



Modulation of the IL-23/IL-17 axis by fenofibrate ameliorates the ovalbumin/lipopolysaccharide-induced airway inflammation and bronchial asthma in rats

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Abstract

The overlapping between asthmatic subtypes, including both CD4+ T helper (TH)2 and TH17 cells, is found in the natural course of allergic asthma, especially in exacerbations and severe and insensitive forms to steroids, which are in need of new molecular therapies. In the TH2-subset mediated asthma, fenofibrate displays therapeutic promises, besides evidenced therapeutic effects on TH17-mediated colitis and myocarditis. Therefore, the effects of fenofibrate versus dexamethasone on IL-23/IL-17 axis in ovalbumin (OVA)/lipopolysaccharide (LPS)-induced airway inflammation and bronchial asthma in rats were explored. The OVA/LPS sensitization and challenge were performed for 28 days in male Wistar rats. After sensitization, fenofibrate (100 mg/kg/day) or dexamethasone (2.5 mg/kg/day) was orally administered from the day 15 to 28. Either fenofibrate or dexamethasone attenuated the severity of OVA/LPS-induced airway inflammation and bronchial asthma through significant ameliorations in the total serum immunoglobulin (Ig)E assay; the total and differential leukocytic counts in the bronchoalveolar lavage (BAL) fluid; the lung inflammatory cytokines such as interleukin (IL)-4, IL-13, IL-17, and IL-23, transforming growth factor (TGF)- β_1 , and tumor necrosis factor (TNF)- α levels; and the lung IL-17 and IL-23 expressions. In addition to the reduction in the inflammatory and fibrotic histopathological scores, fenofibrate significantly ameliorated the BAL neutrophilic count and the lung IL-17 and IL-23 expressions in comparison to dexamethasone. The suppression of IL-23/IL-17 axis could be considered a molecular therapeutic target for fenofibrate in OVA/LPS-induced airway inflammation and bronchial asthma. Combined therapeutic regimens of fenofibrate and steroids should be furtherly investigated in severe and resistant asthma.

Keywords Dexamethasone · Fenofibrate · Interleukin-17 · Interleukin-23 · OVA/LPS-induced airway inflammation and bronchial asthma

Introduction

Allergic asthma is a complex chronic heterogeneous inflammatory disorder of airways (Barnes 2011; Wang and Wills-

Karp 2011; Lambrecht and Hammad 2014). It is associated with cardinal symptoms of paroxysmal airflow obstruction, airway hyperresponsiveness (AHR), chest tightness, wheezing, and coughing (Ke et al. 2010; Kim et al. 2013).

Recently, multiple asthmatic endotypes are explored, which all are dependent on the functional differentiation polarization cascades of the naïve CD4+ T helper (TH) cells into the appropriate TH cells lineages as TH2 and TH17 (Choy et al. 2015; Robinson et al. 2017). The overlapping and reciprocal immunopathogenesis between these differential subtypes, including both TH2 and TH17 cytokines, are found in the natural courses of the allergic asthmatic diseases, especially in exacerbations, severe subtypes, and insensitive steroid subtypes (Simpson et al. 2006; Hastie et al. 2010; Busse et al. 2013; Lambrecht and Hammad 2014; Robinson et al. 2017).

The TH2-subset mediated responses, also called as type 2-high airway inflammation, with the integration of multiple

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released cytokines as interleukin (IL)-4 and IL-13, are well-known pathophysiological asthmatic pathway with final eosinophilic recruitments (Choy et al. 2015; Robinson et al. 2017), while the TH17 cell activation pathway, known as type 2-low airway inflammation, is displaying important cascades with final neutrophilic recruitments (Bi and Yang 2012; Busse et al. 2013; Singh et al. 2014).

Through its essential regulatory and maintenance roles, IL-23 initiates the upregulation and differentiation of TH17 cells (Bettelli et al. 2006), which is controlled by several mediators from which transforming growth factor (TGF)- β_1 displays a chief activation and consequent remodeling effects (Chakir et al. 2003; Li et al. 2010; Bi and Yang 2012; Morishima et al. 2013), and tumor necrosis factor (TNF)- α (Ogura et al. 2008; Chung et al. 2009). The IL-17 cytokine family including IL-17A (IL-17) and IL-17E (IL-25), IL-22, and TNF- α (Wang and Wills-Karp 2011; Morishima et al. 2013; Busse et al. 2013) are produced from the activated TH17 cells. Among these cytokines, the IL-17 is the highly explored in severe and resistant asthma with evidenced elevated expressions in the sputum, lung sections, bronchoalveolar lavage (BAL) fluid, and sera (Park and Lee 2010; Wang and Wills-Karp 2011).

Nowadays, the manipulation of the TH2 or TH17 polarization is considered an innovative therapeutic modality. However, the deactivation of the TH2 subset cytokines as IL-4 and/or IL-13 showed a reduction in the eosinophilic inflammation, but with further enhancement in TH17 cell-derived cytokines with pulmonary neutrophilic inflammatory reactions. Moreover, the lung eosinophilic and neutrophilic inflammatory changes associated with mucus thickening and AHR were ameliorated after IL-13 and IL-17 neutralization. These data are giving rise to the beneficial effects of these combined therapeutic strategies among patients with both TH2 and TH17 endotypes (Morishima et al. 2013; Choy et al. 2015).

The constitutive lung expression of the peroxisome proliferator-activated receptor (PPAR)- α is evidenced, which is inversely regulated in the chronic airway inflammation (Becker et al. 2008).

Fenofibrate is a synthetic PPAR- α agonist. Fenofibrate regulates expression of many enzymes involved in lipid metabolism, ending in a reduction in the triglyceride levels and an increase in the high-density lipoprotein levels. Clinically, it decreases cholesterol levels in the nonalcoholic fatty liver diseases and enhances the insulin sensitivity in type 2 diabetic patients. Moreover, its fibrinolytic, antithrombotic, and anti-inflammatory properties have been evidenced in the cardiac and diabetic patients (McKeage and Keating 2011; Krysiak et al. 2013; Linz et al. 2014; Tarantino et al. 2017). It is also used as a supplementary therapy in patients with hyperuricemia (Khanna et al. 2012).

Moreover, fenofibrate reduced chronic lung inflammation and allergic asthma through the reduction in airway infiltration of neutrophils and macrophage, chemoattractants, and inflammatory cytokines such as IL-4, TNF- α , TGF- β_1 , and matrix metalloproteinases (Delayre-Orthez et al. 2005; Denning and Stoll 2006; Becker et al. 2006, 2008; Samah et al. 2012; Estrela et al. 2017). Interestingly, in colitis and myocarditis as autoimmune diseases with inflammatory backgrounds, fenofibrate could profoundly suppress the expression of IL-23 and IL-17 (Lee et al. 2007; Cheng et al. 2016).

In allergic asthmatics with severe phenotypes, the effect of fenofibrate on IL-23/IL-17 immunopathogenic axis and how this axis manipulates the other TH2 and TH17 regulatory cytokines is considering a new inspire. So, this present study was conducted to explore the effects of fenofibrate versus corticosteroids, as a gold standard in the treatment of allergic severe asthma, on IL-23/IL-17 axis in ovalbumin (OVA)/lipopolysaccharide (LPS)-induced airway inflammation and bronchial asthma in rats.

