

In-vivo antioxidant and anti-inflammatory activity of rosiglitazone, a peroxisome proliferator-activated receptor-gamma (PPAR- γ) agonists in animal model of bronchial asthma

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Keywords

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Abstract

Objectives Peroxisome proliferator activated receptor-gamma (PPAR- γ) has been shown to play an important role in the control of immunological and inflammatory responses. This study aims at investigating the potential role of rosiglitazone, a strong PPAR- γ agonist in a murine model of bronchial asthma.

Methods Adult male guinea pigs were administered ovalbumin 100 mg/kg subcutaneous (SC) and 100 mg/kg intraperitoneal (IP). Treatment with rosiglitazone [5 mg/kg/day, per oral (PO)] was assessed for 21 days. On day 21, the animals were challenged with the same dose of ovalbumin. The forced expiratory volume in 1 s (FEV₁) to forced vital capacity (FVC), FEV₁/FVC, was measured using a spirometer to diagnosis lung obstruction. Serum levels of interleukin-5 (IL-5) and immunoglobulin E (IgE) were assessed. The activity of superoxide dismutase (SOD) and catalase and the level of reduced glutathione (GSH) were determined in lung tissue homogenates.

Key findings Our results demonstrated that treatment with rosiglitazone resulted in a statistically significant improvement in lung function and histopathological features. Significant decrease in the serum levels of IL-5 and IgE were observed. The activity of SOD and catalase as well as the GSH level were significantly increased in the lung tissues of treated animals compared with untreated asthmatic animals. Serum IgE concentrations and IL-5 levels were directly correlated to each other and inversely correlated to the SOD, GSH and catalase levels in the all studied guinea pigs.

Conclusions Our results provide evidence that the PPAR- γ agonist rosiglitazone may have potential in the development of therapies for bronchial asthma.

Introduction

Bronchial asthma is a disease characterized by reversible airway obstruction in response to allergens, chronic eosinophilic airway inflammation and nonspecific airway hyperresponsiveness (AHR).^[1] Typically, airflow obstruction diseases such as bronchial asthma are diagnosed using spirometer by demonstrating a lower than normal ratio of forced expiratory volume in 1 s (FEV₁) to forced vital capacity (FVC), FEV₁/FVC.^[2]

The pathological symptoms and clinical hallmarks of allergic asthma are the result of a Th2 type-dominated cytokine profile with increased levels of interleukin (IL)-4, IL-5 and IL-13.^[3] These cytokines may induce airway inflammation and AHR directly, through effects on airway smooth muscle and bronchial mucosa,^[4] or indirectly, through effector cells such as B-cells, mast cells and eosinophils,^[5,6] and further sustain the Th2 response.^[7]

Among the Th2 cytokines, IL-5 is a major factor that promotes eosinophilia by priming the cells for heightened responsiveness, enhancing their adhesion to the endothelium, increasing eosinophilic cytotoxicity and prolonging the viability of mature eosinophils.^[8]

Moreover, the importance of immunoglobulin E (IgE) in airway inflammation and the development of bronchial asthma have been explored in different animal models and in patients suffering from bronchial asthma. Furthermore, in patients suffering from bronchial asthma, IgE levels in serum or bronchoalveolar lavage fluid are often increased and may correlate with the incidence or severity of the disease.^[1]

Pulmonary cells have several ways of alleviating the effects of oxidative stress and diminishing the expression of the receptor superfamily containing transcription factors that regulate gene expression.^[9] Peroxisome proliferator-activated receptor-gamma (PPAR- γ) is a prototypical ligand-activated nuclear receptor involved in the control of energy balance and both lipid and glucose homeostasis. It controls the transcription of genes encoding proteins that involved in the lipid and glucose metabolism.^[10] Early investigation of PPAR- γ focused on its role in regulating adipocyte differentiation of pre-adipocytes into mature adipocytes in which gene expression, cell morphology and hormone sensitivity change.^[9] Recent studies have demonstrated that this receptor has a pivotal role in regulating the immune response.^[11] PPAR- γ is now being investigated as a potential target in a variety of lung-related diseases.^[12] PPAR- γ interacts with a wide spectrum of natural and synthetic lipophilic ligands. The natural ligands include arachidonic acid, polysaturated fatty acids, e.g. linoleic acid, prostaglandin J2 derivatives and oxidized fatty acids.^[10] Moreover, PPAR- γ ligands have been shown to exert anti-inflammatory effects on immune cells,^[13,14] as well as on specific cells in the lung, such as alveolar macrophages,^[15] airway epithelial cells^[16] and airway smooth muscle cells.^[17] Furthermore, several investigators have demonstrated that rosiglitazone, a member of the thiazolidinedione (TZD) drug class, reduces glucose, fatty acid and insulin blood concentrations. At the same time, rosiglitazone inhibits the growth of many cells, particularly cancerous airway epithelial cells, through binding to PPAR receptors and affecting DNA expression.^[18,19]

Food and Drug Administration reported serious side effects of the most common used anti-inflammatory drugs that lead to bleeding or perforated ulcers and deaths.^[20] Thus, there is a need for new anti-inflammatory drug that characterized by high safety profile and less side effects. Furthermore, there are few studies on anti-inflammatory effect of rosiglitazone; there is limited data regarding their effect on gastrointestinal tract (GIT). Recently, it was reported that rosiglitazone has potential therapeutic effects

on acute lung injury and pulmonary arterial hypertension.^[21,22] These studies suggested that rosiglitazone has protective and remodelling action in asthma, while the specific mechanisms are still unclear.

