

Rosiglitazone Attenuates Allergic Inflammation and Inhibits Expression of Galectin-3 in a Mouse Model of Allergic Rhinitis

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Peroxisome proliferator activated receptor gamma (PPAR- γ) agonists are involved in the regulation of inflammatory responses and recent studies suggest that PPAR- γ ligands may have potential for the treatment of allergic airway disease. This study investigated the effect of the PPAR- γ agonist, rosiglitazone, on nasal mucous allergic inflammation and galectin-3 gene expression in a murine model of allergic rhinitis (AR mice). Rosiglitazone resulted in a statistically significant attenuation of

the increased numbers of inflammatory cells and Th2 cytokine levels in nasal cavity lavage fluid of AR mice. Furthermore, the expression of galectin-3 in the nasal mucosa of AR mice was statistically significantly increased. Over-expression of galectin-3 was markedly suppressed by rosiglitazone treatment. These data suggest that the PPAR- γ agonist, rosiglitazone, may have potential in the development of therapies for allergic rhinitis.

KEY WORDS: ROSIGLITAZONE; PEROXISOME PROLIFERATOR ACTIVATED RECEPTOR GAMMA AGONIST; ALLERGIC RHINITIS; GALECTIN-3; CYTOKINES; MICE

Introduction

Allergic rhinitis is a common inflammatory disease of the airways¹ that affects an estimated 15 – 20% of the population.² Attention has focused on the desirability of avoiding the adverse effects of medicines traditionally used to treat allergic rhinitis (such as intranasal corticosteroids), which has stimulated the search for effective and safe alternatives.

Peroxisome proliferator activated receptors (PPARs) are transcriptional factors

belonging to the ligand-activated nuclear receptor superfamily.³ Previous studies have shown that PPAR- γ is involved in airway inflammation and remodelling in asthma.^{4,5} Activation of PPAR- γ reduces the expression of various cytokines, airway hyper-responsiveness and activation of eosinophils, which are increased by induction of asthma.⁶ Galectins are β -galactoside-binding proteins with a conserved carbohydrate-recognition domain.⁷ Among the 14 galectins, the expression of galectin-3 has

been shown to play a pivotal role in pathological processes as an inflammatory mediator.^{8,9} Recently, the presence of galectin-3 in activated cells that are involved in allergic airway inflammation has been extensively documented.¹⁰

This study aimed to investigate the effect of the PPAR- γ agonist, rosiglitazone, on nasal mucous allergic inflammation and galectin-3 expression in a murine model of ovalbumin (OVA)-induced allergic rhinitis.

Materials and methods

MICE

BALB/c mice, age 8 – 10 weeks, weighing 18 – 25 g, were provided by the Laboratory Animal Centre, China Medical University, Shenyang, Liaoning Province, China, and were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH Publication No. 85-23, revised 1996). Mice were randomly divided into three groups of 10: group C (control mice), group AR (mice with allergic rhinitis), and group R (mice with allergic rhinitis treated with rosiglitazone). On days 0 and 7, all mice were actively immunized by intraperitoneal injection of 100 μ g OVA (Sigma, St Louis, MO, USA). On day 14, mice in groups AR and R were challenged by dripping OVA dissolved in physiological saline (1 mg/ml, 10 μ l/nostril) into the bilateral nasal cavities. On days 14 – 20, rosiglitazone (5 mg/kg, GlaxoSmithKline, Uxbridge, UK) was administered orally to mice in group R 1 h before nasal antigen challenge with OVA. Mice in group C were exposed to an aerosol containing 0.9% saline. This animal model has been described previously.¹¹

NASAL HYPERSENSITIVITY

The severity of allergic rhinitis was determined by measuring the frequencies of nose rubbing or scratching and sneezing for

30 min after the last intranasal challenge with OVA, as described previously.¹²

NASAL CAVITY LAVAGE FLUID

Partial tracheal resection was performed 24 h after the last challenge. The nasal cavity was washed twice with 0.5 ml physiological saline, and nasal cavity lavage fluid was collected in Eppendorf tubes and preserved in ice. Samples were centrifuged at 700 *g* for 5 min at 4°C. After centrifugation the supernatants were stored at –70°C. The deposited cells were smeared, then stained with Wright–Giemsa and classified by standard morphology.

