

Anti-inflammatory effects of ivermectin in mouse model of allergic asthma

Shuhan Yan · Xinxin Ci · Na Chen · Chi Chen · Xiangchao Li ·
Xiao Chu · Jianhua Li · Xuming Deng

Received: 10 February 2010 / Revised: 7 December 2010 / Accepted: 30 December 2010
© Springer Basel AG 2011

Abstract

Background and objective Asthma is an inflammatory disease of the lungs that is characterised by increased inflammatory cell infiltration into the airways and poor respiratory function. Ivermectin is a semi-synthetic derivative of a family of macrocyclic lactones that shows broad-spectrum anti-parasitic activity. This drug has been shown to possess anti-inflammatory activity, but whether it can be used in asthma treatment has not yet been investigated. In this study, we aimed to investigate the inhibitory effects of ivermectin on allergic asthma symptoms in mice. **Methods and results** We used a mouse asthma model, in which allergic airway inflammation and airway remodeling were induced by ovalbumin (OVA) sensitisation and challenge. Ivermectin or PBS treatment was administered 1 h before OVA challenge. Ivermectin at 2 mg/kg significantly diminished recruitment of immune cells, production of cytokines in the bronchoalveolar lavage fluids and secretion of OVA-specific IgE and IgG1 in the serum. Histological studies indicated that ivermectin suppressed mucus hypersecretion by goblet cells in the airway. **Conclusions** This is the first study to demonstrate that ivermectin is an effective suppressor of inflammation and may be efficacious in the treatment of non-infectious airway inflammatory diseases such as allergic asthma.

Keywords Allergic airway inflammation · Asthma · Ovalbumin-induced mouse model of asthma · Ivermectin

Abbreviations

BALF	Bronchoalveolar lavage fluids
Cdyn	Lung compliance
DEX	Dexamethasone
H&E	Hematoxylin and eosin
IVER	Ivermectin
OVA	Ovalbumin
PAS	Periodic acid-Schiff
PBS	Phosphate-buffered saline
RL	Airway resistance

Introduction

Allergic asthma is a complex chronic airway condition that is characterised by airway inflammation, lung eosinophilia, mucus hypersecretion by goblet cells, elevated serum IgE levels and intrapulmonary overexpression of Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13 [1–3]. The prevalence of asthma is rapidly increasing worldwide, especially in young children, and asthma has become a significant cause of morbidity and mortality in developing countries [4]. Although the pathophysiology of asthma has been extensively investigated, there is still no cure for this disease. Dexamethasone is widely used to treat allergic asthma, but long-term treatment has numerous side effects. Therefore, the development of novel anti-inflammatory approaches for asthma treatment is critical.

One potential class of treatments is the macrolides antibiotics, which have been widely used as antibacterial agents due to their ability to reversibly bind the 50S

Responsible Editor: Michael Parnham.

S. Yan · X. Ci · N. Chen · C. Chen · X. Li · X. Chu · J. Li ·
X. Deng (✉)
Department of Veterinary Pharmacology, College of Animal
Science and Veterinary Medicine, Jilin University,
Changchun 130062, Jilin, People's Republic of China
e-mail: xumingdeng@jluhp.edu.cn

ribosomal subunit of susceptible microorganisms and inhibit protein synthesis. In addition to their antibacterial activity, these agents exhibit anti-inflammatory activity in humans and animals and have shown efficacy in the treatment and management of some inflammatory diseases [5–9]. Moreover, reports indicate that macrolides, such as clarithromycin and azithromycin, can attenuate allergic airway inflammation in experimental mouse models of allergic asthma [10, 11].

Ivermectin is a semi-synthetic derivative of a family of macrocyclic lactones. This drug is an effective microfilaricide used in the treatment of strongyloides, scabies, and all types of filariasis except for *Dipetalonema* (Mansonella) perstans infections [12]. In addition to these antimicrobial effects, recent evidence suggests that ivermectin also possesses distinct anti-inflammatory properties. For example, ivermectin has been shown to affect cellular and humoral immune responses [13] and inhibit nitric oxide and prostaglandin E2 production by LPS-stimulated RAW 264.7 macrophages [14]. Thus far, the effects of ivermectin treatment on a non-infectious mouse airway inflammatory model have not been evaluated. Thus, we investigated whether ivermectin has a distinct anti-allergic and anti-inflammatory effect in asthmatic mice.

