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Evaluation of bronchial inflammatory response via expression of NF-κB & IL-1β in mice airways by TAK-242 and LPS administration: Targeting TLR4/MD2 signalling pathway

Swamita Arora
Amity Institute of Pharmacy, A -Block, Amity University, Sector-125, Noida, U.P.-201303, India

Sanjar Alam
Department of Pharmacology, R. V Northland Institute of Pharmacy, Gautam Buddh Nagar, U.P.-203207, India

Tanveer Naved
Amity Institute of Pharmacy, A -Block, Amity University, Sector-125, Noida, U.P.-201303, India

Wasim Akram
Department of Pharmacology, R. V Northland Institute of Pharmacy, Gautam Buddh Nagar, U.P.-203207, India

Harikesh Kalonia
Wockhardt Research Centre, D-4 MIDC, Chikalthana, Aurangabad, Maharashtra-431007, India

Sangeetha Gupta
Amity Institute of Pharmacy, A -Block, Amity University, Sector-125, Noida, U.P.-201303, India
*Corresponding author email: sgupta23@amity.edu

***Abstract*---**Optimization of animal model of asthma via toll like receptors-4 (TLR4) activation for bacterial exacerbation of asthma. LPS contributes to asthma exacerbations. Ovalbumin protein sensitization & challenge were examined after LPS exposure. Present research aimed, TLR4 stimulation mediated TLR4/MD2 complex formation is inhibited by TAK-242, a newer compound, for bacterial asthma management in a mouse model. Swiss Albino mice were induced with OVA (10μg, i.p. and 50 μg, i.n.) and LPS (20 μg, i.p. and 4 μg, i.n.), stimulates TLR4 receptor mediated TLR4-MD2 complex formation in

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toxic groups. Animals were sensitized (i.p.) on 0, 7th and 14th day and challenged (i.n.) followed by TAK-242 treatment (0.1 mg/kg, 1 mg/kg &10 mg/kg i.p.) given on 21st and 22nd days, followed by euthanasia and samples; BALF and lung tissue were collected on 23rd day. Further analysis such as total leukocyte count & differential leukocyte cell count, lung histopathology & immunohistochemistry of NF-κB, proteins like Interleukin-1β in BALF and lungs in toxic, treatment groups in comparison with control group were carried out. TAK-242 reduced TLC/DLC values in BALF, including eosinophil, neutrophil, lymphocyte, macrophage counts, lung histological alterations and immunohistochemical positivity of NF-κB and IL-1β proteins expression. While, these values significantly increased in asthmatic groups treated with OVA and LPS. TAK-242 can be a promising inhibitor of bacterial asthmatic symptoms induced by LPS via ovalbumin induced mouse model of allergic asthma.

***Keywords*---**bronchial asthma, lipopolysaccharide, nuclear factor kappa B, ovalbumin, TAK-242, toll-like receptor.

Introduction

Disease like Asthma is a condition with eosinophil, basophil, neutrophil, monocyte, macrophage and activated mast cells infiltration as in inflammation. Exacerbations of asthma are accompanied by bronchoconstriction, airway inflammation and production of mucus due to various triggering factors such as allergen, bacterial and viral infection and atmospheric irritants. Furthermore, current asthma management predicts future exacerbations[1]. According to recent research, inflammatory infiltration appears to have a crucial part in the development in asthma exacerbations. It result, suppressing cell inflammation and discriminating between eosinophilic and neutrophilic asthma, effective possibly for asthma exacerbation therapeutic management[2,3]. Toll-like receptors (TLRs) as cell surface receptors recognize exogenous or endogenous chemicals produced by microorganisms or host cells that have a role in asthma which is publicly recognized[4].

The endotoxin LPS has sparked influence in the development of allergy diseases. Gram-negative bacteria, which are widespread in our environment, contain LPS in their cell walls. Several studies have found that LPS intensified broncho-constrictive and inflammatory effects in allergic asthma patients and animal models, as well as increased antigen-specific allergic reactions in the airway, and that the severity of asthma is linked to LPS concentration[5]. The most potent microbe-associated molecular patterns (MAMP) molecule is gram-negative bacterial membrane LPS, which promotes host cells by activating the MD2-TLR4 complex[6]. LPS-binding protein (LBP) binds to lipid A moiety of LPS that is expressed on the target cell surface[7]. Excessive production of IgE against aeroallergens, bronchial hyperresponsiveness and the pathogenesis of allergic asthma are all linked to polymorphisms in the TLR4 gene[8].

