Propofol inhibits NF-κB activation to ameliorate airway inflammation in ovalbumin (OVA)-induced allergic asthma mice

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1. Introduction

Asthma is a chronic inflammatory, allergic disorder of the airways, which is characterized by inflammatory cells infiltration, airway inflammation, mucus hypersecretion and airway hyperresponsiveness (AHR) [1,2]. Th2 lymphocyte plays a pivotal role in the pathogenesis process of allergic asthma by producing the type 2 (Th2) cytokines such as IL-4, IL-5, and IL-13. These cytokines induce release of IgE and the infiltration of Eosinophils into the lungs, which lead to the development of allergic asthma [3,4]. The nuclear factor kappa B (NF-κB) activation was observed in both human and animal models of asthma [5,6]. NF-κB, which is a multicellular transcription factor, plays a central role in regulating inflammatory and immune responses by regulating related cytokines and genes expressions [7,8]. Furthermore, inhibiting of NF-κB could protect against OVA-induced asthma [9,10]. So far, it is estimated that 300 million individuals are affected by the disease worldwide, the incidence of asthma and the frequency of its complications are still increasing [11]. However, the current treatments are not sufficient. New effective therapies for asthma are urgently required.

Propofol is one of the most widely used intravenous anesthetic for induction and maintenance of anesthesia or sedation [12]. Recent studies have suggested that propofol takes part in protection of various organs [13]. Propofol has been shown to induce bronchodilation in some species, including asthmatic patients [14–16]. Furthermore, a lot of studies have indicated that propofol has anti-inflammatory effect on neutrophil-mediated responses [17–19]. However, the effects of propofol on allergic eosinophil inflammation remain unclear. Thus, in this study, we aimed to investigate the effects of propofol on OVA-Induced allergic asthma mice.

2. Materials and methods

2.1. Chemicals and reagents

Propofol (Diprivan, 1% propofol, CG411; AstraZeneca, Caponago, Italy), OVA was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Mouse IL-4, IL-5, IL-13 and IgE ELISA kits were obtained from R & D Systems (Minneapolis, MN). NO assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Antibodies against NF-κB, iκBα, LaminB1, and β-actin were obtained from Santa Cruz Biotechnology (Autogen, Bioclear, UK). Antibody against iNOS was from Wuhan Boster (China). All other chemicals were according with

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sensitized by intraperitoneal injection of 10 mg Al(OH)₃ gel in 0.1 mL normal saline or PBS on days 1 and 13. Propofol (50, 100, 150 mg/kg) was given intraperitoneally 1 h before OVA challenge. The control and asthma groups received PBS on the same schedule.

2.3. Establishment of OVA-induced allergic asthma mice

2.3.1. Model and grouping

50 mice were randomly divided into five groups (n = 10) as follows: control group; OVA group; OVA + propofol groups (50, 100, 150 mg/kg); Mouse asthma model was established as previously published [20], with some modifications. Briefly, Mice were sensitized by intraperitoneal injection of 10 μg OVA/20 mg Al(OH)₃ gel in 0.1 mL normal saline or PBS on days 1 and 13. From day 25 to 32, the mice were then challenged by intranasal inhalations with OVA (1 mg/mL) or PBS aerosol challenges for 30 min once a day. Propofol (50, 100, 150 mg/kg) was given intraperitoneally 1 h before OVA challenge. The control and asthma groups received PBS on the same schedule.

2.4. Measurement of airway hyperresponsiveness (AHR) (FlexiVent, SCIREQ, USA)

24 h after the final OVA treatment, Pulmonary resistance (RL) was used to evaluate the airway responsiveness(AHR) and detected by an invasive pulmonary facility for small animals (FlexiVent, SCIREQ, USA). The protocol of invasive airway hyperresponsiveness (AHR) measurement was performed as previously described [21]. Briefly, each mouse was tracheostomized and intubated under anesthesia. Increasing methacholine (Mch) (0, 3.125, 6.25, 12.5, 25 and 50 mg/mL) was administered by nebulization at intervals of 3 min. Data were expressed as a percentage change from the baseline value.

2.5. Bronchoalveolar lavage fluid (BALF) collection and cell counting

Immediately following the assessment of AHR, mice were euthanized. The trachea was cannulated, and the lungs were washed with 0.8 mL cold PBS for 3 times to collect BALF. Then the BALF samples were centrifuged, supernatants were collected and stored at −80 °C for analyzing the levels of cytokines. BALF cell pellets were suspended with PBS for total and differential cell counts. The total number of cells therein was counted, and then the samples were centrifuged onto glass slides to determine the numbers of eosinophils, neutrophils, macrophages and lymphocytes in BALF by Wright–Giemsa staining.