Methods

Experimental animals

Fifty male albino Wistar rats (200–230 g) were obtained from Egyptian Organization for Biological Products and Vaccines. The acclimatization for 1 week, the free access ad libitum to chow diet and water, and the maintenance of rats on normal light-dark schedule and temperature 25 ± 3 °C during the course of the experiment were done. The experimental protocol was approved by the Institutional Animal Care and Use Committee at Suez Canal University, which is following the National Institutes of Health guide for the care and use of laboratory animals (MD, USA).

Drugs and chemicals

Fenofibrate was obtained as a white powder from Minapharm Company, Egypt. Dexamethasone was obtained as an off-white powder from Arab Drug Company (ADCO), Egypt. The OVA was purchased as a bottle containing 1 g of a lyophilized powder, $\geq 90\%$ albumin from hen egg white from Sigma-Aldrich Company, St. Louis, USA. Aluminum hydroxide (Al(OH) $_3$) was attained as a bottle containing 1 g of powder from Sigma-Aldrich Company, St. Louis, USA. The LPS (*Escherichia coli* serotype 0111:B4) was purchased as a white-yellow lyophilized powder from Sigma-Aldrich Company, St. Louis, USA. All other chemicals and solvents were of analytical grades and were obtained from Sigma-Aldrich Company, St. Louis, USA.

Induction of experimental model of airway inflammation and bronchial asthma in rats

An experimental model of airway inflammation and bronchial asthma was induced by OVA/LPS administrations, which mimicked the severe exacerbations of bronchial asthma in human (Kumari et al. 2015). Freshly prepared OVA solution was used before each administration as the previous method prescribed by Dong et al. (2014) and Kumari et al. (2015) with modifications in frequency of challenging. The intraperitoneal sensitization was done on days 0, 7, and 14 with OVA (1 mg) adsorbed on $\text{Al}(\text{OH})_3$ gelatinous (20 mg) in a final volume of 2.5 ml/kg. After that, challenge was done by intratracheal instillation of 1.1% OVA dissolved in normal saline (200 μL) on days 21 (Dong et al. 2014), 23, 25, and 27. Simultaneously, LPS (1.5 mg/kg) was dissolved in normal saline and injected intraperitoneally on days 7, 21, 23, 25, and 27, 1 h before each OVA administration (Haddad et al. 2001; Kumari et al. 2015) (Fig. 1).

Experimental protocol

Rats were randomly allocated into five groups of ten animals each:

Normal control group included normal rats without airway inflammation and bronchial asthma, nor any drug administration.

Vehicle control group included normal rats without airway inflammation and bronchial asthma, where the rats were sensitized and challenged with the same regimens of airway inflammation and bronchial asthma induction using $\text{Al}(\text{OH})_3$ and normal saline,

respectively. From the day 15 to 28, rats were receiving 1% carboxymethyl cellulose (CMC; 5 ml/kg) by oral gavage.

OVA/LPS control group included rats with airway inflammation and bronchial asthma. From the day 15 to 28, rats were receiving 1% CMC (5 ml/kg) through oral gavage (Fig. 1).

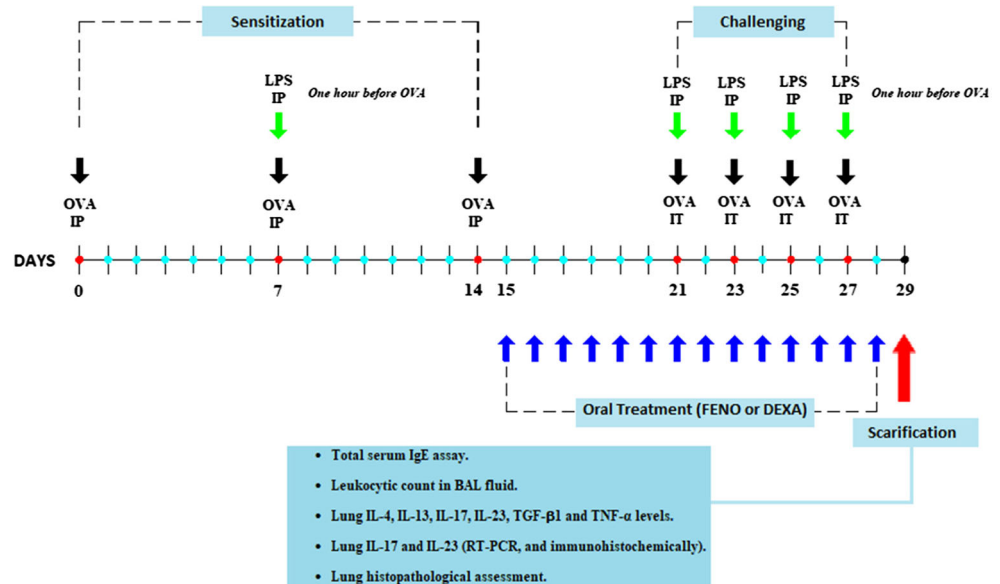
OVA/LPS and fenofibrate-treated group is where rats with airway inflammation and bronchial asthma received fenofibrate by oral gavage at a dose of 100 mg/kg/day dissolved in 1% CMC (5 ml/kg) for 14 days, starting from day 15 (Samah et al. 2012) (Fig. 1).

OVA/LPS and dexamethasone-treated group included rats with airway inflammation and bronchial asthma which received dexamethasone orally at a dose of 2.5 mg/kg/day for 14 days, starting from day 15 (Arora et al. 2017) (Fig. 1).

Blood sampling and total serum immunoglobulin E assay

On the day 29, the day after last administration of treatment/vehicle, blood specimens from the anesthetized rats were sampled through retrobulbar venous plexuses. Sera were obtained by blood centrifugation ($\times 323\text{ g}$, for 20 min at 4°C). Storage at -80°C was done for subsequent total serum immunoglobulin (Ig)E assays. Rat IgE enzyme-linked immunosorbent assay (ELISA) kit (ab157736; Abcam®, UK) was used to measure the total serum IgE levels using an automated ELISA reader (Metertech, M960) following the manufacturer's instructions.

Fig. 1 The experimental protocol of OVA/LPS-induced airway inflammation and bronchial asthma. The treatment modalities and methodological procedures were schemed. DEXA, dexamethasone; FENO, fenofibrate; IgE, immunoglobulin E; IL, interleukin; LPS, lipopolysaccharide; OVA, ovalbumin; RT-PCR, reverse transcription polymerase chain reaction; $\text{TGF-}\beta_1$, transforming growth factor- β_1 ; $\text{TNF-}\alpha$, tumor necrosis factor- α



Determination of total and differential leukocytic count in BAL fluid

After scarification, the trachea of each rat was cannulated and the BAL was done three times with phosphate buffer saline (PBS, 5 ml/time). The BAL fluid was centrifuged at $\times 81g$ for 10 min at 4 °C. After centrifugation, the cell pellets were re-suspended in PBS (1 ml/each cell pellet), then re-centrifuged again on $\times 81g$ for 10 min at 4 °C and stained with Wright-Giemsa solution. In addition, 10 μ L of the solution was used for counting of the total and differential leukocytes with a hemocytometer (Guo et al. 2013).

Lung sample collection and processing

After collection of BAL fluids, extracted lungs were washed with ice-cold PBS. The right lungs were processed for the histopathological and immunohistochemical examinations, and the left lungs were divided into two parts, frozen dried in liquid nitrogen, and kept in -80 °C till further assays of cytokine levels and RNA extractions (Delayre-Orthez et al. 2008).