In this study, airway hyperresponsiveness (AHR) was tested in mice lungs [9]. OVA challenge triggers airway wall remodeling in long-term exposure studies such as smooth muscle hyperplasia, fibrosis, desquamated epithelium and narrowing of lumen[10]. In the context of asthma pathogenesis aggravation, the unfriendly TLR-4 receptor and NF-κB pathways are the most essential therapy mechanisms [11]. TLR4 is inhibited by TAK-242 which connect Toll-interleukin-1 receptor domain & does not allow interaction between TLR4 and adaptor molecules blocking various signalling pathways [12]. LPS challenge reduced NF-κB generation and dampened neutrophil stimulation and its gathering in the lungs. As a result, TAK-242 could be used to disrupt adhesion molecules and inflammatory mediators [13]. The goal of this study was to see TAK-242, had any pharmacological value in the treatment of TLR-4 aggravated bacterial asthma.

Material and Method

Experimental Animals

Experimental animals were Swiss albino mice weighing 25–30 g procured. Resource was central animal house, AIIMS, New Delhi (India). The institutional animal ethical Committee, CPCSEA authorized and gave approval to the protocol for animal experiments with registration number, 1149/PO/Re/S/07/CPCSEA' at R.V. Northland Institute, Greater Noida, Dadri, U.P (India). Animals were housed in plastic cages with 12 h light/12 h dark cycle. Diet given was commercial standard pellets and were acclimatize for one week and kept at a temperature (23±2°C), relative humidity level (60±5%).

Drugs and Chemicals

Ovalbumin (CAS No. 138831-86-4 & Catalog No. S7951), Lipopolysaccride (CAS No. 9025-35-8 & Catalog No. L2630), TAK-242 (CAS No. 243984-11-4 & Catalog No. 614316), Dexamethasone (CAS No. 50-02-2 & Catalog No. D1756) and all additional AR grade compounds taken by Sigma Chemicals, St. Louis, Missouri, USA. NF-κB (Catalog No. PA5-16545,) and IL -1β (Catalog No. P420B) antibodies were procured from Thermo Fisher Scientific, USA.

Treatment Protocol

Seven groups (n = 6) of animals being made and given 22 days of treatment as per Table 1. Animals were weighed and euthanized 24 hours after the last treatment, and their lungs were extracted and cleaned with normal saline shown in Figure 1. A tissue section of lung had been kept in formalin (10%) for histopathology. BALF was collected from the cannulated trachea of animals under ketamine (24 mg/kg, s.c.) for TLC/DLC estimations[14]. OC (10µg i.p./50µg i.n.), LPS (20ug i.p./4µg i.n.), TAK-242 (0.1mg/kg i.p., 1mg/kg i.p., 10mg/kg i.p.) and DEX (1mg/kg i.p.) dose selection and treatment schedule were based on the previous literature[5,15–17].

Table 1
Group wise dosing schedule

S.No.	Groups	Route, Dose, Duration

I	Normal control (NC)	PBS for 22 days
II	OVA Control (OC)	OC on 0, 7th & 14th day (10µg, i.p.) + on 21st & 22nd day (50µg, i.n.)
III	OVA + LPS	OC & LPS on 0, 7th & 14th day (10µg, i.p./20 µg, i.p.) + on 21st & 22nd day (50µg, i.n./4 µg i.n.)
IV	OVA + LPS + TAK-242 (0.1mg/kg)	OC & LPS on 0, 7th & 14th day (10µg, i.p./20 µg, i.p.) + on 21st & 22nd day (50µg, i.n./4 µg i.n.) TAK on 21st & 22nd day (0.1mg/kg i.p.)
V	OVA + LPS + TAK-242 (1mg/kg)	OC & LPS on 0, 7th & 14th day (10µg, i.p./20 µg, i.p.) + on 21st & 22nd day (50µg, i.n/4 µg i.n.) TAK on 21st & 22nd day (1mg/kg i.p.)
VI	OVA + LPS + TAK-242 (10mg/kg)	OC & LPS on 0, 7th & 14th day (10µg, i.p/20 µg, i.p) + on 21st & 22nd day (50µg, i.n/4 µg i.n.) TAK on 21st & 22nd day (10mg/kg i.p.)
VII	OVA + LPS + DEX (1mg/kg)	OC & LPS on 0, 7th & 14th day (10µg, i.p/20 µg, i.p) + on 21st & 22nd day (50µg, i.n/4 µg i.n) DEX on 21st & 22nd day (1mg/kg i.p.)