Based on this evidence, we hypothesized that the potent PPAR- γ agonist rosiglitazone, which has anti-inflammatory and antioxidant activity, would be beneficial in treating respiratory diseases such as bronchial asthma. Therefore, in an experimental model of bronchial asthma we evaluated the role of rosiglitazone in regulation of the allergic immune response associated with bronchial asthma. The effects of rosiglitazone on the inflammatory mediator; IL-5 as well as on oxidative stress were assessed.

Materials and Methods

Animals

Adult male guinea pigs weighing 400–450 g were obtained from the animal house of the Faculty of Medicine, Cairo University (Cairo, Egypt). The animals were housed in a pathogen-free environment in the animal house of October University for Modern Sciences and Arts, Egypt, in a 12/12-h light/dark cycle with food and water available *ad libitum*. Procedures involving animals and their care confirmed to the institutional guidelines and complied with national and international laws on the care and use of laboratory animals. The Research Ethics Committee of Faculty of Pharmacy, October University for Modern Sciences and Arts, Egypt, approved the procedures of this study (1 October 2014).

Chemicals and drugs

Rosiglitazone ($\geq 98\%$) was purchased from Cayman Chemical, Ann Arbor, MI, USA. The concentration of IL-5 was determined using a kit provided by Diaclone Res., Besancon Cedex, France. The concentration of IgE was determined using a kit provided by Diagnostic Automation/Cortez Diagnostics (Calabasas, CA, USA). Other chemicals used in this study were of analytical grade and were purchased from Sigma (St. Louis, USA).

Experimental design

Guinea pigs were used and divided into three groups each containing eight animals. The induction of bronchial asthma was performed according to the method of Piper and Vane with modification.^[23] On day 1, the first and second groups were immunized by injection of grade V chicken ovalbumin [100 mg/kg intraperitoneal (IP) and 100 mg/kg subcutaneous (SC)], while the third group was negative control group (control) and injected with saline only throughout the experiment. We confirmed the success of all sensitized animals by observing the skin allergic

reactions. Starting on the second day of immunization to avoid dampening sensitization, the first immunized group was treated with 5 mg/kg/day per oral (PO) of rosiglitazone (Figure 1) according to previous studies^[24,25] for 21 days (treated group), while the second immunized group received from the second day oral saline for the same period (asthma group).^[26] On day 21, the two immunized groups (asthma and treated groups) were challenged with another dose of ovalbumin, injected by the IP and SC routes. The control group underwent the same schedule for challenge, but received saline rather than ovalbumin (Table 1).

Assessment of respiratory functions

One hour after ovalbumin injection, guinea pigs were anesthetized by IP injection of 1.5 g/kg urethane according to the previous described.^[27] After full anaesthesia, the animal trachea was exposed and cannulated using a Y-shaped cannula. One arm of the Y-shaped cannula was connected to a spirometer (power lab, 4/30; AD Instruments Pty Ltd, Sydney, NSW, Australia). The animal was allowed to breathe freely through the other arm of the cannula while monitoring the following ventilatory parameters: FVC, FEV₁ and FEV₁/FVC.

Quantitative determination of IL-5

The IL-5 level was determined based on the method of Tavernier *et al.*^[28] Briefly, diluted samples or IL-5 standards

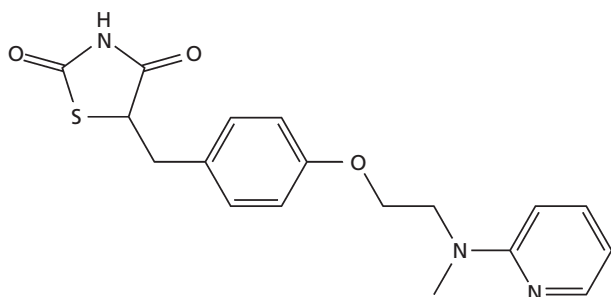


Figure 1 Chemical structure of rosiglitazone.