Preparation of lung tissue homogenates

One part of each left lung was thawed, weighed, and homogenized in 4 ml of cold PBS, and the supernatants were used for further measurement of lung cytokine contents by enzyme-linked immunosorbent assay (ELISA) techniques.

Determination of lung cytokine levels

Rat IL-4 ELISA kits (Quantikine® ELISA, R&D systems, Minneapolis, MN, USA), rat IL-13 and IL-17A ELISA kits (Abcam®, UK), rat IL-23 and TNF- α ELISA kits (Ray Biotech Inc.1, Norcross, USA), and rat TGF- β_1 ELISA kits (BioVendor Laboratory Medicine, USA) were obtained. To measure the lung homogenate concentrations of the mentioned cytokines, an automated ELISA reader (Metertech, M960) was used according to the manufacturers' instructions.

Quantitative reverse transcription polymerase chain reaction (RT-PCR) for detection of IL-17 and IL-23

Total RNA was extracted from frozen lung tissue samples (100 mg) using an RNA extraction kit (Qiagen, Hilden, Germany) and treated with DNase to remove any residual genomic DNA. The concentration of the RNA extracted was measured using a NanoDrop ND-1000 (Thermo Fisher Scientific, Wilmington, DE, USA). Single-stranded cDNA was synthesized from 5 to 10 mg total RNA using Superscript II Reverse Transcriptase (Gibco BRL; Life Technologies, Bethesda, MD, USA). To determine IL-17

and IL-23 subunit alpha (IL-23A) genes in lung tissue, the following specific primers were used: rat IL-17 cDNA fragment, 5'-TCTCCAGAACGTGAAGGTC-3' (primer 1) and 5'-AAGTGGAACGGTTGAGGTAG-3' (primer 2); rat IL-23 cDNA fragment, 5'-ATCCCCAAGGTCTCAAGG-3' (primer 1) and 3'-CCAGTAGGGAGGTATGAAGC-5' (primer 2); and b-actin, 5'-TCCTGTGGCATCCATGAAACTA-3' (sense) and 5'-CCAGGGCAGTAATCTCCTTCTG-3' (antisense) (Silva et al. 2012). All primers were used at a final concentration of 0.5 mmol/L. Quantification of transcripts was performed with the Light Cycler 480 Real-Time PCR System (Roche Diagnostics, Mannheim, Germany), and target sequences were detected using the Universal Probe Library (UPL; Roche Diagnostics GmbH, Roche Applied Science, Mannheim, Germany). Data were analyzed with Light Cycler 480 Software.

Histopathological assessment of lung tissues

The right lungs were fixed in 10% neutral buffered formalin. Each paraffin-embedded formalin-fixed tissue block was sectioned into 4 μ m sections and stained with hematoxylin and eosin (H&E) for assessment of interstitial and peribronchial inflammation and airway smooth muscle thickening and Masson's trichrome for evaluation of the subepithelial fibrosis (basal membrane thickness) using light microscope. The inflammatory changes were graded by the following score: 0, indicates normal appearance; 1, indicates few subepithelial and interstitial inflammatory cellular infiltrates with mild airway smooth muscle thickening; 2, indicates a subepithelial ring consisting of a layer of inflammatory cells, with moderate interstitial inflammatory cellular infiltrates and airway smooth muscle thickening; and 3, indicates subepithelial thick ring of inflammatory cells (> four cell layers), with severe interstitial inflammatory cellular infiltrates and airway smooth muscle thickening. The fibrotic changes were graded by the following score: 0, indicates no fibrotic changes; 1, 2, and 3 indicate mild, moderate, and severe fibrotic changes, respectively (Shoseyov 2005; Dong et al. 2014; Muti et al. 2014; Wang et al. 2014).

Immunohistochemical staining of lung IL-23 and IL-17 expressions

The immunohistochemical staining was on 4 μ m sections by using the streptavidin-biotin immunoperoxidase technique, using 3,3'-diaminobenzidine (DAB) as chromogen and hematoxylin as a counterstain. The immunoreactivity was evaluated by measuring integrated optical density (Wang et al. 2014), using an image analysis system "Image J 1.45F" (National Institute of Health, USA).

Statistical analysis

Results were collected and expressed as the mean $\pm$ standard error of the mean (SEM). Results were analyzed using The Statistical Package for the Social Sciences, version 20 (SPSS Software, SPSS Inc., Chicago, USA). One-way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis was used to test the significance of the difference between quantitative variables. Other qualitative values were assessed by using chi-square test. The p value < 0.05 was considered to be statistically significant.

Results

Effects of fenofibrate on total serum IgE levels of OVA/LPS-sensitized and OVA/LPS-challenged rats

As represented in Fig. 2, a nonsignificant difference ($p > 0.05$) was observed in total serum IgE levels between vehicle control and normal control groups. The OVA/LPS control animals showed a significant ($p < 0.05$) increase in total serum levels of IgE compared to the normal controls. Treatment with both fenofibrate and dexamethasone significantly decreased the total serum levels of IgE ($p < 0.05$) compared to OVA/LPS control group. This decrease was also significantly different from the normal control group (Fig. 2).

Effects of fenofibrate on total and differential leukocyte counts in BAL fluid of OVA/LPS-sensitized and OVA/LPS-challenged rats

Intraperitoneal and intratracheal administration of vehicles to normal control rats resulted in insignificant ($p > 0.05$) change in the total and differential cell count in BAL fluid compared to the normal control group. The OVA/LPS control group showed a significant ($p < 0.05$) increase in the BAL fluid's

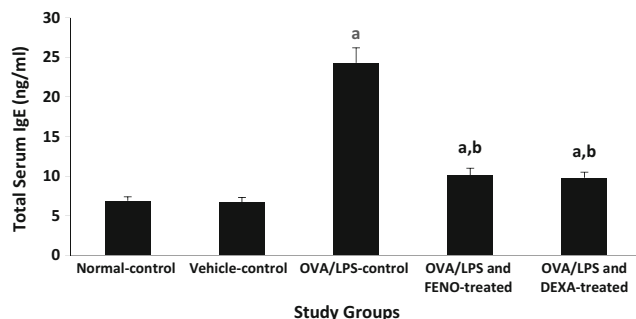


Fig. 2 Total serum IgE levels in different study groups. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared with the OVA/LPS control group. DEXA, dexamethasone; FENO, fenofibrate; IgE, immunoglobulin-E; LPS, lipopolysaccharide; OVA, ovalbumin

total and differential leukocyte count compared to the normal control group. Treatment with either fenofibrate or dexamethasone led to significant ($p < 0.05$) reversals of OVA/LPS-induced increases ($p < 0.05$). Of notice, both drugs almost normalized lymphocytes and macrophages count, while treatment with fenofibrate also caused a significant ($p < 0.05$) decrease in the neutrophilic count in comparison with the dexamethasone-treated group (Fig. 3).