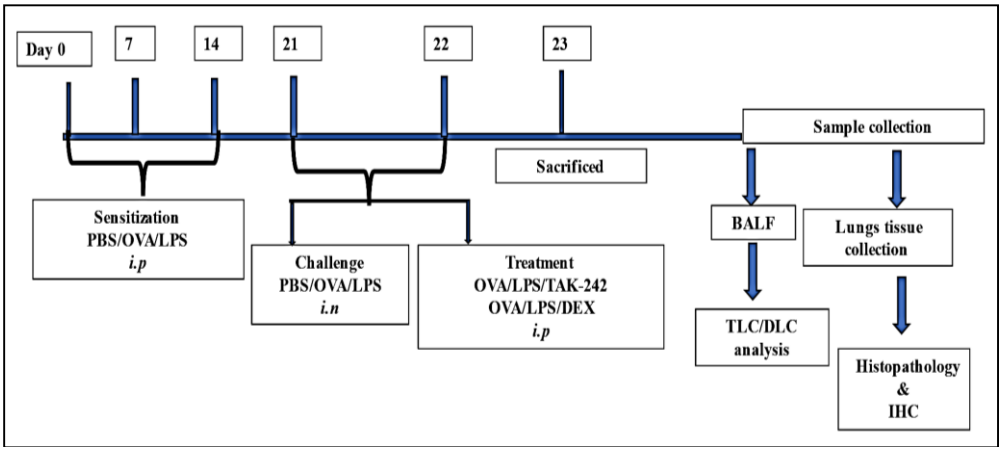

Figure 1. Experimental design and flow diagram (study protocol)

Bronchoalveolar Lavage (Fluid) Collection-BALF

Mice were euthanized post ketamine (24 mg/kg, s.c.) induced anesthesia. After the trachea was exposed, a polypropylene cannula was placed into it. At 37°C, a 10 ml syringe was used to inject 0.9% (w/v) PBS (10 ml) into the lung and were gently squeezed for 5 minutes to recover BALF. Centrifugation of obtained fluid (5 ml) been done on 5000 rpm speed taking 10 minutes maintain temperature of 4°C. The cells which accumulated in lower portion in centrifuge tube got retrieved

after the supernatant was discarded. These pelleted cells immersed in 0.5 ml of saline. TLC/DLC were performed in an automated cell analyzer[18].

Histopathological Examination

Lung tissue being taken out and preserved in formalin solution (10% v/v), dried, immersed in paraffin later cut into 5 µm segments and then treated by xylene to make them paraffin free and hematoxylin and eosin (H&E) were used to stain on glass slides. A pathologist, oblivious to the research groups inspected the stained slides under a light microscope. Lung inflammation; goblet cell hyperplasia, lumen occlusion & smooth muscle bundles were graded on a subjective scale of 0 to 4. Fig. 2. B.[19,20]. Cell counts were conducted to figure out the accumulation of inflammatory cells in intraluminal portion along with alveolar and peribronchial parts as well[21].

Immunohistochemical Analysis

Lung tissue sections were made paraffin free by using acetone and xylene for 5 minutes each. Then, samples were treated with ethanol to rehydrate them. Washing by distilled water conducted followed by retrieval of antigens by Citrate buffer (pH-6). Washing of sections been done three times with a Tris-buffer saline solution over the course of one hour & later incubated using primary antibodies against NF-κB and IL-1β protein (1/200; Thermo Fisher Scientific, USA) at 4°C whole night. Avidin-biotin-peroxidase complex and biotinylated secondary antibodies was used to determine immunoreactivity. For 2 minutes, diaminobenzidine was used as a substrate to generate an immunoreactive signal. The exposed area pictures were taken using Meiji techno microscope coupled to Lumenera camera. The pictures had been analysed blindly using Lumenera analyzer software. The reciprocal intensity approach was used for the semi-quantification of protein expression using the Fiji (Image J) software. The photos pixel intensities ranged from 0 to 250. The 0 value represents the darkest colour shade of the picture, while the 250 value represents the lightest colour shade of the image[22].

Statistical Data Analysis

Results been quantified in mean ± SEM (6 mice/group). One-way ANOVA along with Tukey's Multiple Comparison Test later used for finding the data significance, $p < 0.05$ value selected significant. Graph Pad Prism 3.0 software (San Diego, California, USA) used for graphical representation of the statistical data.

Results

Effect of TAK-242 on OVA/LPS-induced Histopathological Lung Changes

Lung tissue from the control group exhibited natural architecture; normal single cell layer alveoli and bronchioles were enclosed by a thin membrane. On the other hand, administration of OVA/LPS (20µg, i.p./4µg, i.n.) caused marked distortion of lung architecture (yellow arrow), narrowing of alveoli (blue arrow) and

bronchioles, smooth muscle hyperplasia (black arrow) with inflammatory cells count (red arrow) around the bronchial walls. Administration of TAK-242 (10mg/kg) and DEX (1mg/kg) significantly attenuated histological damage while treatment with TAK- 242(0.1mg/kg) showed a nonsignificant protective effect (Figure 2).

