HRP was used as secondary antibody (Santa Cruz, CA, USA) to amplify signal. Protein detection was accomplished with a Super Signal Fluorescence agent (GE).

MMP-2 and MMP-9 Detection by Gelatin Zymography

Whole lung protein was extracted by using lysis buffer. Lung tissue homogenates were analyzed on 7.5% polyacrylamide gels containing 0.1% gelatin (Sigma-Aldrich, St. Louis, MO). The electrophoresis gels were incubated in 2.5% Triton X-100 for 30 min, followed by incubation in 50 mM Tris pH 7.5, 200 mM NaCl, and 10 mM CaCl₂ at 37 °C overnight. Gels were then stained with 0.2% Coomassie blue R-250. Gelatinase activity was detected as clear zones of lyses (negative staining) against the blue background. At last, the gels were scanned and calculated by using the Multi Gauge V3.0 program to make a permanent record of results.

Statistical Analysis

Statistical differences were performed using one-way ANOVA followed by Tukey's *post hoc* tests. The unpaired Student *t* test was used to compare between two groups. Data were expressed as means ± SEM and *P* values < 0.05 were considered statistically significant. Each experiment was repeated at least three times.

RESULTS

PGZ Treatment Suppressed Inflammatory Cell Recruitment in BALF

Firstly, we evaluated the change of total and differential leukocytes in the BALF following OV administration. The results showed that OVA treatment obviously increased the number of total cells, eosinophil, neutrophil, and macrophage in BALF (all *P* < 0.05; Fig. 1a). However, PGZ significantly reduced the numbers of OVA-induced total cells, eosinophil, neutrophil, lymphocyte, and macrophage (all *P* < 0.05) in

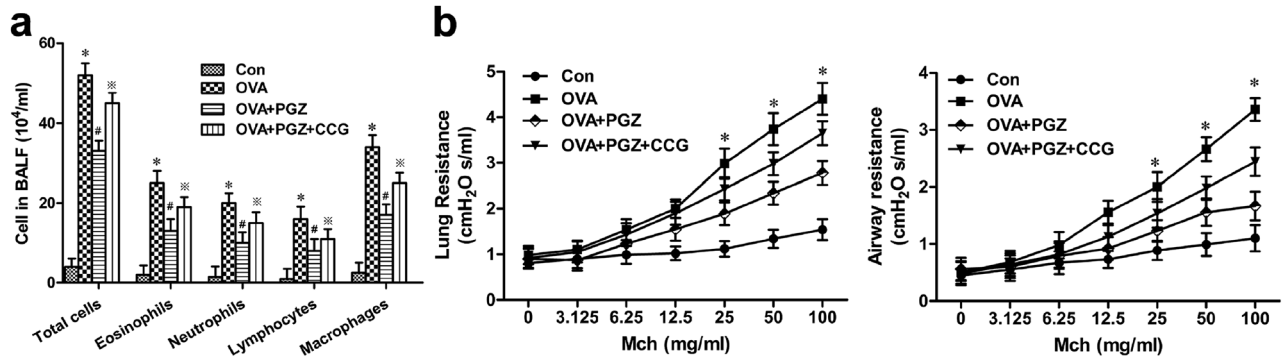


Fig. 1. PGZ suppressed inflammatory cell recruitment in BALF and AHR in chronic OVA-induced asthma. **a** Effect of PGZ on inflammatory cell recruitment in BALF collected from OVA-induced asthma mouse model. **b** Effect of PGZ on lung resistance and airway resistance at baseline and in response to MCh through inhalation in OVA-induced asthma mouse model was recorded and analyzed. Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; # $P < 0.05$ vs. OVA group; $P < 0.05$ vs. OVA + PGZ group.

OVA + PGZ group when compared to OVA group. Notably, CCG 63802 administration dramatically impaired PGZ's inhibitory effects on BALF cell accumulation. These results suggest that PGZ inhibited the OVA-induced inflammatory response in asthma mouse model.