Materials and methods

Materials

Ivermectin and dexamethasone phosphate were purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Ivermectin was dissolved in PBS at a concentration of 0.2 mg/mL and 20 g/0.2 mL of this solution was intragastrically administered to mice. The final dose of ivermectin was 2 mg/kg. Dexamethasone was dissolved in PBS at a concentration of 0.2 mg/mL and 20 g/0.2 mL of this solution was intragastrically administered to mice. The final dose of dexamethasone was 2 mg/kg.

Animals

Female BALB/c mice (4–6 weeks old and weighing 18–20 g) were purchased from Shanghai Jingke Industrial Co., LTD (Certificate SCXK2003-0003) (Shanghai, China), maintained in the animal facility under standard laboratory conditions for 1 week before experiments began and provided water and standard chow ad libitum. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health.

Sensitisation and airway challenge

Mice were divided into the following four groups ($n = 10$): negative control group, ovalbumin (OVA) + IVER group, OVA + DEX group, and OVA group. All mice were sensitised with 20 µg OVA (Grade V; Sigma–Aldrich, St. Louis, MO, USA), adsorbed in 100 µg/mL of Imject Alum (Pierce, Rockford, USA), by i.p. injection on days 0, 7 and 14. On days 25 through 27, anaesthetised mice were challenged with 50 µg of OVA in 50 µL of PBS, and mice from the negative control group were given an equal volume of PBS in place of OVA. On each day of days 25 through 27, ivermectin at 2 mg/kg [15] was administered intragastrically 1 h before OVA challenge. Dexamethasone at 2 mg/kg was administered i.p., as a positive control. PBS was used in place of ivermectin and dexamethasone for the OVA group. All mice were sacrificed with an overdose of pentobarbital on day 28.

Collection of bronchoalveolar lavage fluid (BALF)

Mice were killed 24 h after the final challenge. The chest cavity of each animal was carefully opened, after which the trachea was exposed and catheterised. The BALF was collected by cannulating the upper part of the trachea, and then PBS was infused in three aliquots (0.3, 0.3 and 0.4 mL) in a total volume of 1 mL. Lavaged samples from each mouse were kept on ice until required. Fluids were centrifuged, and pellets were recovered for cellular analysis. Supernatants were stored at -80°C for biochemical analyses.

Inflammatory cell counts in BALF

Cell pellets were resuspended in 1 mL of PBS. Total cell numbers were obtained by counting cells in a hemacytometer. The bronchoalveolar lavage cells were separated by Cytospin and then stained using a modified Giemsa method. Briefly, cytopins were stained for 3 min in May–Grünwald and 1 min in 1:1 May–Grünwald: MilliQ, dipped in MilliQ, stained for 20 min in 1:4 Giemsa: MilliQ, dipped twice in MilliQ, dipped in 0.01% acetic acid until excess stain was removed, dipped four times in 95% ethanol, dipped four times in 100% ethanol and dipped twice in xylene [16]. Differential cell counts were performed using standard morphological criteria. At least 200 cells were counted per slide.

Measurement of serum OVA-specific IgE and IgG1 and BALF cytokines

Blood samples were obtained from the orbital immediately after mice had been killed on day 28 and centrifuged at 2,000g for 10 min at room temperature. The serum was separated and stored at -80°C until analysed. Levels of

OVA-specific IgE and IgG1 were measured using an enzyme-linked immunosorbent assay (ELISA). Microplate wells were coated with 1% OVA in coating buffer (0.05 M sodium carbonate–bicarbonate, pH 9.6) overnight at 4°C. The wells were then incubated with blocking buffer (1% BSA in PBS, pH 7.2) at room temperature for 1 h and washed. Diluted (1/10) serum samples were added to the microplate and incubated at room temperature for 2 h, washed and incubated with biotin-labelled anti-mouse IgE or anti-mouse IgG1. Plates were then incubated with streptavidin-peroxidase at room temperature for 30 min and then with TMB substrate for 30 min. The enzymatic reaction was stopped with 2 M H₂SO₄, and absorbance was read at 450 nm. The units were reported as concentration (mg/mL). The levels of IL-4, IL-5 and IL-13 in BALF were determined by specific mouse IL-4, IL-5 and IL-13 ELISA kits (BioLegend, Inc. Camino Santa Fe, Suite E San Diego, CA, USA).