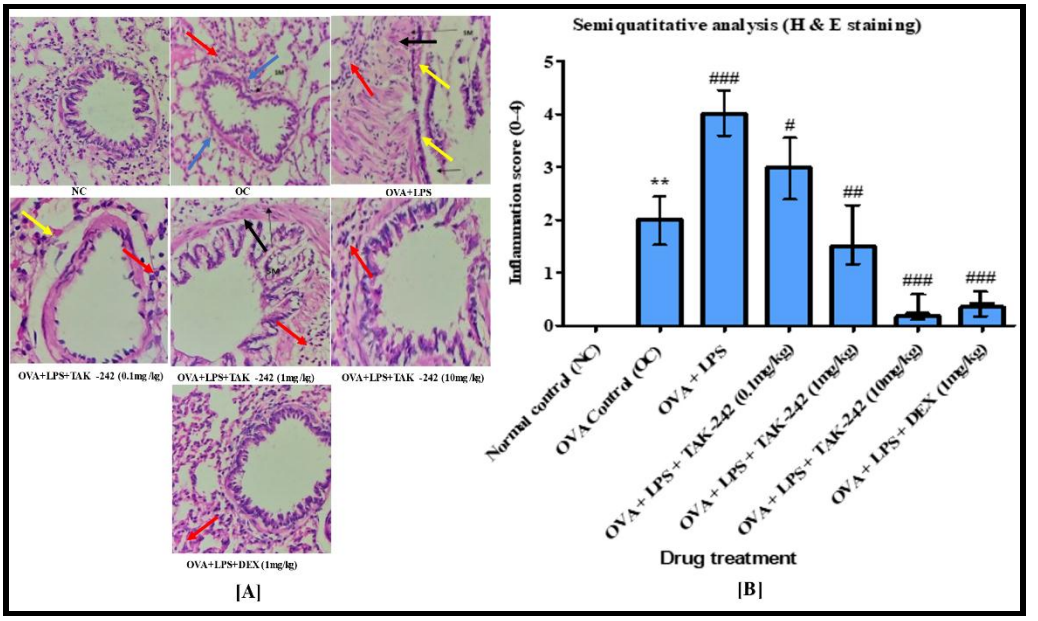

Figure 2. (A) H&E stained slices demonstrate histological changes induced by OVA & LPS groups as compared with normal control (PBS) groups and reversal by TAK-242 (0.1mg/kg), TAK-242 (1mg/kg) and TAK-242 (10mg/kg) doses in lung tissues (scale bar 100 µm). Treatment with OVA & LPS showed marked distortion in the lung architecture, alveolar and bronchial narrowing and smooth muscle thickening with peribronchiolar mild to moderate cells infiltration (Lymphocyte/Neutrophil/Eosinophil/Macrophages). Treatment with TAK-242 (10mg/kg) and DEX (1mg/kg) effectively reduced these histological aberrations whereas TAK-242 (0.1mg/kg) was found to be ineffective. (B) Semiquantitative analysis of lung inflammation was calculated on scale value- 0 to 4. Cell counts were assessed blindly using a four-point grading system: 0: normal, 1: minimal inflammation, 2: moderate inflammation, 3: maximum inflammation, and 4: highly excessive inflammation

TAK-242 Induced TLR4-MD2 Signaling Pathway Antagonism

The TLR4-MD2 complex activates airway inflammation. As a result, we investigated the level of TLR4 receptor expression, resulted their inward signalling & elevation of NF-κB and IL-1β expression in lung tissues. The level of expression of mediators NF-κB as well as IL-1β markedly raised in OVA group and LPS group than normal control animals group, while, TAK-242 (10mg/kg) treatment reversed the inflammation ratio significantly (Figure- 3).

TAK-242 Effect in the Lungs NF-κB & IL-1β Immunohistochemical Expressions

Expression of NF-κB and IL-1β proteins in lung tissues increased in OVA/LPS sensitized and nasally challenged mice than NC group. IL-1β was highly expressed mainly in the bronchial area, luminal membrane, interstitial inflammatory cells, lymphocytes and neutrophils in OC, OVA+LPS, OVA+LPS+TAK-242 (1mg/kg) group and weakly expressed in TAK-242 (10mg/kg) & DEX (1mg/kg) treated groups (Figure. 3), while the NF-κB immuno-histochemistry showed expression on higher side in OC, OVA+LPS, OVA+LPS+TAK-242 (1mg/kg) treated groups. Both LPS+TAK-242 (10mg/kg) and LPS+DEX (1mg/kg) standard treatment groups significantly decreased NF-κB and IL-1β proteins expression among OC & OVA+LPS-challenged mice lung.