PGZ Alleviated AHR in Chronic OVA-Induced Asthma

Airway hyperresponsiveness (AHR) is referred as one of the most significant features of asthma. The lung mechanics were analyzed by using the forced oscillation technique (FlexiVent). It has been demonstrated that the baseline of these four groups was almost the same. While when the concentration of MCh was increased gradually, the lung resistance (LR) and airway resistance (AR) of OVA treatment group generated by MCh administration at doses from 25 to 100 mg/ml were significantly increased in the OVA group when compared to the control group ($P < 0.05$, respectively; Fig. 1b). Pretreatment of PGZ significantly suppressed OVA-induced LR and AR in OVA + PGZ group compared to the OVA group. However, CCG 63802 treatment partially reversed LR and AR in OVA + PGZ + CCG group compared to OVA + PGZ group. These data implied that the inflammation-mediated AHR was alleviated by PGZ treatment.

PGZ Treatment Attenuated Chronic Airway Inflammation

In addition, H&E and alcian blue staining were performed to evaluate airway eosinophilic inflammation and severity of acidity mucus production. The results showed that, compared to Con group, OVA administration

remarkably induced eosinophil infiltration and mucus overproduction in the airway in OVA group ($P < 0.05$, respectively; Fig. 2), which was significantly alleviated by PGZ treatment in OVA + PGZ group ($P < 0.05$, respectively). However, in OVA + PGZ + CCG group, the eosinophil infiltration and mucus hypersecretion were more increased with CCG 63802 pretreatment than those in OVA + PGZ group ($P < 0.05$, respectively).

PGZ Influenced the Levels of Th2 Cytokines in BALF and IgE in Serum

To determine the possible effects on the T cell response associated with asthmatic mice, we evaluated the Th2-type cytokines and chemokine level in BALF. As shown in Fig. 3a, compared with the Con group, OVA-induced group had significantly elevated Th2-type cytokine and chemokine levels (all $P < 0.05$). Compared to OVA challenge group, IL-4, IL-13, IL-17, IFN- γ , and eotaxin were significantly decreased in PGZ pretreatment group (all $P < 0.05$), which was then remarkably abrogated in CCG 63802 treatment group (all $P < 0.05$). But no influence on MCP-1 levels was found under PGZ or CCG 63802 treatments. Furthermore, to confirm Th2-type response involved in OVA-induced asthmatic mice, we measured the total and OVA-specific IgE levels in serum. Total and OVA-specific IgE levels were remarkably increased in the OVA-induced group when compared to Con group ($P < 0.05$, respectively; Fig. 3b). However, the PGZ group significantly decreased total and OVA-specific IgE in serum compared with OVA-induced mice ($P < 0.05$, respectively). Co-treatment with CCG 63802 could partially attenuate the inhibitory effect of PGZ on total and OVA-specific IgE levels ($P < 0.05$, respectively).