Lung histology

After BALF was obtained, lungs were removed by dissection and fixed in 4% paraformaldehyde. Lung tissues were sectioned, embedded in paraffin and cut at 3 µm. Tissue sections were then stained with hematoxylin and eosin (H&E) for general morphology and with periodic acid-Schiff (PAS) for identification of goblet cells in the epithelium.

Measurements of airway hyperresponsiveness (AHR)

AHR was measured 24 h after the final OVA challenge. Mice were anaesthetised by an i.p. injection of sodium pentobarbitone (50 mg/kg), and tracheotomy was performed as described [17]. The internal jugular vein was cannulated and connected to a microsyringe for intravenous methacholine administration. Airway resistance (RL, which refers to the pressure driving respiration divided by flow) and lung compliance (C_{dyn}, which refers to the distensibility of the lung and is defined as the change in volume of the lung produced by a change in pressure across the lung) in response to increasing concentrations of methacholine were recorded using a whole-body plethysmograph chamber (Buxco, Sharon, CT, USA) as described [17]. Results are expressed as the percentage of the respective basal values obtained from PBS-challenged negative control mice.

Statistical analysis

All values were expressed as mean ± standard error of the mean (SEM). Differences between mean values of normally distributed data were assessed by one-way analysis of variance (ANOVA; Dunnett's *t* test) and Student's *t* test. Statistical differences were considered significant at *P* < 0.05.

Results

The effect of ivermectin on the release of IL-4, IL-5 and IL-13 in BALF

The levels of BALF cytokines were determined by ELISA in each experimental group 24 h after the last challenge. OVA sensitisation and challenge induced a significant elevation in the levels of IL-4, IL-5 and IL-13 in the BALF. As shown in Fig. 1, the concentrations of IL-4, IL-5

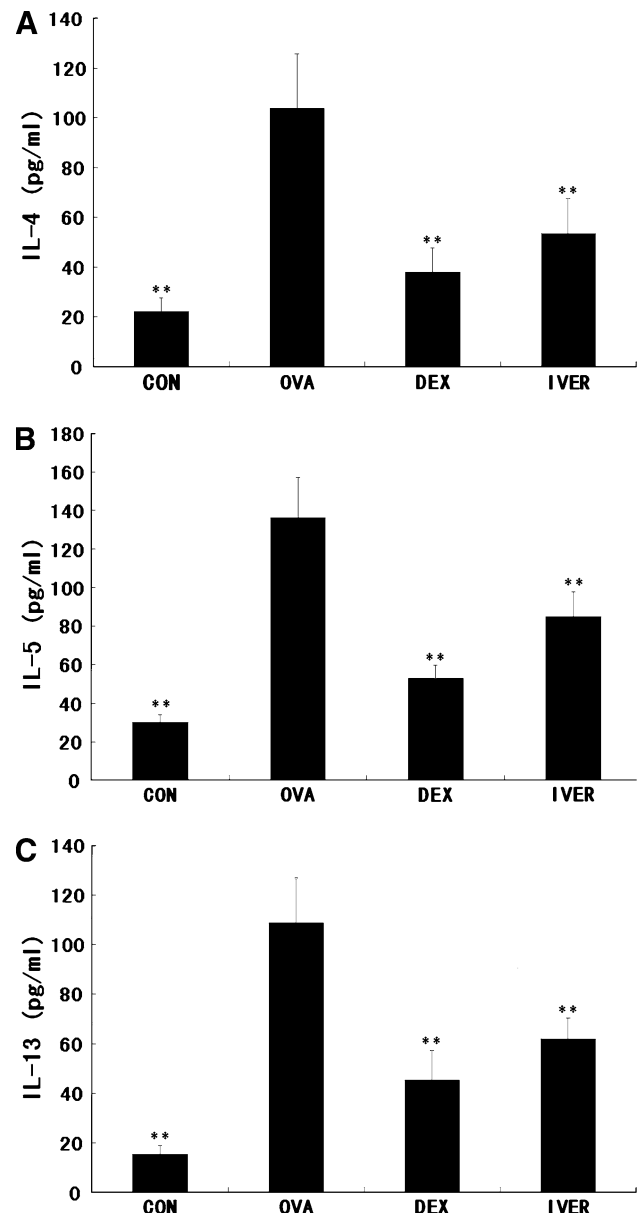


Fig. 1 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on IL-4 (a), IL-5 (b) and IL-13 (c) level in BAL fluid. Levels of Th2 cytokines were analyzed using ELISA (*n* = 10 per group). The values represent the mean ± SEM of three independent experiments. ***P* < 0.01 versus OVA