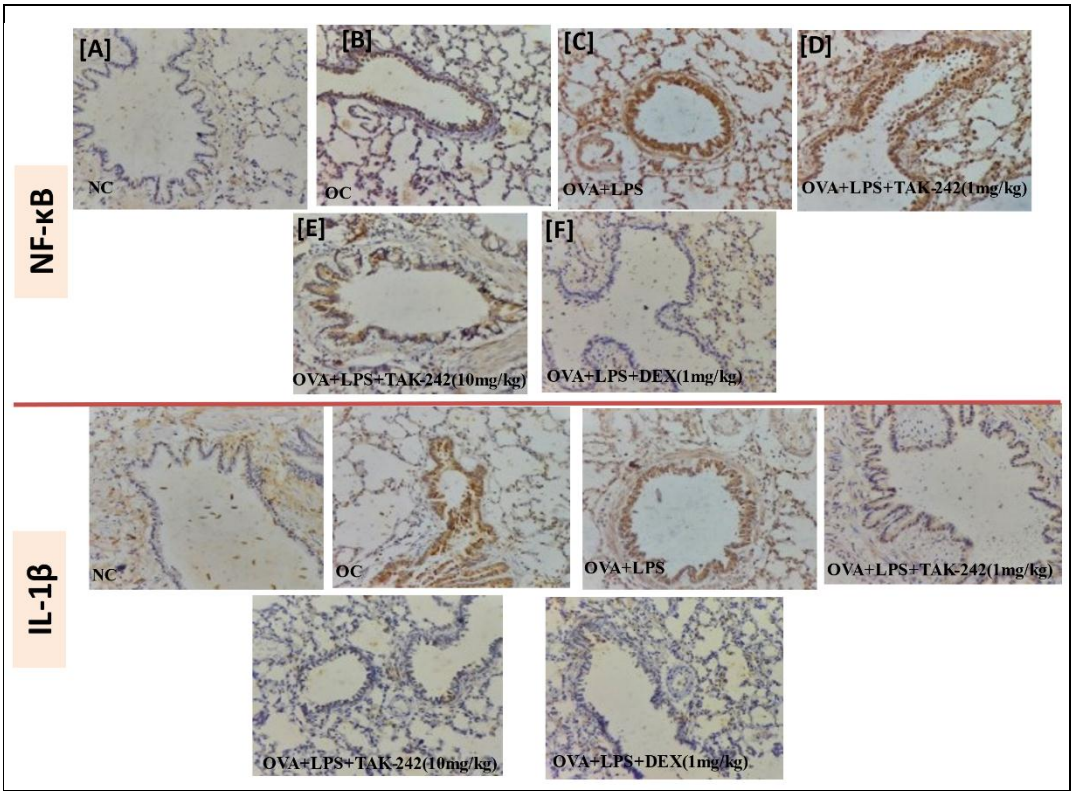

Figure 3. Immunohistochemical analysis of NF-κB & IL-1β proteins in mice lung tissue (scale bar-100 µm). Groups (A) Normal control, (B) OC (10µg i.p., 50µg i.n.), (C) OVA+LPS (20µg i.p., 4µg i.n.), (D) OVA+LPS+TAK-242 (1mg/kg),(E) OVA+ LPS+ TAK-242 (10mg/kg), (F) OVA+ LPS+ DEX (1mg/kg) treated tissues shows a significant increase in bronchial nuclear reactivity for NF-kB & IL-1β protein in toxic groups as compared to mice treated with TAK-242 (10mg/kg), DEX (1mg/kg) treatment & control groups

TAK-242 Effect in the Lungs Total-leukocyte Cell & Differential-leukocyte Cell Count

The TLC count were ($P < 0.05$, $P < 0.01$, $P < 0.001$) raised significantly in OVA and LPS groups, mildly in OC groups than NC groups in efficacy study of TLR4-MD2 complex inhibitor drug in BALF. Whereas, significant reduction in TLC infiltration in TAK-242 (10mg/kg) in comparison with DEX (1mg/kg) & TAK-242 (1mg/kg) groups. TAK-242 (0.1mg/kg) groups shows nonsignificant alterations in inflammatory cell infiltrate. In DLC estimation of animal groups in TLR-4 inhibitor efficacy study shows in OC & OVA+LPS (20µg i.p., 4µg i.n.) treatment groups significant elevation in neutrophil count ($P< 0.001$), eosinophil count ($P< 0.001$) & macrophages count ($P< 0.001$) with slight variation in lymphocyte count (BALF: $p < 0.05$) as compared with NC animal groups shown in Table 2.