ENZYME-LINKED IMMUNOSORBENT ASSAY

Supernatants were analysed by enzyme-linked immunosorbent assay (ELISA). The amount of interleukin (IL)-4, IL-5 and IL-13 in the nasal cavity lavage fluid was measured with a specific mouse IL-4, IL-5 and IL-13 ELISA kit (R&D Systems, Minneapolis, MN, USA).

REVERSE-TRANSCRIPTION POLYMERASE CHAIN REACTION

Reverse-transcription polymerase chain reaction (RT-PCR) was used to examine the expression of galectin-3 mRNA in nasal mucosa tissues. Total RNA was isolated with TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). First-strand complementary DNA synthesis was performed on 0.5 μ g total RNA using the random priming extension method. The primer sequences were galectin-3 (sense): 5'-CCTATGACCTGCCCTTGC-3', galectin-3 (antisense): 5'-CCCAGTTGGC TGATTTC-3'; glyceraldehyde-3-phosphate dehydrogenase (GAPDH; sense): 5'-TTCA CCACCATGGAGAAGGC-3', and GAPDH (antisense) 5'-GGCATGGACTGTGGTCATGA-3'. The predicted size for the PCR products for

each primer pair was 513 base pairs (bp) for galectin-3 and 236 bp for GAPDH. The PCR cycle conditions were: denatured at 94 °C for 3 min; then 36 cycles of 94 °C for 30 s, 55 °C for 1 min, and 72 °C for 1 min; and final extension at 72 °C for 5 min. Amplified products were subjected to electrophoresis in 1% agarose agar. The gels were recorded using a ChemiImager™ 5500 imaging system (Alpha Innotech Corp., San Leandro, CA, USA). Densitometry analysis and measurement of the relative quantities of GAPDH and galectin-3 PCR products were undertaken using FluorChem™ version 2.0 software (Alpha Innotech Corp.). The amount of galectin-3 mRNA gene expression in nasal mucosa was calculated as the ratio of the optical densities of galectin-3 mRNA and GAPDH mRNA measured at 320 nm.

STATISTICAL ANALYSIS

Results were expressed as mean $\pm$ SE. Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA). All data were analysed with SPSS® version 12.0 software (SPSS Inc., Chicago, IL, USA). *P* values < 0.05 were considered statistically significant.

Results

EVALUATION OF NASAL HYPERSENSITIVITY

The frequencies (per 30 min) of nose rubbing or scratching and sneezing, indicating allergic rhinitis, were significantly higher (*P* < 0.05) in the allergic rhinitis group (74.4 ± 11.7 and 42.5 ± 27.2 , respectively) than in the control group (26.9 ± 9.2 and 5.7 ± 1.1 , respectively). After treatment with 5 mg/kg rosiglitazone for 7 days, the frequencies (per 30 min) of nasal rubbing or scratching and sneezing in mice with allergic rhinitis decreased significantly (42.1 ± 12.6 and 8.3 ± 3.9 , respectively) (*P* < 0.05).

CELL COUNT IN NASAL CAVITY LAVAGE FLUID

We examined the effects of rosiglitazone on the infiltration of inflammatory cells into nasal cavity lavage fluid in mice. Total inflammatory cell levels in nasal cavity lavage fluid were significantly elevated in the OVA-challenged mice compared with the control mice (*P* < 0.05). The greatest increase in inflammatory cells was observed in eosinophils followed by macrophages, with smaller increases in neutrophils and lymphocytes. Rosiglitazone treatment (5 mg/kg rosiglitazone for 7 days) significantly reduced total inflammatory cell numbers, and counts of eosinophils, macrophages, neutrophils and lymphocytes compared with cell counts in untreated group AR mice (*P* < 0.05 in all cases; Fig. 1).

TH2 CYTOKINE IN NASAL CAVITY LAVAGE FLUID

Significant elevations of IL-4, IL-5 and IL-13 in nasal cavity lavage fluid were observed following OVA sensitization and challenge (Fig. 2). Levels of IL-4, IL-5 and IL-13 in the nasal cavity lavage fluid of group AR mice were significantly increased compared with levels found in the control group (*P* < 0.05), and were markedly decreased in group R mice (*P* < 0.05). This indicates that treatment with 5 mg/kg rosiglitazone for 7 days inhibits the production of these ILs in the mouse model of allergic rhinitis.