and IL-13 in OVA-challenged mice were 103.87 ± 21.68 pg/mL, 136.21 ± 20.71 pg/mL and 108.73 ± 20.93 pg/mL, respectively. These elevated IL-4, IL-5 and IL-13 concentrations were markedly reduced by ivermectin and dexamethasone treatment ($P < 0.01$).

Effect of ivermectin on the release of anti-OVA IgE and IgG1 in the serum

To determine the effect of ivermectin on OVA-specific IgE and IgG1 release in OVA-challenged mice, ELISAs were performed on serum samples 24 h after the final challenge. As shown in Fig. 2a, OVA-specific IgE production in serum was dramatically elevated in OVA-challenged mice compared with PBS-challenged mice. OVA-challenged mice pretreated with ivermectin and dexamethasone had reduced OVA-specific IgE levels compared with untreated, OVA-challenged mice ($P < 0.01$). In addition, OVA-challenged mice had elevated serum levels of OVA-specific IgG1 as compared with PBS-challenged mice. Ivermectin and dexamethasone at 2 mg/kg strongly suppressed OVA-specific IgG1 levels (Fig. 2b).

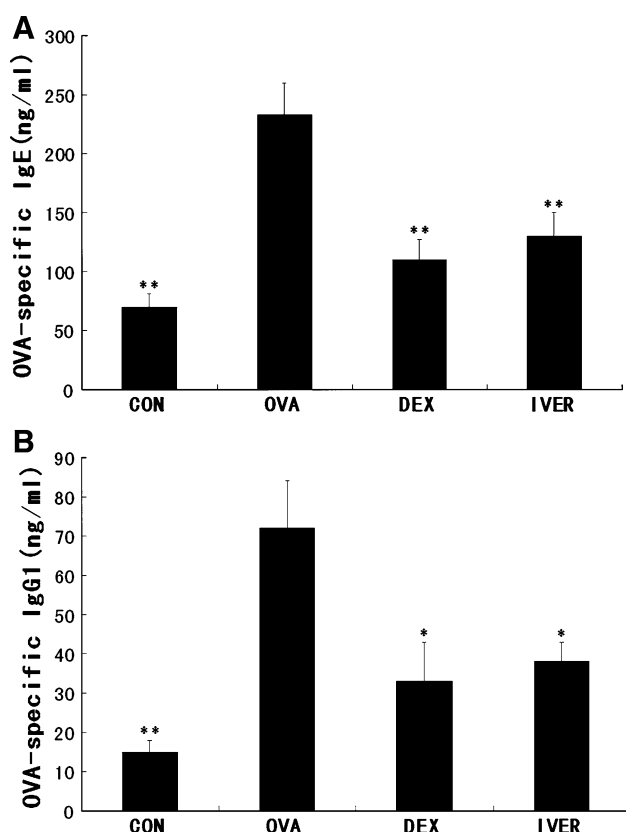


Fig. 2 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on OVA-specific IgE (a) and IgG1 (b) in serum. OVA-specific IgE and IgG1 levels in serum were measured by ELISA ($n = 10$ per group). Values shown are the mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ versus OVA

Effect of ivermectin on infiltration of inflammatory cells in BALF

We examined the effects of ivermectin on the infiltration of inflammatory cells such as eosinophils, neutrophils, lymphocytes and macrophages into the BALF of OVA-challenged mice. OVA challenge induced significantly greater numbers of eosinophils, neutrophils, lymphocytes and macrophages than PBS challenge. Ivermectin and dexamethasone significantly inhibited inflammatory cell infiltration into the BALF (Fig. 3).