(10 µl) were mixed with 100 µl of 50 µg/l anti-IL-5 immunoglobulin G labelled with horseradish peroxidase in 10 mmol/l ethylenediaminetetraacetic acid. A 100-µl aliquot of the mixture was transferred to each of the wells. The plate was then incubated for 60 min at room temperature without shaking and then washed three times with 10 mmol/l phosphate buffered saline (PBS) (pH 7.0) containing 0.1 mol/l NaCl. The micro-plate-bound horseradish peroxidase activity was determined by adding 100 µl of 0.15 mol/l citric acid sodium phosphate buffer (pH 4.9) containing 2.0 g/l of *o*-phenylenediamine and 0.02% (v/v) hydrogen peroxide and incubating for 20 min at room temperature. The reaction was stopped by adding 100 µl of 1 mol/l sulfuric acid, and the absorbance was measured with a micro-plate reader at a wavelength of 450 nm. The level of IL-5 in the serum of guinea pig was calculated from the standard curve.^[28] The results were expressed as picogram/milliliter (pg/ml).

Enzyme immunoassay for the quantitative determination of IgE

IgE molecules from the serum sample were allowed to bind to both immobilized antibodies and the anti-IgE peroxidase conjugate according to the method of Crowther.^[29] Then, the wells were washed with 10 mmol/l PBS wash buffer to remove any material not bound to the inner surface of the wells. The quantity of the bound conjugate is directly proportional to the IgE concentration of the tested sample. Colour appears during incubation with the substrate solution, and the intensity is directly proportional to the IgE concentration in the sample. The enzymatic reaction was stopped by dispensing acidic solution (1 N HCl) into the wells. The results were expressed as nanogram/milliliter (ng/ml).

Determination of SOD activity in lung homogenate

The lung were finely sliced and homogenized in 10% (w/v) phosphate buffered saline, pH 7.8. The homogenates were

Table 1 The descriptive characteristics of studied guinea pigs and their classification into three groups

| | CT (<i>n</i> = 8) | BA (<i>n</i> = 8) | ROSI (<i>n</i> = 8) |
|------------------|--|---|---|
| Type | Adult male guinea pigs | Adult male guinea pigs | Adult male guinea pigs |
| Weight | 400–450 g | 400–450 g | 400–450 g |
| Light/dark cycle | 12/12 h | 12/12 h | 12/12 h |
| Treatment: | Saline (5 ml/kg IP plus 5 ml/kg SC) | Grade V chicken ovalbumin (100 mg/kg IP plus 100 mg/kg SC) | Grade V chicken ovalbumin (100 mg/kg IP plus 100 mg/kg SC) |
| From 2 to 21 day | Saline | Oral saline | Rosiglitazone (5 mg/kg/day, PO) |
| At day 21 | Saline | Grade V chicken ovalbumin (100 mg/kg IP and 100 mg/kg SC), | Grade V chicken ovalbumin (100 mg/kg IP and 100 mg/kg SC), |

BA, bronchial asthma group; CT, control guinea pigs; *n*, number of guinea pigs; ROSI, rosiglitazone treated.

centrifuged at 300 g at 4°C for 20 min using a Remi C-24 high-speed, cooling centrifuge. The clear supernatant was used for assaying the levels of antioxidant enzymes (superoxide dismutase (SOD) and catalase (CAT)) and reduced glutathione (GSH) using a spectrophotometer (UV-PC 1601; Shimadzu, Tokyo, Japan).

SOD activity was measured using the method of Nishikimi *et al.*^[30] Briefly, freshly prepared phenazine methosulfate (0.93 μM) was added to a cuvette containing 0.1 M sodium pyrophosphate buffer (pH 8.3), nitro blue tetrazolium (0.3 mM), nicotinamide adenine dinucleotide hydrogen (NADH) (0.47 mM) and the sample. The increase in absorbance at a wavelength of 560 nm was measured. The SOD activity was expressed as unit/gram (U/g) wet tissue.

Determination of CAT activity in lung homogenate

CAT activity was measured as described by Clairborne.^[31] In brief, the supernatant of the previously prepared lung homogenate was added to a cuvette containing 50 mM phosphate buffer (pH 7.8), and H₂O₂ was added to a final concentration of 10 mM. The disappearance of H₂O₂ was determined by measuring the absorbance at 240 nm. The difference in the absorbance per unit of time is a measure of CAT activity. Activity was expressed as micromoles/seconds/gram (μmol/sec/g) wet tissue.

Determination of lung GSH content

The reduced GSH level was determined using the method of Prins and Loose.^[32] Briefly, 0.5 ml of previously prepared homogenate was added to 0.5 ml of tungstate solution after centrifugation at 150 g for 5 min. The supernatant (200 μl) was added to a tube containing Tris buffer followed by the addition of 0.2 ml of 5,5-dithio-bis-(2-nitrobenzoic acid reagent. After 30–60 s, the colour had developed and the optical density was measured at wavelength of 412 nm. GSH content was expressed as milligrams/gram (mg/g) wet tissue.