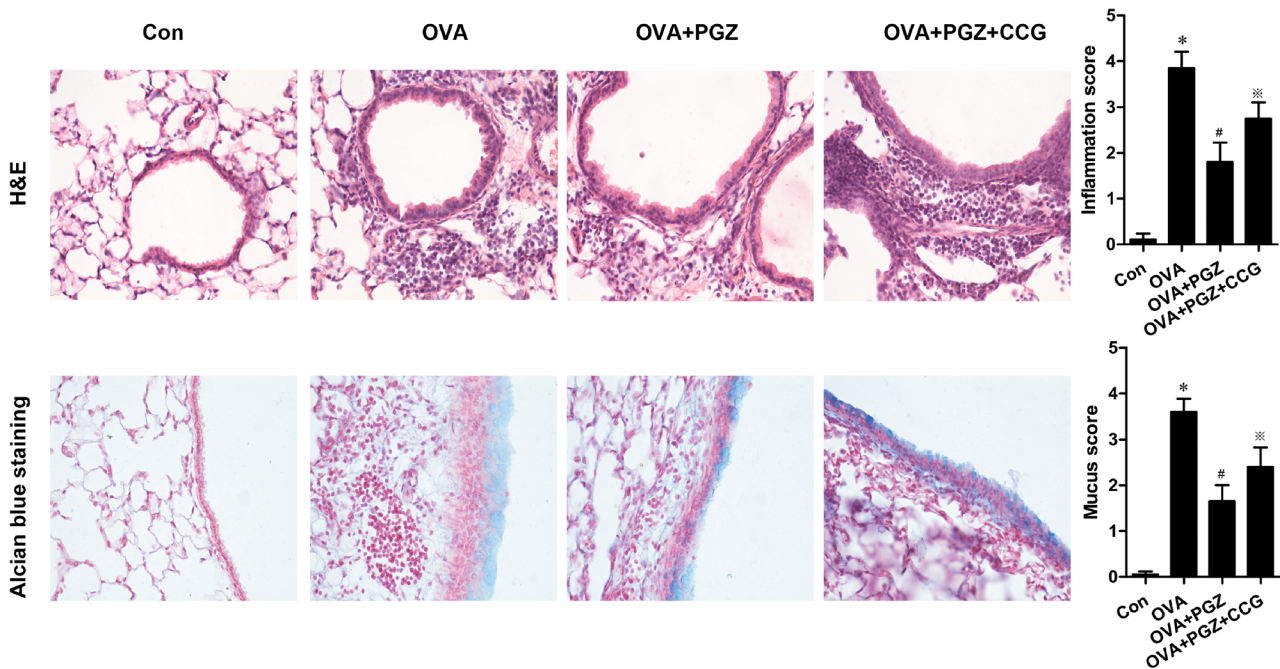


Fig. 2. PGZ treatment attenuated OVA-induced chronic airway allergic inflammation. Inflammatory cell infiltration and mucus production in the lung were assessed by H&E and alcian blue staining in OVA-induced asthma mouse model with PGZ treatment ($\times 200$). Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; # $P < 0.05$ vs. OVA group; $P < 0.05$ vs. OVA + PGZ group.

PGZ Treatment Inhibited OVA-Induced Airway Remodeling

Deposition of collagen and airway smooth muscle cell proliferation were measured by Masson's trichrome staining and immunohistochemistry (IHC) for α -actin, respectively, to evaluate OVA-induced airway remodeling in this study. Compared to Con group, OVA treatment noticeably enhanced collagen deposition and airway smooth muscle cell proliferation in OVA group ($P < 0.05$, respectively; Fig. 4), but PGZ significantly alleviated OVA-induced airway remodeling in OVA + PGZ group when compared to OVA group. CCG 63802 partially impaired PGZ's effect on collagen deposition and airway smooth muscle cell proliferation in OVA + PGZ + CCG group ($P < 0.05$, respectively).

Effects of PGZ and CCG 63802 on PPAR γ and RGS4 Protein Expressions

To determine the PPAR γ and RGS4 protein expressions associated with asthmatic mice, we evaluated the PPAR γ and RGS4 protein expression by IHC. As shown in Fig. 5, the expression level of PPAR γ was significantly increased in PGZ treatment group when compared to OVA group ($P < 0.05$). However, there were no significant

changes of PPAR γ expression after CCG 63802 administration in OVA + PGZ + CCG and OVA + PGZ groups. In addition, it was found that the RGS4 expression was also significantly upregulated in OVA + PGZ treated mice when compared to OVA group ($P < 0.05$). However, in OVA + PGZ + CCG group, the RGS4 inhibitor CCG 63802 treatment significantly reduced RGS4 expression when compared to OVA + PGZ group ($P < 0.05$).

Signaling Pathway Involved in the RGS Protein Mediated Negative Regulation of OVA-Induced Asthma

In previous studies, RGS4 protein has been proved to have more close relationship with cell proliferation [11], and the possible downstream signal of RGS4-mediated negative regulation of OVA-induced asthma by PGZ was investigated. Our results showed that p-ERK, p-Akt, and p-mTOR expressions were markedly upregulated by OVA but downregulated by PGZ (all $P < 0.05$). However, CCG 63802 administration could partially restore the expressions of p-ERK, p-Akt, and p-mTOR expressions when compared to OVA + PGZ group (Fig. 6a). Total ERK, m-TOR, and Akt expressions did not change among different treated groups. The balance of matrix metalloproteinases (MMPs) and tissue inhibitor of