GALECTIN-3 mRNA EXPRESSION IN NASAL MUCOSA TISSUES

We found that galectin-3 mRNA expression in the AR group, expressed as the ratio of the optical densities of galectin-3 mRNA and GAPDH mRNA at 320 nm, was significantly higher than the expression in the control group (0.67 ± 0.15 vs 0.13 ± 0.04 , *P* < 0.05; Fig. 3). Over-expression of galectin-3 in OVA-

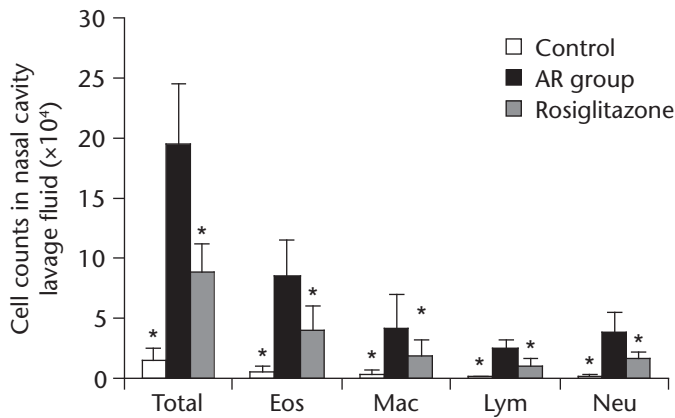


FIGURE 1: Inflammatory cell counts in nasal cavity lavage fluid of control mice, mice with allergic rhinitis (AR group) and AR mice treated with rosiglitazone (5 mg/kg for 7 days). Total, total cells; Eos, eosinophils; Mac, macrophages; Lym, lymphocytes; Neu, neutrophils. Data show mean $\pm$ SE for 10 mice per group in three separate experiments. * $P < 0.05$ compared with the AR group

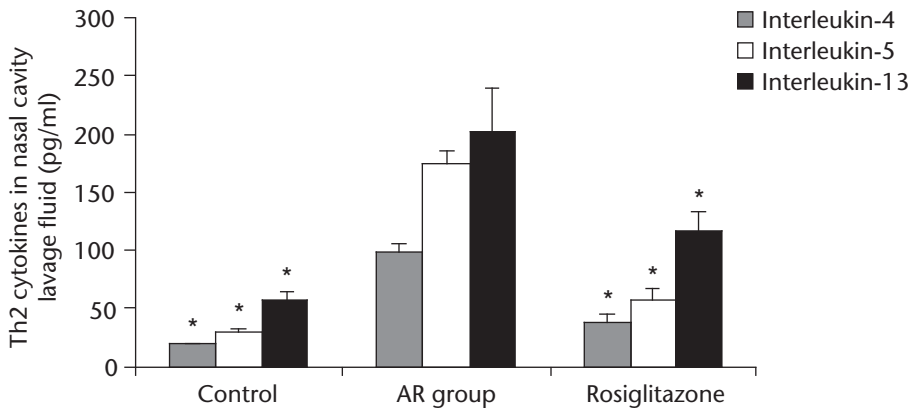


FIGURE 2: Effect of rosiglitazone (5 mg/kg for 7 days) on Th2 cytokine levels in nasal cavity lavage fluid after ovalbumin challenge for control mice, mice with allergic rhinitis (AR group) and AR mice treated with rosiglitazone (5 mg/kg for 7 days). Nasal cavity lavage fluid was collected 24 h after the last ovalbumin challenge and levels of Th2 cytokines were analysed using enzyme-linked immunosorbent assay. Data show mean $\pm$ SE for 10 mice per group in three separate experiments. * $P < 0.05$ compared with the AR group

sensitized and challenged mice was significantly suppressed by treatment with 5 mg/kg rosiglitazone for 7 days (0.29 ± 0.18 , $P < 0.05$).