Effects of fenofibrate on OVA/LPS-induced changes in lung cytokine levels

Nonsignificant differences ($p > 0.05$) were observed between normal and vehicle control groups regarding lungs' cytokine levels (Fig. 4). In the OVA/LPS control rats, significant increases in lung levels of IL-4, IL-13, IL-17, IL-23, TGF- β_1 , and TNF- α were revealed compared to the normal control group ($p < 0.05$, Fig. 4). Oral administration of either fenofibrate or dexamethasone to OVA/LPS control rats induced significant ameliorations in the above measures towards normal ($p < 0.05$). Obviously, the fenofibrate-treated group led to significant decreases in lung levels of both IL-17 and IL-23 when compared to dexamethasone-treated group ($p < 0.05$, Fig. 4).

Effect of fenofibrate on lung IL-17 and IL-23 gene expressions in OVA/LPS-challenged rats

The mRNA levels of IL-17 and IL-23 were determined using RT-PCR analysis (Fig. 5). Vehicle control group showed the insignificant difference in IL-17 and IL-23 mRNA levels as compared to normal control group ($p > 0.05$). Compared with the normal control group, the IL-17 and IL-23 mRNA levels were significantly higher in the OVA/LPS control group ($p < 0.05$). Treatment with either fenofibrate or dexamethasone significantly reduced these higher levels, with more significant reductions in the fenofibrate-treated group in comparison to the dexamethasone-treated group ($p < 0.05$).

Effect of fenofibrate on OVA/LPS-induced histopathological lung changes

In normal control and vehicle groups, lung tissue showed the preserved architecture; alveoli had thin walls that were lined by single layer of cells, and bronchioles were lined by pseudostratified columnar epithelium with a thin wall, thin muscle layer, surrounded by loose connective interstitial tissue with thin-walled vessels. On the other hand, lung tissue in OVA/LPS control group showed a significant distortion of architecture, narrowing of alveoli and bronchioles, thickening of the bronchial and alveolar walls and the muscle layer, with marked inflammatory cell recruitments mainly around the bronchial walls, infiltrating the bronchial epithelia with

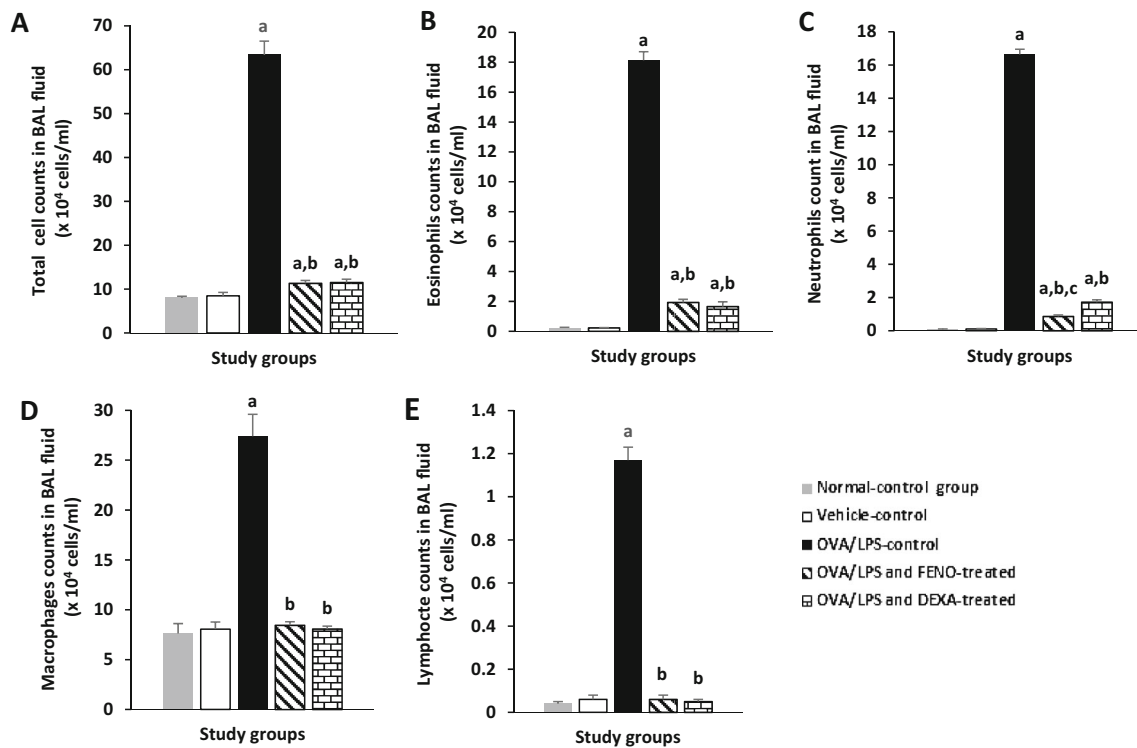


Fig. 3 Effects of fenofibrate and dexamethasone on the **A** total, **B** eosinophils, **C** neutrophils, **D** macrophages, and **E** lymphocytes counts in BAL fluid ($\times 10^4$ cells/ml). Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group.

^b $p < 0.05$ compared with the OVA/LPS control group. ^c $p < 0.05$ compared with the OVA/LPS and DEXA-treated group. BAL, bronchoalveolar lavage; DEXA, dexamethasone; FENO, fenofibrate; LPS, lipopolysaccharide; OVA, ovalbumin

shedding foci, and in the alveolar walls, with congested blood vessels ($p < 0.05$, Figs. 6 and 8), with moderate to severe bronchiolar subepithelial fibrosis, and alveolar septal collagen deposition, ($p < 0.05$, Figs. 7 and 8). Both fenofibrate and dexamethasone significantly ameliorated the encountered OVA/LPS-induced histopathological and fibrotic changes ($p < 0.05$). The marked improvements were recorded after fenofibrate treatment ($p < 0.05$) in comparison to the dexamethasone-treated group (Figs. 6, 7, and 8).

Effect of fenofibrate on OVA/LPS-induced alterations on the lung IL-23 and IL-17 immunohistochemical expressions

Lung IL-17 and IL-23 expressions were significantly increased in OVA/LPS-sensitized and OVA/LPS-challenged rats. IL-17 was highly expressed mainly in the alveolar septa, alveolar macrophages, interstitial inflammatory cells as lymphocytes and neutrophils, and weakly to bronchiolar epithelium cells

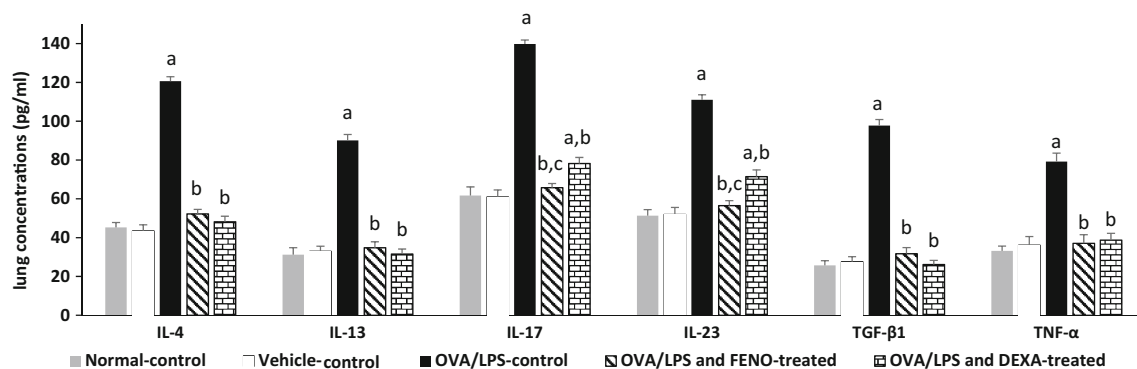


Fig. 4 Lung IL-4, IL-13, IL-17, IL-23, TGF- β_1 , and TNF- α levels in different study groups. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared with the OVA/LPS control group. ^c $p < 0.05$ compared with

the OVA/LPS and DEXA-treated group. DEXA, dexamethasone; FENO, fenofibrate; IL, interleukin; LPS, lipopolysaccharide; OVA, ovalbumin; TGF- β_1 , transforming growth factor- β_1 ; TNF- α , tumor necrosis factor- α