Histopathological examination of the lung

The right lung was removed for histopathological evaluation, fixed overnight in 10% formalin and embedded in paraffin. The tissues were sliced into 4 μm sections and stained with haematoxylin and eosin. The degree, extent and distribution of lung inflammation were evaluated as follows: mild (1), moderate (2) and severe (3) for degree of inflammation; focal, patchy and diffuse for the extent of inflammation; and interstitial, perivascular and peribronchial for the distribution of inflammation.

Blood glucose and plasma lipids analyses

Standard, commercially available assays were used to evaluate the plasma levels of glucose, total cholesterol, triglycerides low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol in the studied guinea pigs.

Statistical analysis

Data are expressed as the mean ± standard deviation of the mean. Comparisons between different groups were carried out by one-way analysis of variance followed by the Kruskal–Wallis test. The level of significance was set at $P < 0.05$. Graphpad Prism software (version 5, GraphPad Software, Inc., San Diego, CA, USA) was used to carry out statistical analysis.

Results

Effect of rosiglitazone on respiratory functions

The FEV₁/FVC ratio ranged from 87.7% to 97.8%, with an average of $95.06\% \pm 3.6$, in the control group and from 21.2% to 29.9%, with an average of $24.7\% \pm 3.029$, in the asthma group. Rosiglitazone treatment significantly improved the deteriorated respiratory function tests compared with untreated asthmatic animals. The FEV₁/FVC ratio ranged from 41% to 56.6%, with an average of $49.8\% \pm 5.799$, in the rosiglitazone treatment group.

Effect of rosiglitazone on serum levels of IL-5 and IgE

Quantitative determination revealed that the serum levels of IL-5 and IgE were significantly elevated in asthmatic animals compared with control animals. The inhibitory effect of rosiglitazone treatment on IL-5 and IgE were indicated when rosiglitazone administered resulted in significantly decreased serum levels of IL-5 and IgE compared with the asthma group (Figure 2a and 2b, respectively; $P < 0.001$).

Effect of rosiglitazone on the tissue SOD and CAT activity

The mean activity of SOD and CAT in the lung tissue homogenates of the asthma group were significantly lower than in the control group. Our results showed that the rosiglitazone treatment significantly elevate the SOD and CAT enzymes activity compared with the asthma group (Figure 3a and 3b, respectively; $P < 0.001$).

Effect of rosiglitazone on the tissue GSH level

The mean level of reduced GSH in lung tissue homogenates of the asthmatic animal group was significantly lower

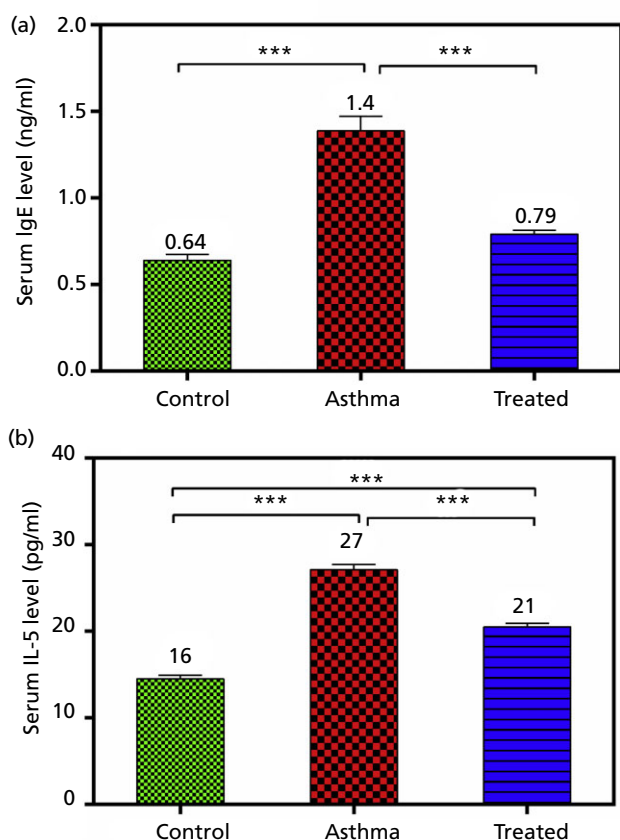


Figure 2 Serum levels of IgE level (A) and IL-5 level (B) in ovalbumin-sensitized guinea pigs with bronchial asthma (BA) and ROSI (5mg/kg/day for 21 days) treated groups. The level of IL-5 and IgE in guinea pigs with BA was significantly higher than that of the control group and significantly decreased in ROSI-treated group (** $P < 0.001$).

compared with the control group. However, in the animal group, treated with rosiglitazone elicited significant increase in the level of reduced GSH compared with untreated asthmatic animal group (Figure 3c; $P < 0.05$).