2.6. Cytokines and NO assay

The levels of IL-4, IL-5, IL-13 and eotaxin in the BALF, OVA-specific IgE in the serum were measured by using ELISA kits according to the manufacturer’s instructions. The level of NO in BALF was tested by using NO assay kit according to the manufacturer’s protocols.

2.7. Hematoxylin and eosin (HE) and periodic acid Schiff (PAS) staining

After the BALF collection, lungs from each animal were collected and immersed in 10% neutral-buffered formalin, embedded in paraffin and sliced. The sections were cut into 5 μm sections. Then the sections were stained with hematoxylin and eosin (H&E) for general morphology. Pathologic changes were observed and captured with a light microscope. Periodic acid Schiff (PAS) to examine mucus production and identify the goblet cells in lung tissues. The histological changes in the lungs were scored according to previously reports [22]. Each histological characteristic was scored 0 to 5 (Fig. 5C). PAS stained goblet cells in the lung sections were performed as previously described [23]. Briefly, to estimate the severity of mucus secretion, the numbers of goblet cells were evaluated using a 5-point scoring system (Fig. 5D).

2.8. In vitro study

A549, a human type II lung epithelial cell line, was purchased from the American Type Culture Collection (ATCC, USA). Cells were maintained in DMEM supplemented with 10% fetal bovine serum, 100 mg/mL streptomycin, and 100 U/mL penicillin. Cells were cultured with complete medium at 37 °C in 100% humidified atmosphere of 5% CO₂ in air. All experiments were performed when cells were 80–90% confluent.

2.9. Western blot analysis

The lung tissues were homogenized, and tissue proteins were extracted using a protein extract reagent kit (Thermo) according to the manufacturer’s instructions. Then, the equal samples (50 μg) of proteins were separated on SDS-PAGE and transferred on to PVDF membranes (Millipore, Billerica, MA, USA). After transferring, the membranes were blocked with 5% skim milk for 2 h at room temperature. Then, the membranes were probed with primary antibodies: iNOS NF-kB, p-NF- κB, LaminB1, and β-actin at 4 °C overnight. After washing with tris-buffered saline containing 0.1% Tween-20 (TBST) three times, incubated the membranes with HRP-labeled secondary antibodies for 1 h at room temperature. Finally, membranes were detected by ECL Western blotting reagents.

2.10. Immunofluorescence

A549 cells were washed with PBS, fixed at 4 °C ice-cold paraformaldehyde solution for 30 min, washed three times in PBS, and then permeabilized with 0.5% Triton X-100 at room temperature for 15 min and blocked with 5% bovine serum albumin (BSA) for 30 min. The primary antibody NF-κB p65 (1:200) were incubated at 4 °C overnight. Subsequently, the Dylight 488-conjugated donkey anti-rabbit immunoglobulin G (IgG) antibody was incubated for 1 h at room temperature. In the dark. The incubated cells were washed in PBS, and DAPI (Thermo Fisher) was used to visualize nuclei.

2.11. Statistical analysis

All values were presented as mean ± standard error of mean (S.E.M.). The statistical significance among different treatments in each individual experiment was compared by ANOVA. Values of p < 0.05 were considered to be statistically significant.

3. Results

3.1. Propofol reduced OVA-induced airway hyperresponsiveness

The effects of propofol on OVA-induced AHR were detected by measuring RL in response to increased doses of methacholine. The results showed that RL value was significantly increased in response to methacholine inhalation in OVA-Induced allergic asthma mice than the control group. However, treatment of propofol dose-dependently dramatically reduced RL induced by OVA, suggesting that propofol treatment reduced OVA-Induced AHR. (Fig. 1).
3.2. Effects of propofol on the number of inflammatory cells in BALF of OVA-induced airway inflammation model

The effects of propofol on OVA-induced inflammatory cell infiltration in BALF were detected in this study. As shown in Fig. 2. The total numbers of cells, eosinophils, neutrophils and macrophages in BALF were considerably increased in OVA-sensitized and challenged mice. Propofol administration markedly suppressed the total number of cells and the numbers of eosinophils, neutrophils, and macrophages in BALF, indicating that propofol treatment attenuated the infiltration of inflammatory cells in airways challenged with ovalbumin.

3.3. Propofol reduces OVA-induced Th2 cytokine and NO levels in BALF and suppresses iNOS expression in the lungs

ELISA was applied in this study to investigate the effects of propofol on OVA-induced Th2 cytokine production. Compared with control mice, OVA sensitization and challenge significantly induced the production of IL-4 (Fig. 3A), IL-5 (Fig. 3B), IL-13 (Fig. 3C) and eotaxin (Fig. 3D) in BALF. But these increased Th2 cytokines were strongly reduced by the treatment of propofol (50, 100, 150 mg/kg), and the level of NO in BALF markedly increased in OVA-induced mice compared with the control mice, and NO levels significantly decreased in propofol-treated mice (Fig. 3E). Propofol markedly suppressed iNOS expression in the lungs of OVA-induced mice (Fig. 3F).