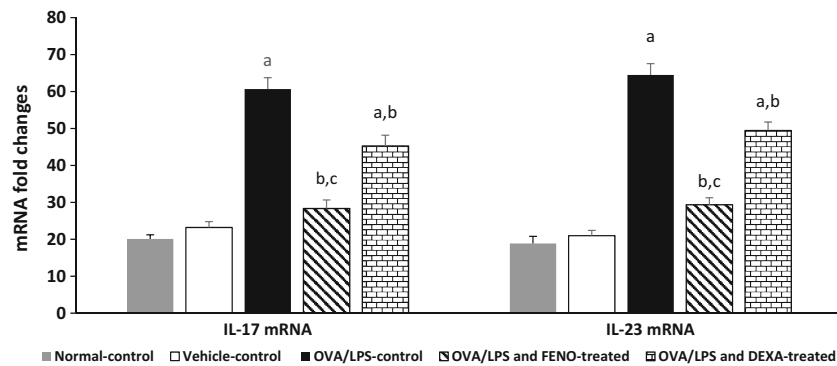


Fig. 5 mRNA levels of IL-17 and IL-23 in lung tissues in different study groups. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared

with the OVA/LPS control group. ^c $p < 0.05$ compared with the OVA/LPS and DEXA-treated group. DEXA, dexamethasone; FENO, fenofibrate; IL, interleukin; LPS, lipopolysaccharide; OVA, ovalbumin

(Fig. 9), while the IL-23 was confined to the alveolar macrophages, lymphocytes, neutrophils, alveolar septa, and bronchial epithelial cells (Fig. 10). Both fenofibrate and

dexamethasone treatments significantly decreased the lung expression of IL-17 and IL-23 in OVA/LPS-challenged rats, with the higher ameliorating effects to fenofibrate (Figs. 9 and 10).

Fig. 6 Histopathological lung changes in different experimental groups; (H&E $\times 100$, $\times 400$). Normal and vehicle control groups showed normal lung architecture. The OVA/LPS control group showed marked distortion in the lung architecture, with alveolar and bronchiolar narrowing and wall thickening showing marked inflammatory infiltrates with shedding foci, thickened muscular layer, and vascular congestion. Fenofibrate and dexamethasone ameliorated these changes, with marked improvements after fenofibrate treatment. Bronchial wall, red arrows; alveoli, black arrows; inflammatory infiltrates, green arrows; blood vessels, blue arrows. DEXA, dexamethasone, FENO, fenofibrate; LPS, lipopolysaccharide; OVA, ovalbumin

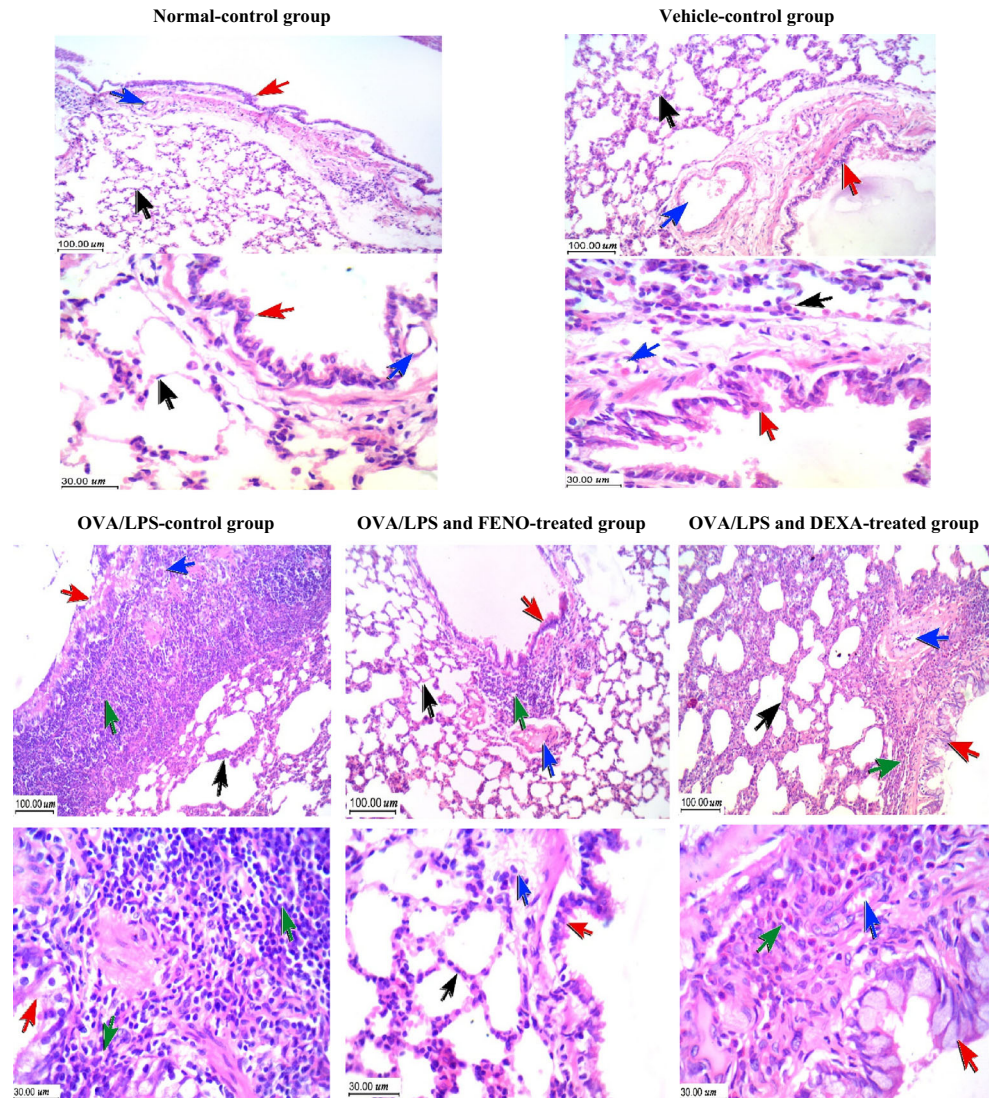
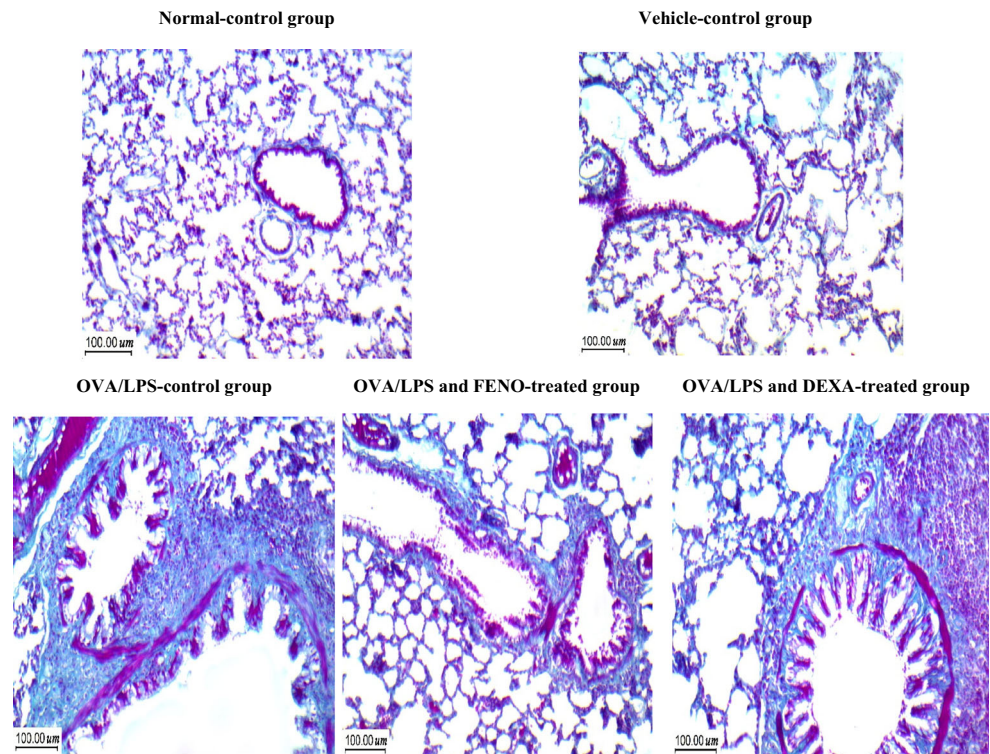