The correlation between studied parameters

Figure 4 shows the scatter plots of significantly correlated parameters in the serum and in lung tissue homogenates of all guinea pigs. The solid lines represent the linear regression and correlation coefficient (r), P is the correlation significant significance level and n is the total number of samples. There were positive correlations between each pair of the studied parameters: IgE/IL-5, SOD/CAT, SOD/GSH and GHS/CAT. While negative correlation of both IgE and IL-5 with each parameter of SOD, GSH and CAT was observed, respectively.

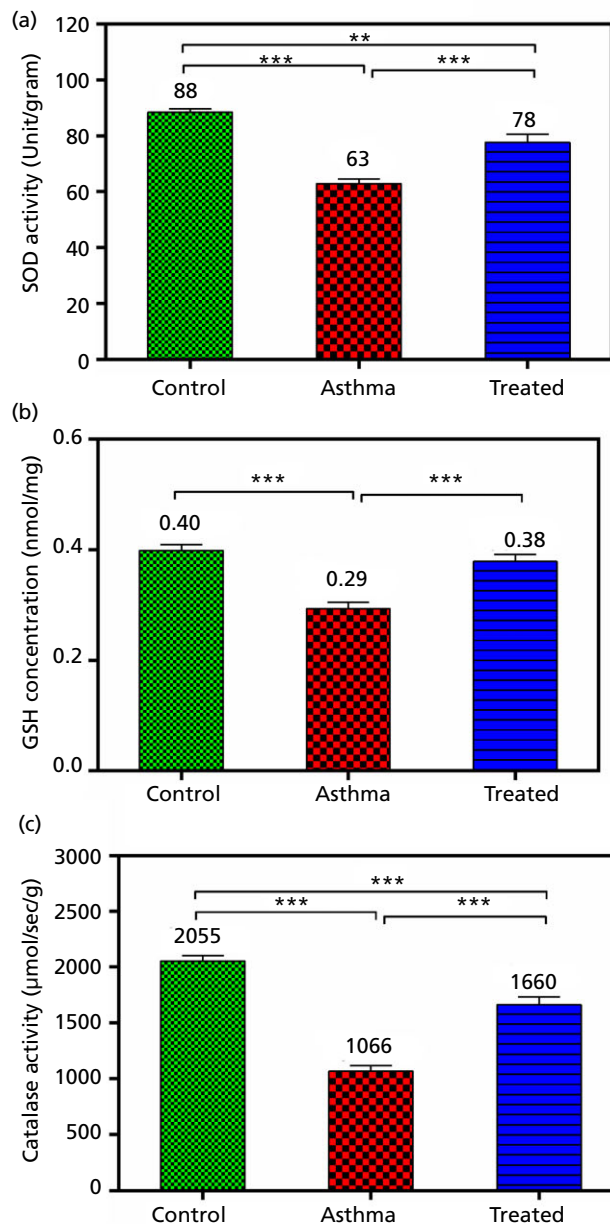


Figure 3 The activity of superoxide dismutase activity (SOD) (a), glutathione concentration (GSH) (b) and catalase activity (c) in lung tissue homogenates of ovalbumin-sensitized guinea pigs with bronchial asthma (BA) and ROSI (5 mg/kg/day for 21 days)-treated groups. The activity of SOD, GSH and catalase in BA group were significantly lower than control group and significantly increased by treatment with ROSI (** $P < 0.01$ and *** $P < 0.001$).

Histopathological examination of the lung tissue

The histopathological study was performed in a randomized, blinded manner to prevent observer bias. In