Effect of ivermectin on inflammatory cell infiltration into lung tissue

To evaluate the anti-inflammatory effects of ivermectin, histopathological studies were performed. Figure 4a shows a photomicrograph of normal lung parenchyma from PBS-challenged negative control mice, which lacks inflammatory cell infiltration. In contrast, inflammatory cell infiltration was observed in the peribronchial and perivascular areas of the lungs of OVA-challenged mice (Fig. 4b). Treatment with either dexamethasone or ivermectin diminished inflammatory cell infiltration into the peribronchial and perivascular areas of the lungs of OVA-challenged mice (Fig. 4c, d).

Effect of ivermectin on airway goblet cell hyperplasia and mucus production

To determine the effect of ivermectin on goblet cell hyperplasia and mucus production by lung tissues, we stained lung sections with PAS. Histological analysis showed no signs of mucus or PAS-positive, mucus-producing cells in the airways of mice in the PBS-challenged negative control group

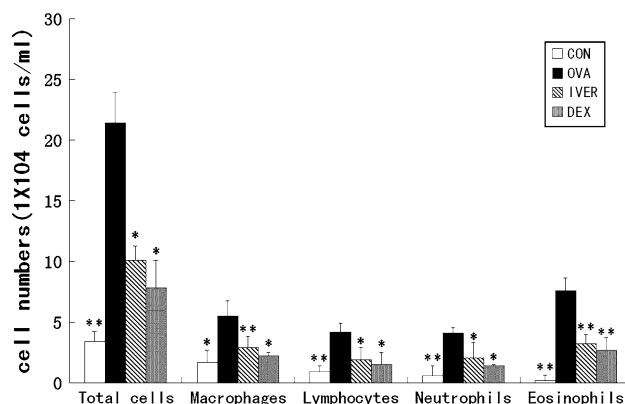
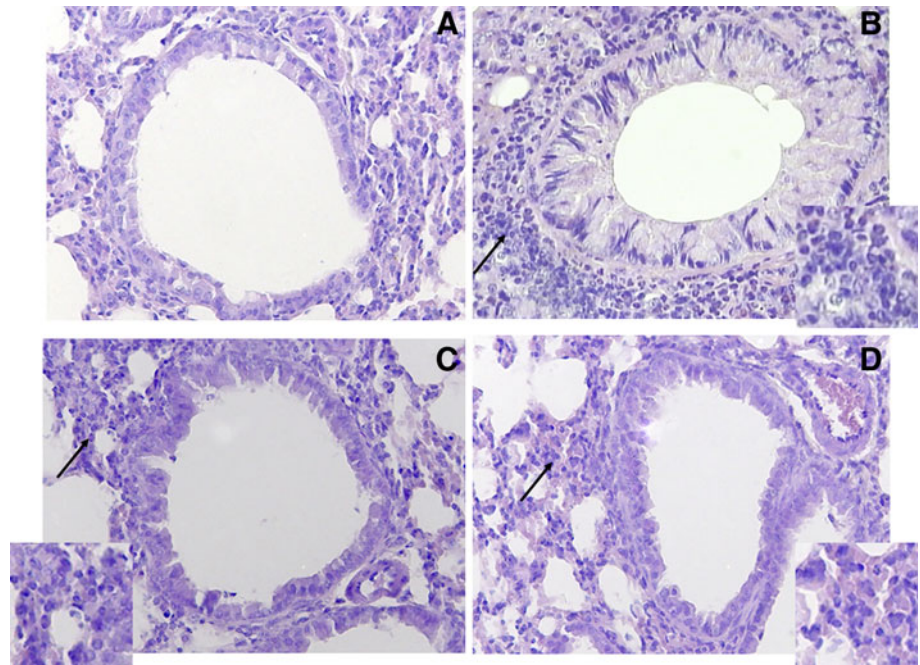


Fig. 3 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on the recruitment of inflammatory cells in BALF. Cell counts were measured as described in “Materials and methods” ($n = 10$ per group). The values represent the mean $\pm$ SEM of three independent experiments. * $P < 0.05$, ** $P < 0.01$ versus OVA

Fig. 4 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on the release of inflammatory cells in lung tissue. Representative haematoxylin–eosin stained sections of lung from: **a** PBS-challenged mice; **b** OVA-challenged mice; **c** asthmatic mice treated with ivermectin (2 mg/kg); **d** asthmatic mice treated with dexamethasone (2 mg/kg). Arrows indicate eosinophilic infiltration. The panel is magnified $\times 400$