Fig. 7 Distribution of collagen in lung sections stained with Masson's trichrome ($\times 100$), which obtained from different experimental groups. Normal distribution patterns were observed in normal and vehicle control groups. In the OVA/LPS control group, marked subepithelial and alveolar septal collagen depositions were observed. Treatment with either fenofibrate or dexamethasone decreased these changes with noticed normal distribution patterns after fenofibrate regimens. DEXA, dexamethasone; FENO, fenofibrate; LPS, lipopolysaccharide; OVA, ovalbumin



Discussion

Previous evidence concluded that upon challenge with OVA/LPS, sensitized rats developed aggravated airway inflammatory responses with the elaboration of various cytokines and chemokines and infiltration of numerous inflammatory cells including eosinophils, neutrophils, macrophages, and lymphocytes. This exaggerated inflammatory pattern mimics the inflammatory response encountered in patients with severe asthma, which are displaying limited responses to typical therapeutic regimens (Delayre-Orthez et al. 2008; Morishima et al. 2013; Kumari et al. 2015).

In the present study, OVA challenging was scheduled into days 21, 23, 25, and 27 of the experiment and scarification was done on day 29. Earlier studies revealed that in actively sensitized animals, repeated challenges were associated with the persistence of the airway inflammatory cascades and infiltrates. Moreover, the lung parenchymal accumulation of eosinophils is peaked after 48 h, while in the BAL fluid is peaked after 72 h (Elwood et al. 1991; Bolton et al. 1997; Szelenyi 2000; Kurucz and Szelenyi 2006; Jung et al. 2007).

In the current experiment, LPS was given to enhance the OVA sensitization and challenging process. Evidently, during sensitization phase, LPS administration upregulates markedly

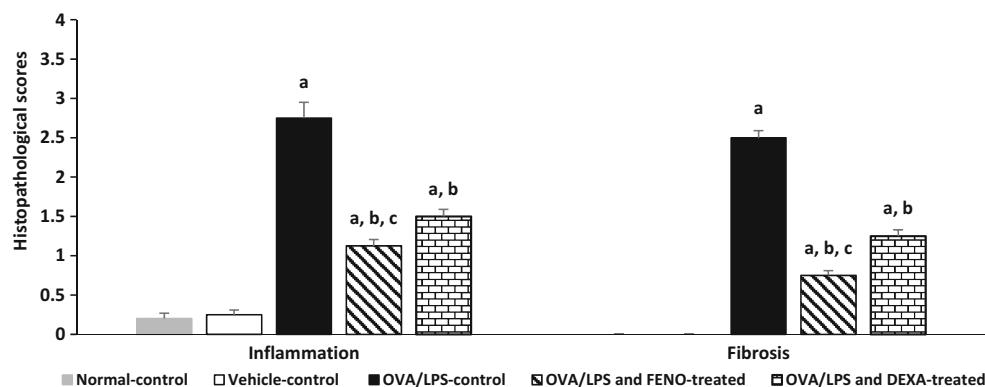


Fig. 8 Lung histopathological scores including the inflammatory and fibrotic changes in different study groups. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared with the OVA/LPS control group.

^c $p < 0.05$ compared with the OVA/LPS and DEXA-treated group. DEXA, dexamethasone; FENO, fenofibrate; LPS, lipopolysaccharide; OVA, ovalbumin