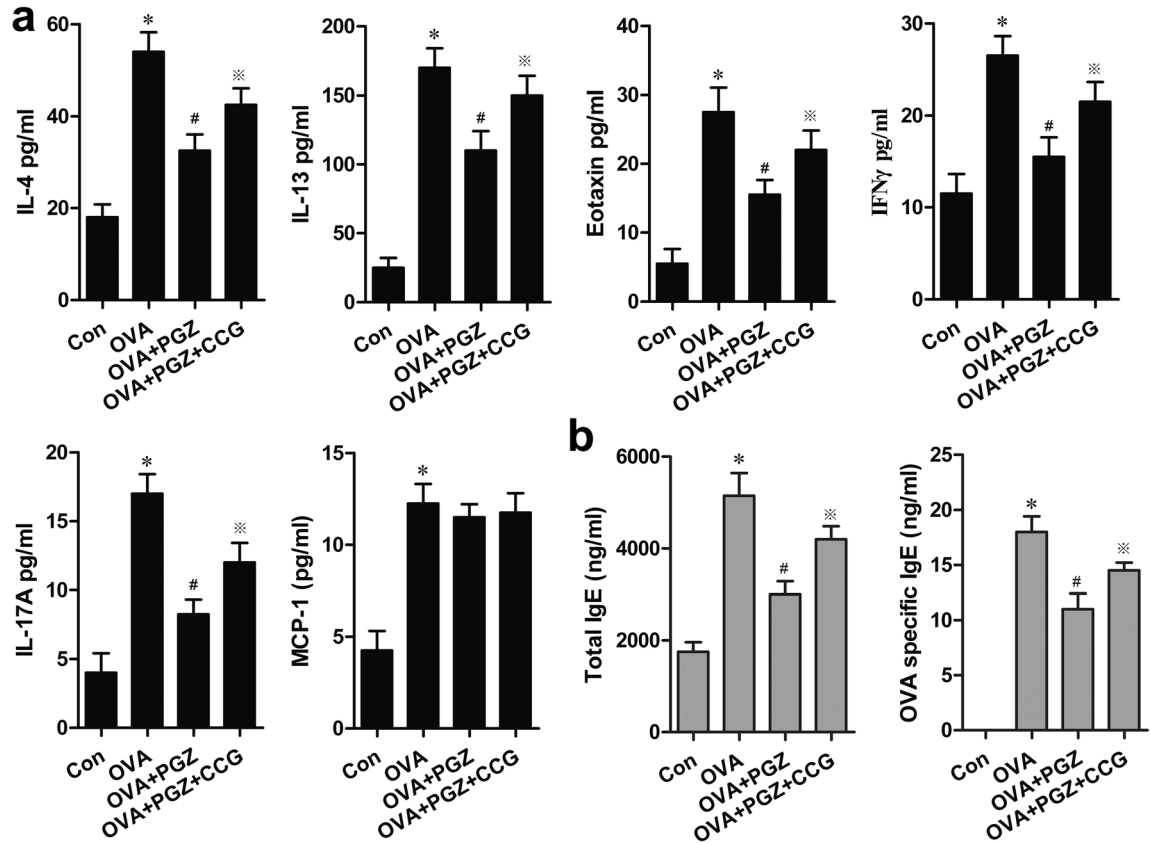


Fig. 3. PGZ influenced the levels of Th2 cytokines in BALF and IgE in serum. **a** The levels of cytokine and chemokine in BALF from OVA-induced asthma mouse model were determined by ELISA kits. **b** Total and OVA-specific IgE levels in serum from OVA-induced asthma were measured by ELISA kits. Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; # $P < 0.05$ vs. OVA group; ** $P < 0.05$ vs. OVA + PGZ group.

metalloproteinases (TIMPs) is very vital to airway remodeling formation. Western blot also showed that PGZ significantly increased OVA-induced downregulation of TIMP protein expression in OVA + PGZ group when compared to OVA group. In contrast, compared to OVA + PGZ group, CCG 63802 reversely decreased TIMP expression in OVA + PGZ + CCG group. Moreover, MMP zymography were performed to detect the enzymatic activity of MMP-2 and MMP-9. The results revealed that MMP activities significantly reduced in OVA + PGZ treatment group but increased in OVA + PGZ + CCG treatment group ($P < 0.05$, respectively; Fig. 6b).

DISCUSSION

Airway inflammation, airway remodeling, and airway hyperresponsiveness are the fundamental components of pathogenesis that lead to symptoms and lung function damage in asthma [17]. In our study, mouse model of

asthma was successfully established through sensitizing and challenging mice with OVA repeatedly. Th2-dominant inflammation during allergic inflammation formation and histocyte proliferation such as eosinophil, neutrophil, lymphocyte, and macrophage during airway remodeling phase were both observed. The representative cytokines and chemokine of Th2, IL-4, IL-13, IL-17, IFN- γ , and eotaxin obviously increased in airway allergic inflammation, of which IL-17A was elevated much higher in airway remodeling model accompany with airway smooth cell proliferation and collagen deposit.