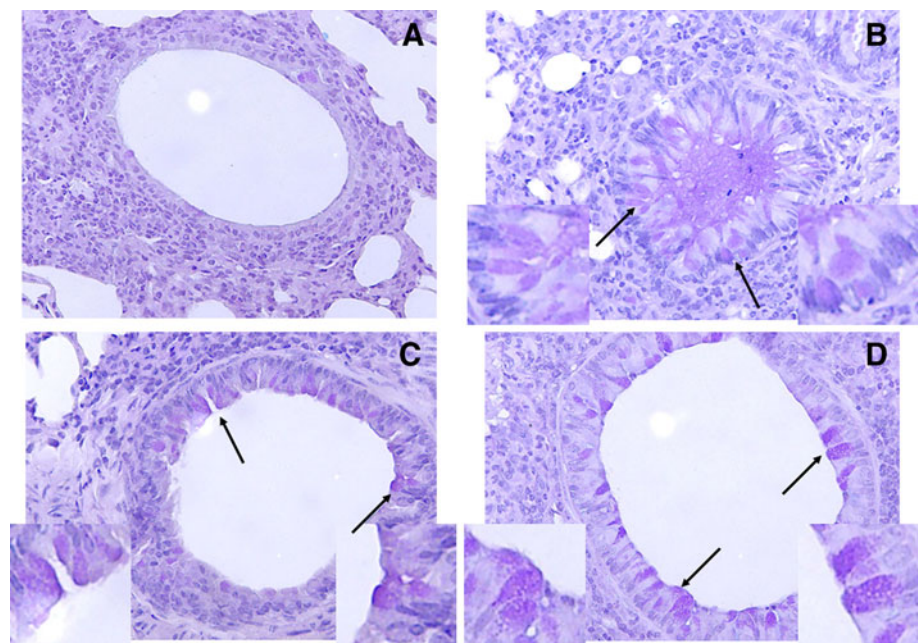
(Fig. 5a). PAS-positive, mucus-producing cells and epithelial cell disruption in the airways were markedly attenuated by dexamethasone and ivermectin treatment (Fig. 5c, d).

Effect of ivermectin on AHR

To examine the inhibitory effect of ivermectin on AHR, we measured RL and Cdyn in response to increasing doses of methacholine administered to mechanically ventilated mice.

RL levels were dramatically elevated in OVA-challenged mice compared to levels in PBS-challenged negative control mice, and treatment with ivermectin or dexamethasone reduced RL levels in OVA-challenged mice (Fig. 6a). Cdyn was dramatically diminished in OVA-challenged mice compared to PBS-challenged negative control mice. However, treatment with ivermectin or dexamethasone significantly reduced Cdyn levels in OVA-challenged mice (Fig. 6b).

Fig. 5 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on airway goblet cell hyperplasia and mucus production. Representative periodic acid-Schiff stained sections of lung from: **a** PBS-challenged mice; **b** OVA-challenged mice; **c** asthmatic mice treated with ivermectin (2 mg/kg); **d** asthmatic mice treated with dexamethasone (2 mg/kg). Arrows indicate mucus within the respiratory epithelial goblet cells. The panel is magnified $\times 400$