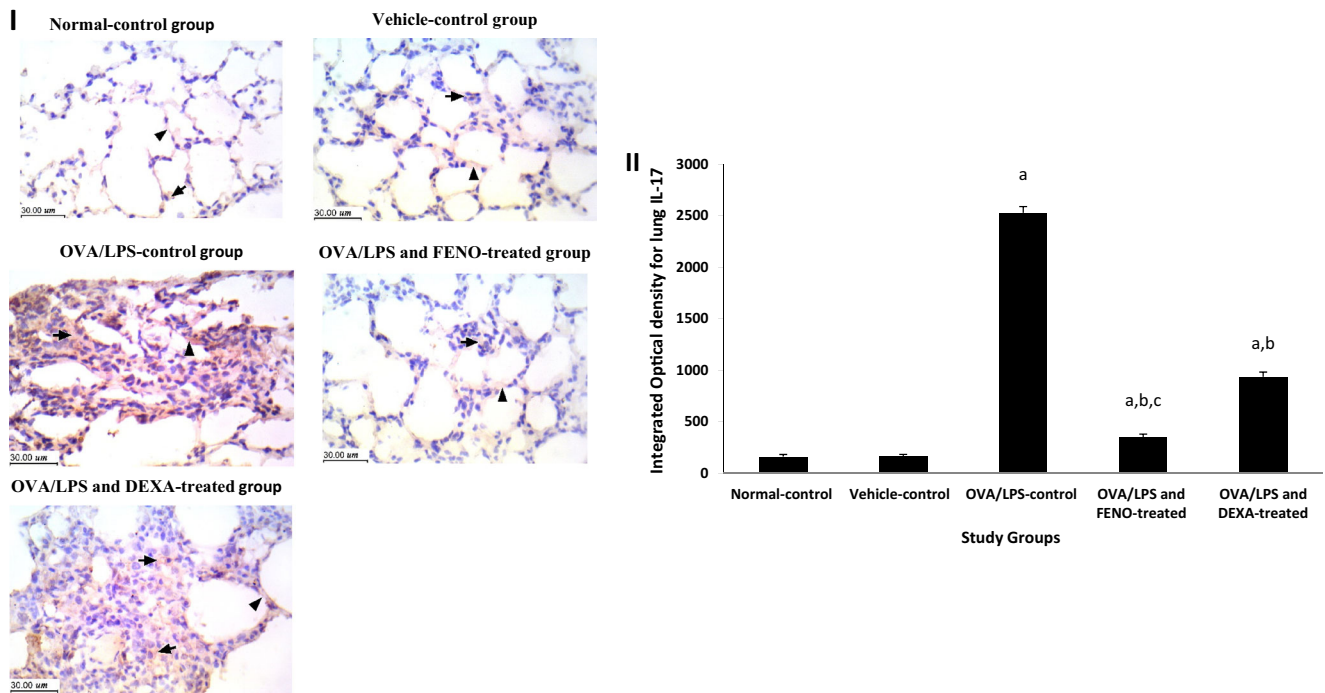


Fig. 9 (I) The immunohistochemical staining of lung IL-17 in different experimental groups at a magnification of $\times 400$. In the OVA/LPS control group, the lung IL-17 immunoreactivity was considerably increased, with high expression mainly in the alveolar septa, alveolar macrophages, interstitial inflammatory cells such as lymphocytes and neutrophils, and faintly to bronchiolar epithelium cells. Fenofibrate or dexamethasone treatment decreased these higher expressions with marked reduction with fenofibrate. (II) The integrated optical density for lung IL-17 positive tissues in different study groups. OVA/LPS induced significant

higher integrated optical density compared to the normal control group, while treatments improved it with the marked improvement with fenofibrate. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared with the OVA/LPS control group. ^c $p < 0.05$ compared with the OVA/LPS and DEXA-treated group. DEXA, dexamethasone; FENO, fenofibrate; IL, interleukin; LPS, lipopolysaccharide; OVA, ovalbumin. Black arrows were indicating the areas of positivity in alveolar macrophages or inflammatory cells. Black arrows heads were indicating alveolar septa staining

the production of the TH2 cytokines including IL-4 and IL-5 and the allergen-specific IgE (Steinke and Borish 2001; Sharma et al. 2006; Huang and Boushey 2013). Moreover, LPS exposure extensively upregulates the production of the TH17 cytokines including IL-17, IL-23, TNF- α , IL-1 β , and several angiogenic factors as nitric oxide in lung tissues and activates the alveolar macrophage Toll-like receptors (TLR)-4 signaling pathway. These events are ending in a marked increase in the lung vascular permeability, prolongation of the early asthmatic responses and histamine-induced bronchoconstriction, and the manifested eosinophilic and neutrophilic infiltrations (Dong et al. 2009; Rehnberg et al. 2015; Lowe et al. 2017).

There has been increasing evidence for important pathophysiological roles of TH17 cells in severe and steroid-resistant forms allergic asthma. The systemic blockade/deficiency in IL-17 inhibited the allergen-induced accumulation of neutrophils in the airways and the AHR (Wang and Wills-Karp 2011; Mizutani et al. 2012). In addition, the eosinophilic and neutrophilic recruitment in severe asthma could be explained by the discovery of the IL-17-producing TH2 memory cells. The TH2- and TH17-released cytokines could be secreted in a dual pattern from the IL-17-producing TH2

memory cells, with intense synergistic promotions on the mixed inflammatory leukocyte enrolments (Wang and Wills-Karp 2011). Moreover, the IL-23 is crucial for the maintenance and function of TH17 cells (Mizutani et al. 2012).

Evidently, through its anti-inflammatory effects (Becker et al. 2012), fenofibrate effectively ameliorates the airway allergic inflammatory reactions (Delayre-Orthez et al. 2008). Moreover, in spontaneous colitis (Lee et al. 2007) and autoimmune myocarditis, fenofibrate possessed anti-IL-17 and anti-IL-23 at the gene expressional levels (Cheng et al. 2016).

In the present work, the OVA/LPS-induced rat airway inflammation and bronchial asthma were therapeutically challenged with fenofibrate versus dexamethasone, highlighting the effects on IL-23/IL-17 axis. The rats sensitized and challenged with OVA/LPS showed significantly higher total serum IgE levels, BAL inflammatory cells infiltrate, lung concentrations of IL-4, IL-13, IL-17, IL-23, TGF- β_1 , and TNF- α , and the lung IL-17 and IL-23 expressions, besides the lung histopathological inflammatory and fibrotic changes.

Elevated allergen-specific IgE level in serum is a hallmark of allergic asthma. The IgE plays a primary role in triggering the early asthmatic response to the inhaled antigens with

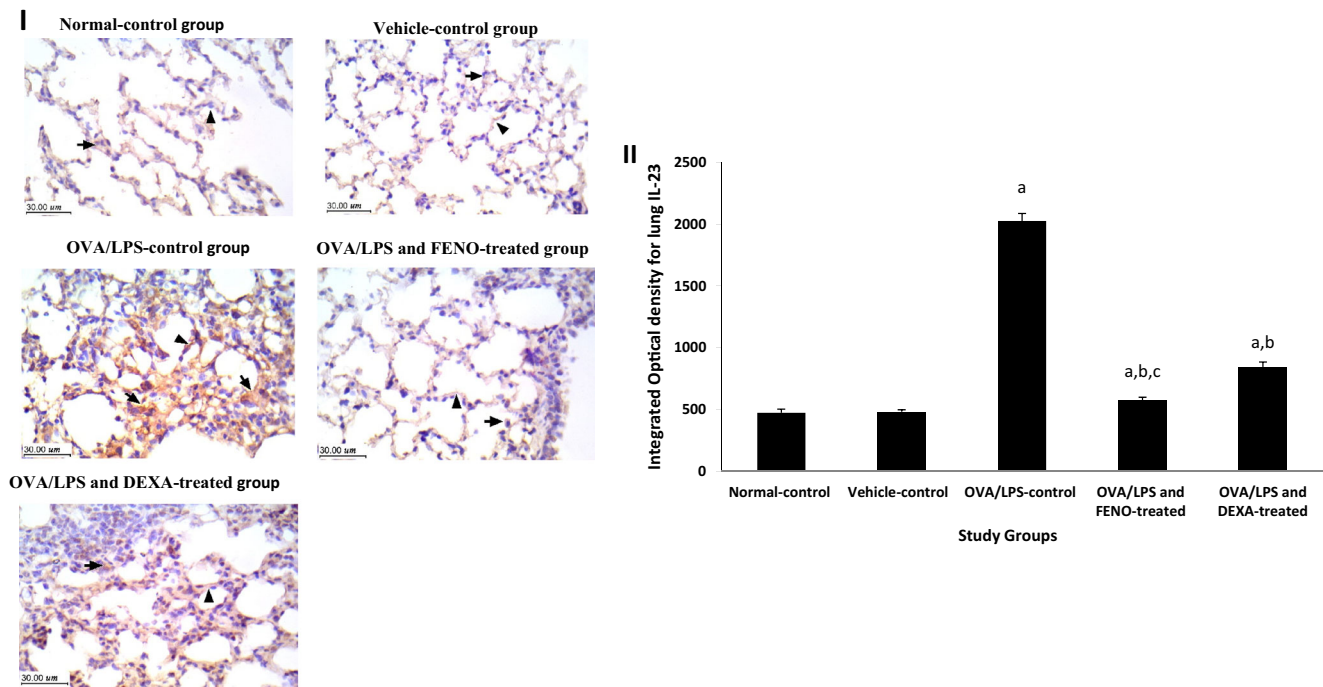


Fig. 10 (I) The immunohistochemical expression of lung IL-23 in different experimental groups ($\times 400$). The sensitization and challenge with OVA/LPS exhibited a marked increase in lung tissue expression of IL-23 mainly in the alveolar macrophages, interstitial inflammatory cells such as lymphocytes and neutrophils, alveolar septa, and bronchial epithelial cells, which ameliorated by therapeutic regimens of fenofibrate or dexamethasone. Fenofibrate treatment displayed the marked amelioration. (II) The integrated optical density for lung IL-23 positive tissues in different study groups. The significant higher integrated optical density