Diverse immune cells, such as neutrophils, macrophages, dendritic cells, T cells, and mast cells, have been evidenced to be regulated by PPAR γ agonists [18]. Furthermore, PPAR γ activation could inhibit the expressions of large amounts of transcription factors, such as nuclear factor of activated T cells, signal transducers and activators of transcription 1 (STAT1), activator protein 1 (AP-1), and nuclear factor-kappa B (NF- κ B), which are directly linked

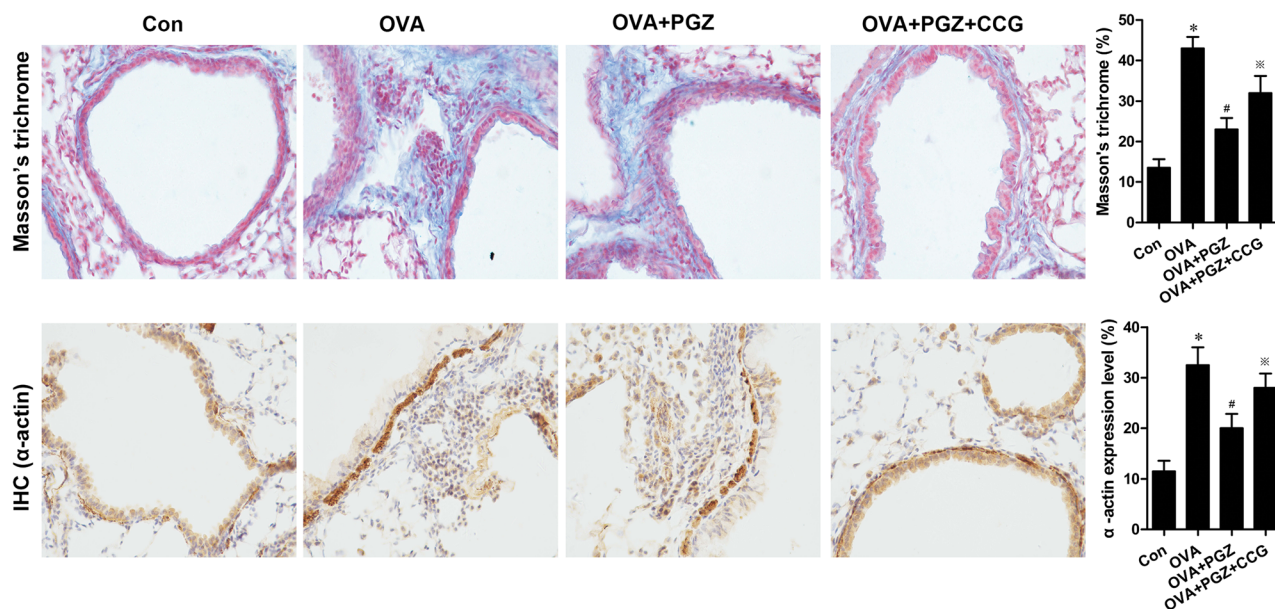


Fig. 4. PGZ treatment inhibited OVA-induced airway remodeling. Collagen deposition and airway smooth muscle cell proliferation were measured by Masson's trichrome staining and IHC for α -actin in OVA-induced asthma mouse model ($\times 200$). Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; # $P < 0.05$ vs. OVA group; $P < 0.05$ vs. OVA + PGZ group.

to several inflammatory factor gene transcriptions [19, 20]. In this study, PPAR γ agonist PGZ substantially ameliorated the pathophysiological features of asthma, including airway inflammation, airway AHR, eosinophil infiltration and mucus hypersecretion, collagen deposition and airway smooth muscle cell proliferation, and the increased levels of Th2 cytokines and chemokine, impaired inflammatory cell accumulation, and decreased total and serum OVA-specific IgE, as well as upregulated the RGS4 expression. Therefore, these data indicated that PGZ exerted its therapeutic effect against asthma through activating PPAR γ and some specific downstream pathways might also involve.