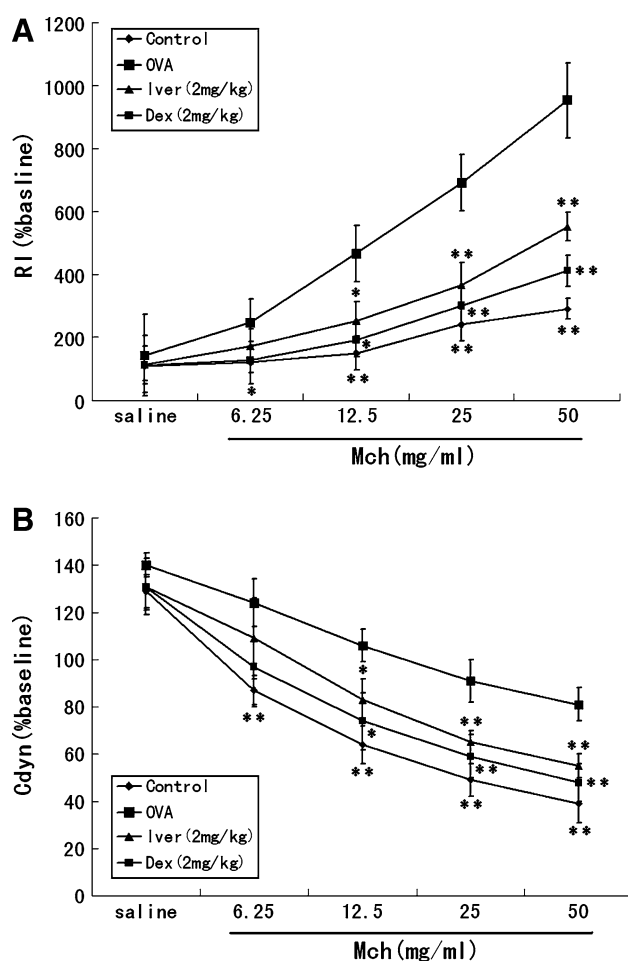


Fig. 6 Effect of ivermectin (2 mg/kg p.o.) and dexamethasone (2 mg/kg i.p.) on airway hyperresponsiveness (AHR) to inhaled methacholine (**a** RL level and **b** Cdyn level). AHR was measured as in “Materials and methods” ($n = 10$ per group). Values are expressed as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$ versus OVA

Discussion

Allergic asthma is one of the most common diseases encountered in the clinic, and mortality from this disease has increased worldwide over the past 20 years [18]. Dexamethasone is an effective drug for treating allergic asthma, but numerous side effects, including weight gain, upset stomach, osteoporosis, psychiatric disturbances, glaucoma, and cataracts occur with long-term treatment. Therefore, alternative drugs to replace dexamethasone are necessary. In the present study, we demonstrated that ivermectin not only effectively suppresses allergic inflammation, as characterised by infiltration of inflammatory cells into regions around the airways, increased number of goblet cells within the airways and elevation of Th2 cytokines in the BALF, but also inhibits AHR in a mouse model of asthma. These effects are likely attributed to ivermectin-mediated decreases in OVA-specific IgE and

IgG1 and BALF cytokines. This study is the first to provide experimental evidence demonstrating that ivermectin has anti-inflammatory properties in a non-infectious mouse model of allergic airway inflammation.

Cytokines are the chemical means by which cells communicate with each other. Allergic asthma is a T cell-dependent disease that is characterised by overproduction of Th2 cytokines [19–21]. These Th2 cytokines induce inflammatory responses, such as airway infiltration and eosinophil activation, IgE production and mucus secretion [22–24]. In this study, we observed that IL-4 and IL-13 levels in the BALF, which are elevated in OVA-challenged mice, as well as OVA-specific IgE levels, decreased following ivermectin treatment. IL-4 may be responsible for the production of IgE and IL-13, and IL-13 plays a pivotal role in causing asthma symptoms such as airway inflammation, goblet cell hyperplasia and airway luminal narrowing [25]. Consistent with our study, ivermectin not only reduced IL-4 and IL-13 levels but also decreased the number of PAS-positive, mucus-producing cells in the airways. Collectively, these data suggest that the anti-inflammatory effects of ivermectin on allergic asthma may be attributed to its inhibition of IL-4 production.

In mice, IgG1 is an anaphylactic antibody that is co-regulated with IgE via the IL-4/Th2 pathway, which may explain the use of IgG1 to assess immunogenicity or adjuvant effects in several murine systems [26, 27]. Our data also show that ivermectin inhibited OVA-specific IgG1 production. These results support the hypothesis that ivermectin treatment has anti-allergic effect through the decrease in IgG1 production.

Eosinophilia is a hallmark of allergic and parasitic diseases. Eosinophils release four principal cationic proteins (major basic protein, eosinophil-derived neurotoxin, eosinophil cationic protein and eosinophil peroxidase), each of which can damage lung tissue [28]. IL-5 is important for eosinophil growth, survival and function. Previous studies have shown that Th2 cell-derived IL-5 stimulates the maturation of eosinophils from CD34+/IL-5Ra+ precursor cells and the mobilisation of mature cells from the bone marrow into the blood [29–32]. Our results showed that ivermectin strongly inhibited IL-5 production induced by OVA challenge to levels comparable to those of PBS-challenged negative control mice. Furthermore, ivermectin diminished eosinophil numbers in the BALF of OVA-challenged mice. In addition to eosinophils, neutrophils, lymphocytes and macrophages are also important mediators of the inflammatory response through their production of cytokines. Our results showed that OVA challenge increased neutrophil, lymphocyte, and macrophage infiltration into the BALF, and ivermectin treatment reduced the recruitment of these cells.