BALF cells of the similar study group when investigated enhancement of macrophage, neutrophil & lymphocyte count in OVA + LPS exposed mice being noticed than control group of mice. However, BALF differential leukocytes count estimation by TLR4/MD2 complex inhibitor TAK-242 (0.1mg/kg, 1mg/kg, 10mg/kg) & DEX (1mg/kg) standard groups along with OC & OVA+LPS groups shows a significant reduction in neutrophil & eosinophil count. Inflammatory cell infiltrates such as eosinophil, neutrophil & macrophages count was significantly reduced after OVA+LPS+TAK-242 (10mg/kg) treated animals. A nonsignificant reduction in lymphocyte infiltration rate evident with OVA+LPS+TAK-242 (0.1mg/kg) groups as compared with TAK-242 (1mg/kg) & DEX (1mg/kg) groups (Figure 4).

Table 2
Efficacy study of TLR-4 inhibitor

Groups	TLC	Eosinophils	Lymphocytes	Macrophages	Neutrophils
Normal control (NC)	55.53 ± 1.40	3.12 ± 0.23	0.40 ± 0.01	58.33 ± 2.17	0.92 ± 0.02
Ovalbumin control (OC)	87** ± 2.32	5.38** ± 0.19	1.10* ± 0.06	99.75*** ± 6.29	1.55* ± 0.13
OVA+LPS	119.75## ± 2.97	8.44### ± 0.19	3.68 ### ± 0.22	117.5# ± 3.81	5.26### ± 0.95
OVA+LPS+TAK 242 (0.1mg/kg)	99.95@ ± 2.39	4.66@@ ± 0.28	3.74@ ± 0.14	94.17@@ ± 1.42	4.27ns ± 0.19
OVA+LPS+TAK-242 (1mg/kg)	79.48 ns ± 1.85	4.11 ns ± 0.20	2.54 ± 0.14	53.8@@@ ± 1.86	3.35@ ± 0.14
OVA+LPS+TAK 242 (10mg/kg)	34.41@@@ ± 1.05	3.69@@ ± 0.09	0.37 @@ ± 0.08	39.17@@ ± 1.51	1.2@@ ± 0.10
OVA+LPS+DEX (1mg/kg)	39.2@@ ± 1.05	2.97@@ ± 0.08	1.34 @ ± 0.03	47.18@ ± 1.12	1.9@ ± 0.13

Observed values are shown as Mean ± SEM (n=6). One-way ANOVA followed by Tukey's test was used for data analysis. The significance of data denoted by, ***$P < 0.001$ significant value, Vs normal control (NC); #$P< 0.05$ significant versus OC; @@$P< 0.01$, @@@$P < 0.001$ significant value, Vs OVA+LPS and 'ns' is non-significant Vs OVA+LPS.