PPAR activation has been verified to upregulate the expression of B/R4 subfamily members of RGS protein and subsequently inhibits GPCRs/G α signal pathway [21]. According to previous studies, RGS4 was upregulated by activation of PPAR α agonist, which resulted in attenuating the diabetic cardiomyopathy aroused from streptomycin, and upregulation of RGS4 by PPAR β could also relieve the arteriosclerosis induced by angiotensin II [15, 22]. Recently, some researchers have found the aberrant RGS4 expression in the airway smooth muscle cells, for example, RGS4 overexpression has been reported to delay and alter lung epithelial cell tubulation through selectively inhibiting G protein-mediated

p38 MAPK activation, and consequently, reducing epithelial cell proliferation, migration, and expression of vascular endothelial growth factor (VEGF) [23]. Furthermore, RGS4 could inhibit GPCR-mediated bronchoconstriction; unexpectedly, RGS4 was required for PDGF-induced proliferation and sustained activation of PI3K, a mitogenic signaling molecule that regulates lung airway smooth muscle proliferation [21]. However, there is still no evidence about its role and the downstream signals in the regulation of airway allergic inflammation and remodeling induced by PPAR γ agonist. In our experiments, RGS4 expression was significantly upregulated by PGZ administration. Whereas after treated by RGS4 inhibitor CCG 63802, RGS4 expression was dramatically reduced but CCG 63802 did not influence the PPAR γ expression. These findings demonstrated that RGS4 directly participated in the regulation of PPAR γ signaling pathway against OVA-induced asthma. The specificity of CCG 63802 for RGS4 has been previously validated by a multiple flow cytometry protein interaction assay [24], and CCG 63802 has been successfully administered by intrathecal catheter for inhibiting RGS4 in spinal treatment [25]. In this study, CCG 63802 was given intratracheally during OVA challenge and