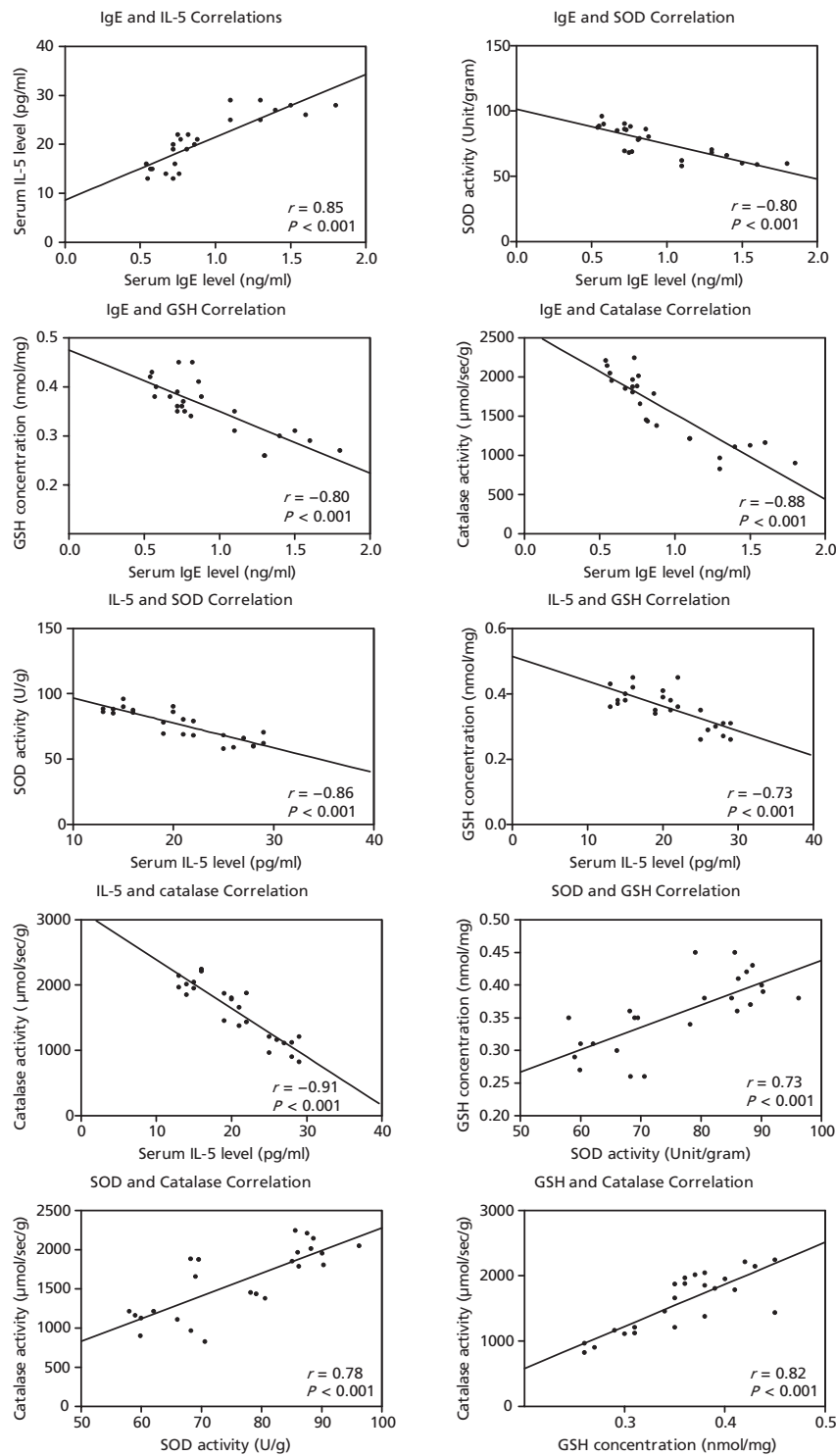


Figure 4 Scatter plots of significantly correlated parameters in the serum and in lung tissue homogenates of all guinea pigs. The solid lines represent the linear regression and correlation coefficient (r), P is the correlation significant significance level and n is the total number of samples.