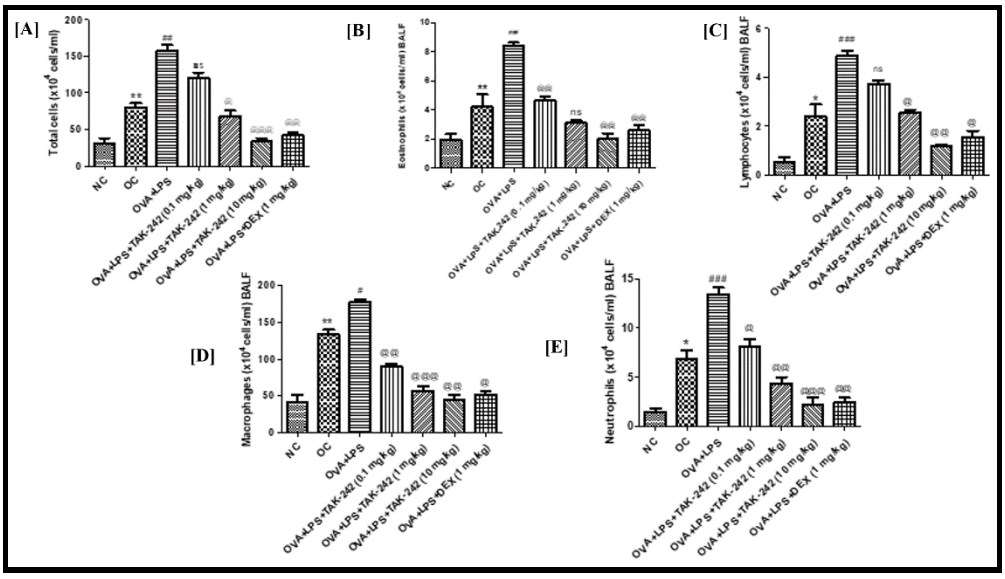

Figure 4. Total cells count (A), Eosinophils (B), Lymphocyte (C), Macrophages (D) & Neutrophils (E) were counted in BALF samples of controls, OC, OVA+LPS exposure (20µg) groups, and followed by TAK-242 (TLR4/MD2 complex inhibitor) treatment OVA+LPS+TAK-242 (0.1mg/kg, 1mg/kg, 10mg/kg) at low, mid & high doses. Low-dose TAK-242 (0.1mg/kg) showed nonsignificant difference, mid dose TAK-242 (1mg/kg) slightly protective effect. High dose of TAK-242 (10mg/kg) and DEX (1mg/kg) with OVA & LPS associated with eosinophils & neutrophils infiltration reduction subsequently in bacterial exacerbation treatment groups. Data calculated in Mean ± SEM, the total leukocytes and differential cell count in BALF samples were significantly decreased ($p< 0.01$) in treatment groups. Treatment with TAK-242 (10mg/kg) dose reduced total leukocytes count ($p< 0.001$), eosinophils ($p< 0.001$) and neutrophils ($p< 0.05$), except lymphocytes as compared with the OC group. One way ANOVA followed by Tukey's test determines the significance of data. ###P< 0.001 significant value, Vs OC, *P < 0.05, **P < 0.01, significant Vs NC; @@p< 0.01, @@@p< 0.001 significant, Vs OVA+LPS and 'ns' is non-significant Vs OC.

Discussion

The ovalbumin induced bronchial asthma model in mice is a frequently used paradigm for testing medication anti-asthmatic potential. This model's most distinguishing trait is airways hyperresponsiveness by certain agents, which produces bronchial allergy. In our investigation, mice sensitized with OVA developed bronchial asthma symptoms, including an increase in neutrophils, eosinophils, and other leukocytes. Increased proinflammatory cytokine expression, NF-κB and IL-1β in lung tissues and BALF were also found, exhibiting symptoms like that of due to allergic reaction of airways medium and bronchial asthma, IgE release and inflammatory cell accumulation[23]. Previous investigations also supporting and showing similarity to these findings. Standard drug i.e. dexamethasone being taken in the investigation as a comparison tool has

been utilized earlier several times broadly when the model with OVA induced asthmatic is used [24].

Pulmonary infection developed in mice with eosinophilia, goblet cell hyperplasia, and enhanced airways hyperresponsiveness after antigen exposure, which is linked to the synthesis of antigen specific IgE. Transgenic strains and many techniques are present for immune system alterations, thereby animal models given potential value to study immunology and inherited characteristics of asthma infection. As a result, mouse models may be more useful for examining pathways that lead to airway sensitization rather than pathogenic cascades upon antigen challenge[25].

Recent research has shed a lot of light on the LPS/TLR4-MD2 signalling pathway. This pathway's information can also be used to simulate how other TLRs downstream signalling are regulated. Because inappropriate control of LPS/TLR4-MD2 signalling has the potential to produce severe inflammation and chronic inflammatory disorders, it's critical to learn more about this pathway and identify new targets to combat these diseases [26]. Since TLR4 stimulation can cause severe reactions like sepsis, inhibitory mechanisms are required to protect the host from inflammation-induced damage. Several negative regulators can influence TLR4 activation on numerous levels. TLR4 activation is often amplified in animals deficient in these crucial regulators. TLRs signalling must be strictly controlled. The negative regulatory mechanisms that have been emerged which reduces TLRs signalling giving immunological balance are taken up here[27].

The combination of LPS and OVA could exacerbate bacterial asthma. Although LPS has been demonstrated in earlier research to aggravate allergies, few studies have looked at the inflammatory impact of both lipopolysaccharide & ovalbumin administration in mice airways, as well as the inflammatory role of toll-like receptors. Present findings suggest that the mice lung infection worsened by LPS and OVA administration which is mediated by the TLR4-MD2 complex signalling pathway. To begin with, LPS treated BALF samples found increased TLC- DLC cell level along with OVA, and proliferation of goblet cells, smooth muscle hyperplasia, lumen occlusion, and recruitment of inflammatory cell infiltrates in airways of Swiss albino mice. Furthermore, due to ovalbumin, the impact of lipopolysaccharide is amplified. Lung allergic disorders, such as asthma, are characterized by eosinophil buildup. In allergic asthma, IL-1β produced by T-helper 2 cells induces eosinophils and neutrophils activation, causing structural damage [28]. Airways infection triggers mediators and cytokines release by IgE and mast cells, resulting in an acute asthmatic response [29]. TAK-242 treatment significantly reduced ovalbumin-lipopolysaccharide induced elevated cytokines in lung histology and BALF inflammatory cell infiltration in the mice airways, indicating that TAK-242 anti-inflammatory activity is mediated by TLR4/MD2 complex inhibition.