induced by OVA/LPS was decreased after administration of fenofibrate and dexamethasone, with marked amelioration with fenofibrate. Results were expressed as mean $\pm$ SEM. $n = 10$ rats/group. ^a $p < 0.05$ compared to the normal control group. ^b $p < 0.05$ compared with the OVA/LPS control group. ^c $p < 0.05$ compared with the OVA/LPS and DEXA-treated group. DEXA, dexamethasone; FENO, fenofibrate; IL, interleukin; LPS, lipopolysaccharide; OVA, ovalbumin. Black arrows were indicating the areas of positivity in alveolar macrophages or inflammatory cells. Black arrow heads were indicating alveolar septa staining

degranulation of mast cell and basophils, the liberation of numerous chemical mediators, and severe airway narrowing (Mizutani et al. 2012). Evidently, when the lung resident TH2 cells are exposed to a specific allergen, they initiate the TH2 cytokines release with subsequent immunopathological cascades of asthma. One of the TH2 cytokines is the IL-4, which is critical for the TH2 cells and allergen-specific B lymphocyte generation with further isotype class switching to IgE, whereas the IL-13 mediates the induction of hyperplastic goblet cells, mucin production, and AHR (Wang and Wills-Karp 2011), with later eosinophilic, lymphocytic, and macrophages/monocytes infiltrates (Choy et al. 2015; Robinson et al. 2017). Chronically, the TH2-type cytokine production, the development of airway inflammation, and AHR are induced by IgE (Wang and Wills-Karp 2011; Mizutani et al. 2012).

Of notice, Mizutani et al. investigated the relationship between IgE-mediated asthmatic responses and IL-17 secretion. They found that IgE mediated the increase in the lung TH17 cells through complement C3a production, which through its receptor signaling promoted the IL-23 production by resident dendritic cells, monocytes, and macrophages. These cascades were ending in more IL-17 production, with neutrophilic chemotaxis, and final severe asthmatic phenotypes (Mizutani

et al. 2012). These findings support our results regarding the OVA/LPS-induced significant higher total serum IgE levels, the BAL total and differential leucocytes count, the lung concentrations of IL-4, IL-13, TGF- β_1 , and TNF- α , and the mRNA expressions of IL-17 and IL-23.

Moreover, in severe asthma, TGF- β_1 produced mainly by eosinophils causes airway remodeling, with various structural alterations as subepithelial fibrotic changes with increased airway smooth muscle masses (Halwani et al. 2011). In addition to TGF- β_1 , TNF- α enhances the differentiation of the TH-17 cells with further production of IL-17, IL-25, and autocrine TNF- α (Morishima et al. 2013). In accordance, the present finding showed higher lung TGF- β_1 and TNF- α concentrations with various histopathological inflammatory and fibrotic changes after OVA/LPS sensitization and challenge.

In the present study, after each therapeutic regimen of fenofibrate and dexamethasone, the total serum IgE concentrations were significantly decreased, with an amelioration of the BAL inflammatory infiltrates, the lung cytokines, and pro-inflammatory and fibrotic mediators. Previous evidence showed that the reduction in serum and BAL IgE concentrations is leading to the avoidance of allergen-linked mediator release and is disabling the IgE-facilitated allergen presentation

in dendritic cells and TH2 cells, with final reduction in the release of IL-4, IL-13, TGF- β_1 , and TNF- α and the activation of the IL-23/TH17 cells/IL-17 axis (Delayre-Orthez et al. 2008; Belvisi and Mitchell 2009; Mizutani et al. 2012; Palomares et al. 2017).

Presently, fenofibrate displayed more reductive effects on the BAL neutrophilic count, lung IL-17, and IL-23 levels than dexamethasone. These observations could be explained as fenofibrate significantly decreased IL-17 which is an important regulator for the neutrophilic accumulation via enhancing the lung IL-8 production, which is considering an effective chemoattractant for neutrophils (Park and Lee 2010). Moreover, the neutrophilic inflammation tends to respond poorly to steroid therapy, as steroids increase neutrophil release from the bone marrow and prevent neutrophil apoptosis (Morishima et al. 2013).

Our results of the anti-inflammatory effect of fenofibrate were in agreement with these previously mentioned mechanisms, which explain how fenofibrate reduced the inflammatory and fibrotic histopathological changes. Further, Belvisi and Mitchell reported that PPAR- α mediated the anti-inflammatory activity of fenofibrate. Fenofibrate suppressed the OVA-induced inflammatory cell infiltrates in PPAR- α +/- mice, while it failed to modify the cell influx triggered by allergen in PPAR- α -/- animals (Belvisi and Mitchell 2009). Also, Becker et al. investigated the time course of changes in PPAR- α mRNA levels in animals exposed to LPS, demonstrated that PPAR- α downregulation preceded the inflammatory cell infiltration, and postulated that PPAR- α downregulation in response to inflammatory stimuli may represent a mechanism contributing to the development of airway inflammation (Becker et al. 2008). Additionally, PPAR- α has anti-inflammatory properties by suppressing inflammatory/inflammatory response gene activation, the induction of expression of genes encoding proteins that are involved in the catabolism of pro-inflammatory lipid mediators (Moraes et al. 2006), and the interference with the transcription factors such as nuclear factor- κ B, activator protein-1, and the signal transducer and activator of transcription (STAT) proteins, which display important roles in the inflammatory response gene induction by cytokine. This repressive effect may be caused by direct interaction of the ligand-bound PPAR- α with the target transcription factor and/or interference with the clearance of the corepressor NCoR from promoters of inflammatory response genes (Lee et al. 2007).

Of notice, Vazquez-Tello et al. reported that the cytokines IL-17/IL-23 through their pro-inflammatory actions could suppress the glucocorticoids' anti-inflammatory and immunosuppressant properties (Vazquez-Tello et al. 2013). This idea could explain the cause of the steroid insensitivity in severe asthma phenotypes.

In conclusion, PPAR- α agonist fenofibrate was effective in attenuating airway inflammation and remodeling in OVA/

LPS-sensitized and OVA/LPS-challenged rats as a model of the severe asthmatic phenotype. This effect was mediated partially through suppression of TH17-IL-23/IL-17 axis. The superior therapeutic effects of fenofibrate over dexamethasone were through amelioration of both TH2 and TH17 cells-derived cytokines. Further studies are required to investigate the effect of combined treatment of steroids and PPAR- α agonists on severe phenotypes of bronchial asthma and airway inflammation. Modulation of the IL-23/IL-17 axis may hold promise for the treatment of severe forms of asthma that are difficult to be treated with existing therapies.

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Compliance with ethical standards

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

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Statement on welfare of animals All animal care and experimental procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (MD, USA) and were approved by the Institutional Animal Care and Use Committee at the Faculty of Medicine, Suez Canal University, Egypt.

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