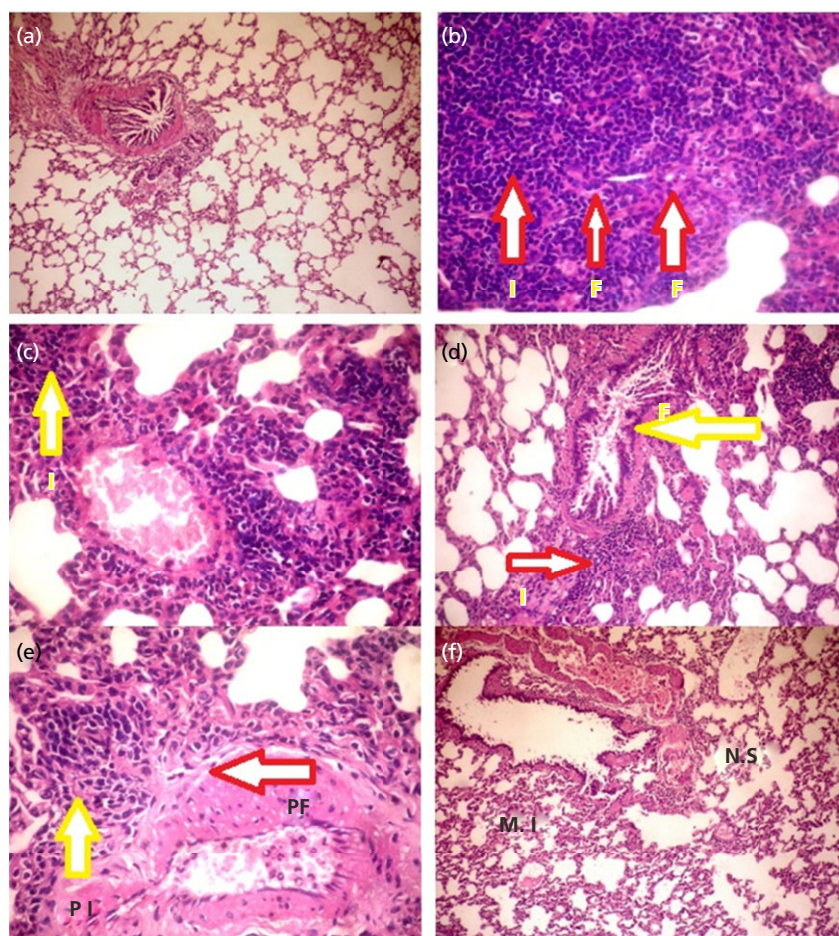


Figure 5 Histopathological examination of the inflammation of the right lung tissues stained with haematoxylin and eosin (H&E) (40 \times). Control (a) without inflammation, ovalbumin-sensitized guinea pigs with bronchial asthma (b–d), and ROSI treated groups (e–f). The extent of inflammation indicated by I: inflammatory infiltration F: overt features of fibrosis with PI: partial peri-vascular fibrosis, NS: no signs of fibrosis, and MF: features of minimal monocytic inflammatory infiltrate.

comparison to control group (Figure 5a), histological examination of the degree, extent and anatomical distribution of allergic inflammation revealed moderate and diffuse interstitial inflammation in the lungs of the untreated asthmatic guinea pig group (Figure 5b). Figure 5b shows inflammation infiltration (I) and overt features of fibrosis (F). This inflammation was also observed in the perivascular and peribronchial zones and was composed mainly of lymphocytes, neutrophils and eosinophils (Figure 5c and 5d). Although the resolution of inflammation in the rosiglitazone-treated group was only partial peri-vascular fibrosis (PI) (Figure 5e) to no signs of fibrosis (NS) and features of minimal monocytic inflammatory infiltrate (MF) observed in Fig. 5f. The overall histopathological lesion was lower in this group compared with the untreated asthmatic guinea pig group (Figure 5e and 5f). Rosiglitazone-treated animals exhibited a mild degree of

interstitial inflammation, which was only observed focally in the lung (Figure 5b).

Blood glucose and plasma lipids

Table 2 summarized the glucose levels and lipids parameters in the studied groups. There were non-significant increases in glucose, triglyceride (TG), total cholesterol, LDL and HDL in Rosiglitazone (ROSI)-treated groups as compared with control group.

Discussion

In this study, treatment of ovalbumin-sensitized guinea pigs with rosiglitazone resulted in a significant decrease in the elevated level of IL-5 compared with the untreated asthmatic animal group (Figure 2a). In accordance with our observations, several studies have reported a significant

Table 2 The glucose levels and lipid profiles of studied guinea pigs

| | CT (n = 8) | BA (n = 8) | ROSI (n = 8) |
|---------------------------|---------------|---------------|---------------|
| Glucose (mg/dl) | 143.38 ± 7.32 | 139.67 ± 8.11 | 146.13 ± 7.25 |
| TG (mg/dl) | 66.31 ± 12.42 | 67.42 ± 14.33 | 69.28 ± 11.16 |
| Total cholesterol (mg/dl) | 58.13 ± 11.13 | 56.41 ± 10.11 | 61.16 ± 10.25 |
| LDL (mg/dl) | 39.20 ± 9.12 | 39.67 ± 8.11 | 41.16 ± 11.25 |
| HDL (mg/dl) | 5.21 ± 1.12 | 4.67 ± 1.11 | 5.56 ± 1.25 |

BA, bronchial asthma group; CT, control guinea pigs; n, number of guinea pigs; ROSI, rosiglitazone treated.

decrease in the level of IL-5 in different asthma models upon activation of PPAR- γ .^[24] Furthermore, PPAR- γ agonists reduce the ability of IL-5 to induce eosinophil survival and chemotaxis.^[33]

Recently, a direct inhibitory effect of PPAR- γ ligands on the expression of IgE heavy chain germline transcripts in the human B cell line DND39 was described.^[34] Similarly, Ruhl *et al.*^[35] reported a significant inhibition of IgE production by the PPAR- γ ligand ciglitazone *in vitro* using purified peripheral blood mononuclear cells and *in vivo* using ovalbumin-sensitized mice that were treated with ciglitazone. Similarly, the increased level of IgE in ovalbumin-sensitized mice was significantly reduced by administration of rosiglitazone or pioglitazone.^[24]

Interestingly, several reports have also demonstrated that local administration of PPAR- γ agonists had similar beneficial effects on pathological conditions, including serum levels of IgE, AHR and lung eosinophilia.^[33]

Considering the results of these studies and our *in-vivo* findings in ovalbumin-sensitized guinea pigs, which show a significant decrease in the serum level of IgE after treatment with rosiglitazone (Figure 2b), we propose that PPAR- γ plays an important role in decreasing IgE production, which significantly contributes to the pathogenesis of bronchial asthma.