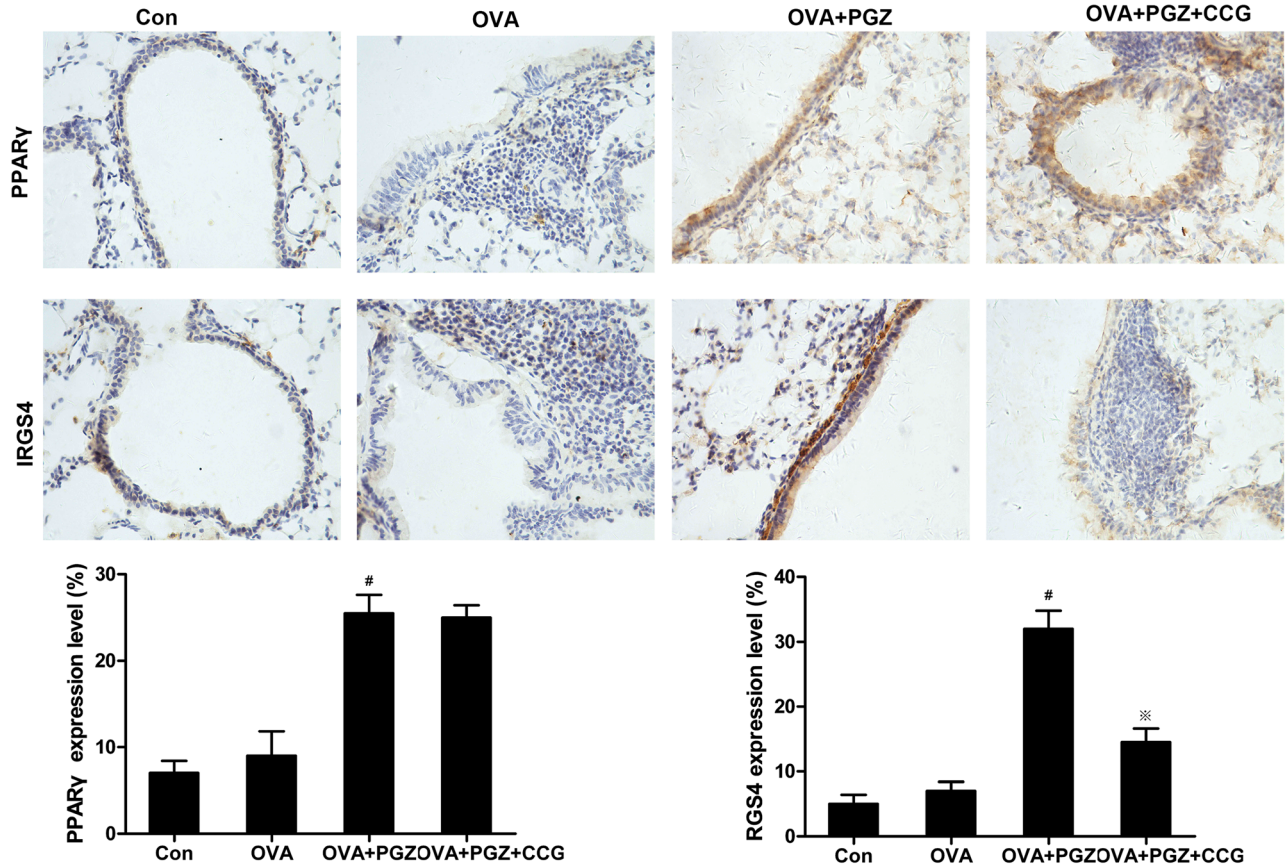


Fig. 5. Effects of PGZ and CCG 63802 on PPAR γ and RGS4 protein expressions. The protein expression levels of PPAR γ and RGS4 in OVA-induced asthma were measured by IHC ($\times 200$). Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; [#] $P < 0.05$ vs. OVA group; [※] $P < 0.05$ vs. OVA + PGZ group.

received favorable inhibitory effect, suggesting the protective role of RGS4 on OVA-induced asthma.

Extracellular regulated kinases (ERK) and protein kinase B (Akt) are intermediaries in relaying extracellular growth. It has been demonstrated that ERK and Akt signaling pathways are mainly engaged in cell proliferation, and phosphorylation is one of the most important mechanisms of above signal molecules playing their roles [26]. It has been demonstrated that different inflammatory signal pathways can be inactivated by different RGS subfamily members upon inactivation of G protein-coupled receptors mediated by Gq and Gi/o proteins [27]. In our experiments, PGZ treatment significantly reduced the expression of ERK, Akt, and its down-stream signaling mTOR in OVA-induced asthma. Whereas once RGS4 expression was

inhibited by CCG 63802, the activations of ERK, Akt, and mTOR signaling were significantly induced. Consistent with our findings, previous study has reported that silence of RGS4 could increase the activation of ERK in human atherosclerosis [15]. Furthermore, in colonic smooth muscle, upregulation of RGS4 expression by IL-1 had been proved to be inhibited by the PI3K/Akt/GSK3 pathway [28]. Therefore, we supposed that ERK and Akt/mTOR may be involved in the RGS4-mediated negative regulation of PGZ on OVA-induced asthma.

Another phenomenon that happened in OVA-induced airway remodeling was collagen over-deposition in the basal membrane of airway wall followed by that airway would turn into more stiff and less elastic [29]. Since matrix metalloproteinases