Airway hyperresponsiveness is linked to higher levels of eosinophils as well as neutrophils in both the lungs and the bronchoalveolar lavage fluids in allergy pathways. TLR4/MD2 complex blocker TAK-242 suppresses eosinophil levels in lung tissues and BALF, suggesting that the newer formulation of the TLR4/MD2 complex inhibitor plays a significant role in the treatment of LPS-induced

bronchial inflammation. The oxidant-antioxidant disequilibrium is also a feature of asthma aetiology. Allergic asthma may lead to cellular and tissue destruction and in sequence reactive oxygen species (ROS) been released by leucocytes. Which enhance the sensitivity of respiratory tract along with mucus secretion[30]. Epithelial loss, enhanced respiratory tract smooth muscle mass, mucus excess release, fibrosis, and intrusion of inflammatory species itself been the histological features to indicate asthma. This respiratory inflammation and modification can affect the central & small airways, as well as the lung parenchyma, causing lung hyperinflation due to mucus clogging [31].

H&E staining demonstrated lung congestion including inflammatory cell intrusion in pulmonary alveoli in OVA-induced animals challenged by inhalation. In mice exposed to intranasal OVA and LPS, inflammation was predominantly found in the peri-bronchial areas. In the bronchial epithelium of the toxic groups, there was more Hematoxylin and Eosin staining than in the TAK-242 treatment groups. Our findings imply that distinct histopathological patterns and BALF leukocyte count are suppressed at different doses of TAK-242 antagonist therapeutic agent that are well proven in animal models of asthma. Nuclear factor-kappa B is a transcriptional mediator, host immunological reaction, cellular proliferation, its activation is triggered by inflammatory cytokines, T lymphocytes and B lymphocytes, LPS, dsRNA, free radicals, physical exercise, and certain anticancer agents.

Asthma, lung cancer, fibrosis & infectious pulmonary disorders such as COVID-19, TB all include NF-κB as a key component. As a result, NF-κB could become a crucial target for therapeutic development in the case of inflammatory lung illness [32]. To be released as a physiologically active cytokine, IL-1β is produced as a preform that must be cleaved via the inflammasome-caspase-1 axis. The inflammatory mediators IL-1β, cytokines species involvement in asthmatic airway inflammation has resurfaced as a topic of research. The presence of cytokine IL-1β were used to assess alveolar macrophage activity in asthma patients. Alveolar macrophages produce IL-1β in the airways, suggesting that IL-1 β could be a mediator in asthma [33]. Our findings revealed revealed about the high density of NF-κB & IL-1β in OVA-LPS challenge groups lung tissues than in the control groups, although IL-1β plays a significant part in the induction of asthma and that their expression was decreased following TAK-242 medication treatment.

Conclusion

Finally, the findings of this study shows that TAK-242 treatment reduces the severity of Th2-mediated asthma symptoms in mice airways induced by LPS via ovalbumin induced asthma mouse model, possibly because it lowers the expression of NF-κB and IL-1β in lung tissues. According to the findings, TAK-242 therapy reduces Th2 immune responses by decreasing the generation of inflammatory cells in bronchoalveolar lavage fluid after antigen stimulation and shows protective effect on lung architecture after H&E staining. TAK-242 appears to be a promising candidate for the management of lipopolysaccride induced bacterial inflammatory response in mice airways through inhibition of TLR4/MD2 complex signalling pathway.

Conflict of Interest

The authors are having no conflict of interest.

Funding

None

Author Contributions

- Conceptualization: Swamita Arora, Sangeetha Gupta, Sanjar Alam
- Data curation: Swamita Arora, Sanjar Alam, Wasim Akram, Harikesh Kalonia, Sangeetha Gupta, Tanveer Naved
- Formal analysis: Swamita Arora, Sangeetha Gupta, Tanveer Naved, Mohammad Khalid, Wasim Akram, Harikesh Kalonia. Sanjar Alam
- Investigation: Swamita Arora, Sangeetha Gupta, Sanjar Alam, Mohammad Khalid, Wasim Akram, Harikesh Kalonia
- Methodology: Swamita Arora, Sangeetha Gupta, Sanjar Alam
- Validation: Swamita Arora, Sangeetha Gupta, Sanjar Alam, Mohammad Khalid, Wasim Akram, Harikesh Kalonia, Tanveer Naved
- Writing-original draft: Swamita Arora, Sangeetha Gupta, Sanjar Alam
- Writing-review & editing: Swamita Arora, Sangeetha Gupta, Sanjar Alam, Mohammad Khalid, Wasim Akram, Harikesh Kalonia, Tanveer Naved

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Abbreviation List

AHR - Airway hyperresponsiveness
BALF - Bronchoalveolar lavage fluid
DEX - Dexamethasone
DLC - Differential leukocyte count
IL-1β - Interleukin 1 beta
LPS - Lipopolysaccride
MD2 - Myeloid differentiation protein-2
NF-κB - Nuclear factor kappa B
OVA - Ovalbumin
ROS - Reactive oxygen species
TAK-242 - Resatorvid
TLC - Total leukocyte count
TLR - Toll like receptor

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