3.4. Effects of propofol on OVA-specific IgE levels in the serum

Allergic airway inflammation in asthma is associated with Th2-related responses, including the production of IgE. We determined the effectiveness of propofol against OVA-specific IgE in the serum. As shown in Fig. 4, OVA-specific IgE markedly increased in OVA-induced mice compared with the control mice, whereas these levels significantly decreased in propofol-treated mice when compared with the OVA-induced mice (Fig. 4).

3.5. Effects of propofol on OVA-induced allergic asthma mice lung histological changes

To evaluate the effects of propofol on the histopathology of OVA-induced allergic asthma, histological changes of lung tissues with or without propofol treatment were detected by H & E and PAS staining analyses (Fig. 5). As shown in Fig. 5HE, in contrast to the histological features of lungs in control group mice, lung tissues from OVA group were significantly damaged with irregular structure, airway epithelial thickening, airway mucous membrane edema, airway lumen narrowing. In addition, the tissues exhibited infiltration of inflammatory cells into the perivascular and peribronchial lesions in the lung tissue, mainly including eosinophils and lymphocytes, with goblet cells proliferation increased. Furthermore, the results of the PAS staining showed that the over-production of mucus and goblet cells hyperplasia in OVA group (Fig. 5PAS). However, treatment of propofol remarkably ameliorated OVA-induced lung injury and inflammatory cell infiltration, significantly inhibited mucus hypersecretion and the number of goblet cells.

3.6. Effects of propofol on NF-κB activation in OVA-induced allergic asthma mice and TNF-α induced A549 cells

The effects of propofol on OVA-induced phosphorylation levels of IkBα and NF-κB p65 transcription activity were detected by western blot and immunofluorescence analysis in this study. As shown in Fig. 6. WB showed that the levels of p-p65 and p-IkBα were dramatically increased in OVA-challenged mice. However, treatment of propofol dose-dependently inhibited OVA-induced p-p65 and p-IκBα expression (Fig. 6A). Compared to the control groups, the OVA group showed a significant increase in the translocation of NF-κB to the nucleus in the lung tissue. In contrast, propofol treatment inhibited OVA-induced activation of NF-κB (Fig. 6B). Moreover, to further investigate the effects of propofol on the NF-κB p65 activation, we use immunofluorescence to detect the transcriptional activity of NF-κB p65. The results indicated that TNF-α treatment considerably promoted the translocation of NF-κB to the nucleus in A549 cells, while the activation of NF-κB was markedly ameliorated by propofol treatment (Fig. 6C). Overall, these results demonstrated that propofol attenuated the activation of NF-κB in OVA-Induced allergic asthma mice and A549 cells.
4. Discussion

Apart from its multiple anesthetic advantages, propofol exerts a number of non-anesthetic effects [13]. Propofol has been demonstrated to induce bronchodilation by attenuating smooth muscle contraction in both healthy and asthmatic patients [16,24]. However, airway inflammation is another central contributor to asthma pathogenesis. In this study, we investigate effects of propofol on allergic airway inflammation not only in vivo but also in vitro. The main findings of our experiments were that propofol could protect against OVA-induced allergic asthma mice by inhibiting NF-κB activation. Propofol has potential as a therapy in the treatment of asthma.

Asthma is one of the most prevalent respiratory diseases worldwide and characterized by a Th2-type immune response [25]. Inflammation has been reported to play a critical role in the development of asthma. OVA exposure in the airway produces a Th2-dominant response. A large of studies showed that in patients with asthma and OVA-induced mice asthma, TH2 lymphocytes infiltrated the lungs, increased the number of inflammatory cells and produced inflammatory cytokines [26,27]. Th2 cytokines, including IL-4, IL-5, IL-13, and eotaxin played critical roles in the pathogenesis of allergic airway inflammation [28]. IL-4 is one of the most important cytokines for the regulation of Th2 inflammatory responses. It induces B cell maturation and switches from IgG to IgE [29]. IL-5 is essential for differentiation, maturation, and survival of eosinophils, which plays an important role in allergen-induced eosinophilic airway inflammation. IL-5 could enhance IL-4-induced IgE production. Another key cytokine in asthma is IL-13, which promotes B-cell differentiation. IL-13 is able to directly lead to airway inflammation and AHR [30]. In addition, eotaxin is a chemokine that is involved in eosinophil recruitment and Th2 cell infiltration [31]. Recent studies suggested that inhibition of these Th2 cytokines might attenuate allergic asthma [32]. In our study, the concentrations of IL-4, IL-5, IL-13 and eotaxin were strikingly increased in Ova-treated mice, while the production of these Th2 cytokines was significantly suppressed after propofol treatment (Fig. 3). Nitric oxide (NO) is produced by T-lymphocytes and epithelial

Fig. 3. Propofol reduces OVA-induced Th2 cytokine and NO levels in BALF and suppresses iNOS expression in the lungs. The values presented are the means ± SEM of three independent experiments. *p < 0.05 vs. control group; †p < 0.05, ††p < 0.01 vs. OVA group.