In the last decade, increases in oxidative stress and oxidant-antioxidant imbalance have emerged as major contributors to the pathogenesis of bronchial asthma.^[36,37] Lung epithelia are being increasingly damaged by free radicals. To treat this damage, free-radical scavengers must be present at the site of free radical production and in adequately high concentrations over sufficiently long periods of time.^[38] Consequently, localized increases in the levels of enzymatic and non-enzymatic antioxidants in pulmonary tissue could be beneficial in the treatment of bronchial asthma.

In our study, treatment of ovalbumin-sensitized guinea pigs with rosiglitazone significantly improved the deteriorated activity of both SOD and CAT enzymes (Figure 3a and 3b, respectively). In accordance with our findings, PPAR- γ ligands have been shown to reduce oxidative stress by stimulating the activity and expression of both SOD and CAT.^[39] Similar to the improvement of SOD and CAT activ-

ity, the level of GSH in lung tissue homogenates was significantly increased in rosiglitazone-treated, ovalbumin-sensitized guinea pigs (Figure 3c). The improvement in the GSH level was previously reported with various PPAR- γ agonists.^[40]

In addition to our present findings, it appears that there is a negative correlation between antioxidant (SOD, CAT and GSH) levels and the FEV₁/FVC ratio in the asthma group, suggesting that increased antioxidant activity in the pulmonary tissue leads to improved respiratory function. These data agree with other studies in which the correlation between oxidant or antioxidant levels and disease severity was investigated. In this regard, Wood *et al.*^[41] reported that SOD activity was negatively associated with asthma severity in patients with mild disease. In addition, Kanazawa *et al.*^[42] reported that enhanced production of superoxide anion by neutrophils in asthmatics was inversely correlated with FEV₁. Ovalbumin-sensitized guinea pigs treated with rosiglitazone showed significant improvement in the FEV₁/FVC ratio, which appeared to be positively correlated with the improvement of antioxidant (CAT, SOD and GSH) levels. Based on these observations, we hypothesize that rosiglitazone has a beneficial effect on bronchial asthma induced in ovalbumin-sensitized guinea pigs, in part through the improvement of antioxidant activity in pulmonary tissue.

The beneficial effects of rosiglitazone on ovalbumin-sensitized guinea pigs were confirmed by histopathological findings because lung sections from ovalbumin-sensitized guinea pigs treated with rosiglitazone showed a profound improvement of the degree, extent and distribution of inflammatory cells in the lung. PPAR- γ expression has been reported to be dramatically upregulated in several inflammatory cell types during inflammatory responses and can be induced by immune regulatory molecules.^[42,43] Overexpression of PPAR- γ potentiates the ability of diverse PPAR- γ agonists to inhibit the expression of inflammatory response genes, which is consistent with the mediating anti-inflammatory effects.^[42,43] As bronchial asthma is characterized by infiltration of pulmonary tissue with activated inflammatory cells,^[1] we propose that rosiglitazone, through activation of overexpressed PPAR- γ receptors on activated inflammatory cells, can inhibit the expression of

several inflammatory mediators, chemo-attractants and survival factors.^[44] These inhibitory effects could subsequently decrease the infiltration of inflammatory cells into the lung and improve the pathophysiological symptoms of asthma. In accordance with our hypothesis, the potential anti-inflammatory effects and clinical benefits of PPAR- γ agonists in various pathological conditions are based on their abilities to inhibit the transcriptional activation of inflammatory response genes through activation of overexpressed PPAR- γ in activated inflammatory cells.^[43,45–47]

Figure 4 gives Pearson's correlation co-efficient (r) between serum IgE concentrations and IL-5 levels with the antioxidant parameters (SOD, GSH and CAT). Serum IgE concentrations and IL-5 levels were inversely correlated to the SOD ($r = -0.80$, $r = -0.86$), GSH ($r = -0.80$, $r = -0.73$) and CAT ($r = -0.88$, $r = -0.91$) levels in the all studied groups, while the antioxidant parameters were directly correlated to each other. These correlations suggested that the deficiency in the antioxidant productions may be related to the allergic reaction in the system. The IgE concentrations were correlated to the severity of the inflammatory conditions as seen by the direct correlation with IL-5 (Figure 4). Crestani *et al.* reported that IL-5 production was associated directly with total IgE levels in the in-vivo study.^[48]

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Conclusion

In conclusion, our results together with the results of previous studies have demonstrated that administration of PPAR- γ agonists improves the asthmatic features, through downregulation of the inflammatory reaction, associated with bronchial asthma. Moreover, PPAR- γ agonists enhance the antioxidant potential of pulmonary tissue. Therefore, it is anticipated that PPAR- γ ligands, particularly TZDs, will be able to serve as effective treatments for chronic respiratory diseases such as bronchial asthma. Clinical trials and further studies are needed to evaluate the definite clinical effects of PPAR- γ ligands based on abundant experimental studies.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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