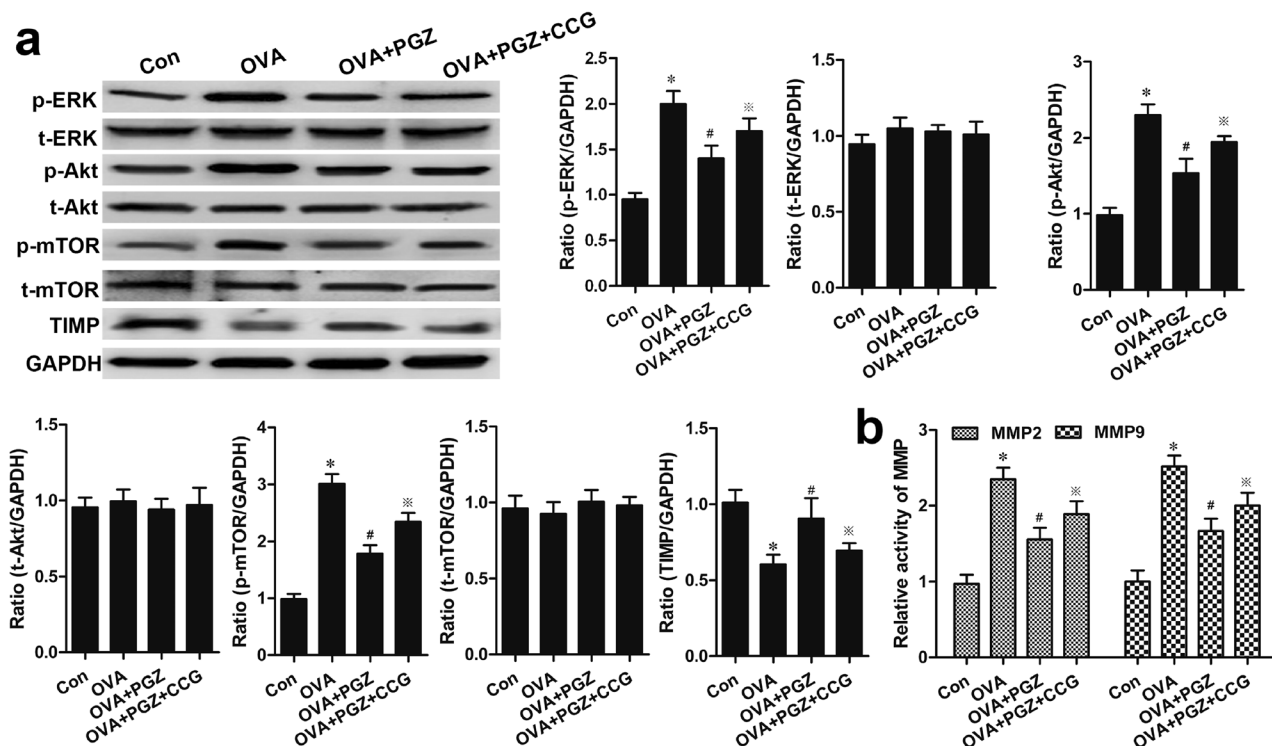


Fig. 6. Signaling pathway involved in the RGS protein mediated negative regulation of OVA-induced asthma. **a** The expression levels of ERK, Akt/mTOR, and TIMP proteins in OVA-induced asthma were measured and quantified by Western blot. **b** The enzymatic activity of MMP-2 and MMP-9 in OVA-induced asthma was determined by MMP zymography. Data were expressed as means $\pm$ SEM. * $P < 0.05$ vs. Con group; # $P < 0.05$ vs. OVA group; ** $P < 0.05$ vs. OVA + PGZ group.

(MMPs) belong to the group of ECM degradation enzymes, the balance of MMPs and tissue inhibitor of metalloproteinases (TIMPs) is very vital to maintain the connective tissue in normal condition, and degradation of basement membrane and extracellular matrix (ECM) is responsible for the formation of airway remodeling [30]. In this study, we found that RGS4 reversed the inhibitory effect of PGZ on collagen deposition by increasing MMP2/9 activities and decreasing TIMP expression. These findings demonstrated that MMP2/9 and TIMP were both associated with PPAR γ /RGS4 pathway in inhibiting OVA-induced airway remodeling in asthma. Though we determined that the protective effect of PPAR γ agonist PGZ on OVA-induced asthma was through regulating RGS4 expression in the current study, but other crucial RGS family members or signaling pathways may also be involved in its protective effects on asthma. For instance, RGS1, 2, 3, 5, and 10 all have been reported to be related to

asthma and suggested to be the potential targets for treatment of allergic inflammatory diseases [31]. Thus, further studies are required to elucidate the precise molecular mechanism underlying the protective effect of PPAR γ agonist PGZ on OVA-induced asthma.

CONCLUSIONS

In conclusion, PPAR γ agonist PGZ exhibited protective effect on OVA-induced asthma by reversing the pathophysiological features of asthma and upregulating RGS4 expression in lungs. The attenuating effect of PPAR γ agonist on airway inflammation and remodeling was abolished by administration of RGS4 inhibitor. These findings suggest that, to some extent, the protective effect of PPAR γ in asthma might partially through regulating RGS4 expression. We also found that ERK/Akt/mTOR signaling

pathways and MMP/TIMP were also involved in the regulation of PPAR γ /RGS4 in OVA-induced asthma. Therefore, we suggested that the activation of PPAR γ /GRS4 and its down-stream signaling played a crucial role in attenuating airway inflammation and remodeling in OVA-induced asthma.

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COMPLIANCE WITH ETHICAL STANDARDS

Ethical Approval. All procedures performed in studies involving animals were in accordance with the Animal Research Ethics Committee of Xi'an Jiaotong University.

Conflict of Interest. The authors declare that they have no conflict of interest.

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