Discussion

Allergic rhinitis is an immune disorder, characterized by a high level of infiltration of eosinophils and other inflammatory cells

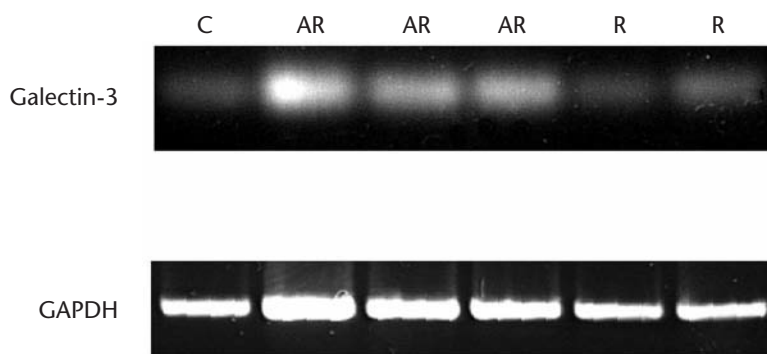


FIGURE 3: Galectin-3 mRNA expression compared with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression in nasal mucosa tissues following electrophoresis on 1% agarose agar C, control mice; AR, allergic rhinitis mice; R, allergic rhinitis mice treated with rosiglitazone (5 mg/kg for 7 days)

into nasal tissue, that is caused by the predominant production of Th2 cytokines such as IL-4, IL-5 and IL-13.¹³ In this study, we have shown that inflammatory cell counts and the levels of IL-4, IL-5 and IL-13 in nasal cavity lavage fluid were significantly increased in mice with allergic rhinitis.

The PPAR- γ agonists are involved in the regulation of inflammatory responses.^{3,14} Studies suggest that PPAR- γ ligands may have potential in the treatment of allergic airway disease.^{4,5} In animal models of allergic airway disease, PPAR- γ activation induces an inhibition of eosinophil and lymphocyte influx and decreases antigen-induced airway hyper-responsiveness, lung inflammation, eosinophilia and *GATA3* expression.^{13,15,16} Consistent with these observations, our current study with an OVA-induced murine model of allergic rhinitis has revealed that administration of the PPAR- γ agonist, rosiglitazone, attenuated increases in inflammatory cells and reduced levels of Th2 cytokine in nasal cavity lavage fluid, airway inflammation and airway hyper-

responsiveness. We conclude, therefore, that PPAR- γ regulation may play an important role in the induction and maintenance of allergic rhinitis in mice, but the role of PPAR- γ in regulating these changes remains unclear.

Galectin-3 is a pro-inflammatory molecule in a murine model of allergic airway disease. Galectin-3 released into the extracellular space under inflammatory conditions activates inflammatory cells associated with allergic responses or contributes to the binding of these cells to extracellular matrix glycoproteins at the sites of inflammation.^{17,18} Recently, galectin-3 was shown to support eosinophil rolling and adhesion *in vitro*.¹⁹ Galectin-3 is also known to have anti-apoptotic properties, which could render the inflammatory cells less sensitive to apoptotic stimuli, thereby enhancing their survival in the inflamed airways.^{20–22} The present results suggest that galectin-3 may contribute to the regulation of the Th1/Th2 response: *gal3*^{-/-} mice have previously been shown to mount a lower airway Th2 response, but a higher Th1 response compared with *gal3*^{+/+} mice.¹⁰

In the present study, we found that the expression of galectin-3 mRNA in the AR group was significantly increased compared with expression in the control group. Our data, therefore, suggest that galectin-3 may play an important role in inducing and maintaining AR. In addition, it was demonstrated that the PPAR- γ agonist, rosiglitazone, reduced the expression of galectin-3 in nasal mucosa tissues of AR mice. This suggests that rosiglitazone ameliorates OVA-induced allergic rhinitis, at least in part, through down-regulating the expression of the galectin-3 gene.

In conclusion, our data suggest a new therapeutic approach using oral

administration of rosiglitazone in the management or treatment of AR. Further studies are needed to identify the molecular mechanism of its anti-inflammatory action. We plan to explore the anti-allergy action of rosiglitazone in other allergic rhinitis models, using different species.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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