Fig. 4. Effects of propofol on OVA-induced OVA-specific IgE levels in the serum were detected by ELISA. The values presented are the means ± SEM of three independent experiments. *p < 0.05 vs. control group; †p < 0.05, ††p < 0.01 vs. OVA group.
The level of exhaled NO, which has been proposed as a marker of airway inflammation in asthma, may reflect airway eosinophilia and the severity of asthma [33]. It appears that the NO level is associated with the iNOS expression of patients with asthma [34]. Our results show that propofol markedly suppressed level of NO in BALF and iNOS expression in the lungs of OVA-induced mice. (Fig. 3) Elevated IgE levels are a hallmark of the Th2 immune response. Our data showed that serum levels of OVA-specific IgE were substantially reduced by propofol in OVA-challenged mice (Fig. 4).

AHR is a hallmark of asthma, and the development of AHR was associated with inflammatory cytokines [35]. In our study, we demonstrated that propofol significantly ameliorated AHR (Fig. 1). These data are consistent with our hypothesis that propofol reduces Th2 cytokine levels. Thus, the observed reduction of AHR by propofol may be associated with the reduction in Th2 cytokine production, tissue eosinophilia and serum IgE level.

Histopathological changes, mucus hypersecretion and goblet cell hyperplasia in the lung are the major characteristics of allergic inflammation. Eosinophils play an important role in the pathogenesis of allergic inflammation [36]. Th2 cytokines are involved in recruiting eosinophils to the lung and play a critical role in goblet cell hyperplasia during allergic inflammation [37]. Here, our findings showed that propofol suppressed inflammatory cell infiltration into the airways as shown by the decreases of total cell and eosinophil counts in BALF (Fig. 2). In addition, propofol reduced eosinophil infiltration and ameliorated many pathological signs of inflammation in histological analysis (Fig. 5A). Moreover, PAS staining showed the over-production of mucus and goblet cell hyperplasia in the airways of Ova-induced mice were decreased by propofol (Fig. 5B). These data are in accordance with our measurements of the Th2 cytokine levels.

Therefore, our results revealed that propofol had protective effects on allergic airway inflammation in OVA-induced allergic asthma mice.
Investigation into the anti-inflammatory effects of propofol so far has been focused mainly on neutrophil-mediated responses [38–41]. The effects of propofol on other types of inflammation have not been studied before. Interestingly, our studies found that propofol has anti-inflammatory effects against TH2-type allergic responses. Allergic airway inflammation is type 2T cell-mediated eosinophilic inflammation, which is different from neutrophil-mediated inflammation in both cellular mechanisms and mediators. Our findings extend the anti-inflammatory effects of propofol from previous studies by demonstrating that propofol can ameliorate allergic airway inflammation.

NF-κB, an essential transcription factor, has been reported to play pivotal roles in various pathophysiological conditions of cell and animal models [42–44]. Once allergen stimulates, IkB detaches from NF-κB, the phosphorylation of IkB protein is markedly elevated, p65 dissociates from the cytoplasm to the nucleus, leading to the synthesis and release of pro-inflammatory cytokines [45,46]. NF-κB has been reported to regulate Th2 cell differentiation and Th2 cytokines expression in allergic asthma [47]. Therefore, it has been demonstrated that NF-κB is a promising target for the therapeutic of asthma [48,49]. To further investigate the underlying mechanism of propofol treatment in the amelioration of allergic airway inflammation, the effects of propofol on NF-κB activation and IkBα degradation were measured. In our study, propofol obviously inhibited the phosphorylation of IkBα and NF-κB p65 in OVA-induced allergic asthma mice. Moreover, propofol inhibited TNF-a-induced transcriptional activity of NF-κB in A549 cells. These results indicated that propofol exerted protective effects in OVA-induced allergic asthma mice and A549 cells by inhibiting the NF-κB signaling pathway.

In summary, our study showed that propofol effectively attenuated airway inflammation in the OVA-sensitized mice. In addition, propofol inhibited the activation of NF-κB in vitro and in vivo. These results suggest that propofol may provide a therapeutic potential for effectively prevention and treatment of asthma.

Conflict of interest

The authors have no conflict of interest to declare.

Acknowledgments

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