AHR, a useful marker of airway abnormality in asthma, has been used in epidemiological studies and is predictive of the subsequent course of disease [33, 34]. Our data demonstrated that ivermectin inhibited OVA-induced AHR caused by inhaled methacholine. IL-13 has been established as an important mediator of AHR. Transgenic mice with high pulmonary expression of IL-13 exhibit extensive AHR and remodelling [35]. Thus, ivermectin-mediated inhibition of AHR might be attributed to the reduction in IL-13 levels.

NF- κ B regulates the expression of many cytokines and mediators that are involved in the inflammatory response [36]. MAPKs play critical roles in the regulation of cell growth and differentiation and in the control of cellular responses to cytokines and stress [37]. Previous studies have suggested that ivermectin-mediated inhibition of inflammatory cytokine production may occur through the suppression of the MAPK and NF- κ B pathways [14, 15], and we speculate that ivermectin suppresses inflammation in our asthma model via a similar mechanism.

In conclusion, intragastric administration of ivermectin at a dose of 2 mg/kg significantly inhibits important features of allergic asthma. Given that ivermectin can be used at low doses and causes mild side effects, this drug may be effective as a therapy for patients with allergic airway inflammation such as asthma.

References

- Elias JA, Lee CG, Zheng T, Ma B, Homer RJ, Zhu Z. New insights into the pathogenesis of asthma. *J Clin Invest*. 2003;111:291–7.
- Wills-Karp M. Immunologic basis of antigen-induced airway hyperresponsiveness. *Annu Rev Immunol*. 1999;17:255–81.
- Anderson GP, Coyle AJ. Th2 and 'Th2-like' cells in allergy and asthma: pharmacological perspectives. *Trends Pharmacol Sci*. 1994;15:324–32.
- Braman SS. The global burden of asthma. *Chest*. 2006;130:4–12.
- Bryskier A, Agouridas C, Chantot JF. New medical targets for macrolides. *Exp Opin Invest Drugs*. 1994;3:405–10.
- Tarayre JP, Aliaga M, Barbara M, Villanova G, Ballester R, Tisne-Versailles J, et al. Cutaneously applied erythromycin base reduces various types of inflammatory reactions in mouse ear. *Int J Tiss Reac*. 1987;4:77–85.
- Mikasa K, Kita E, Sawaki M, Kunitatsu M, Hamada K, Konishi M, et al. The anti-inflammatory effect of erythromycin in zymosan-induced peritonitis of mice. *J Antimicrob Chemother*. 1992;30:339–48.
- Agen C, Danesi R, Blandizzi C, Costa M, Stacchini B, Favini P, et al. Macrolide antibiotics as antiinflammatory agents: roxithromycin in an unexpected role. *Agents Actions*. 1993;38:85–90.
- Giamarellos-Bourboulis EJ. Macrolides beyond the conventional antimicrobials: a class of potent immunomodulators. *Int J Antimicrob Agents*. 2008;31:12–20.
- Hrvčić B, Bošnjak B, Bosnar M, Ferencić Ž, Glojnarčić I, Eraković Haber V. Clarithromycin suppresses airway hyperresponsiveness and inflammation in mouse models of asthma. *Eur J Pharmacol*. 2009;15:236–43.
- Beigelman A, Gunsten S, Mikols CL, Vidavsky I, Cannon CL, Brody SL, et al. Azithromycin attenuates airway inflammation in a noninfectious mouse model of allergic asthma. *Chest*. 2009;136(2):498–506.
- Caumes E, Danis M. New indications of ivermectin. *Rev Med Interne*. 2001;22:379–84.
- Stankiewicz M, Cabaj W, Jonas WE, Moore LG, Millar K, Ng Chie W. Influence of ivermectin on cellular and humoral immune responses of lambs. *Vet Immunol Immunopathol*. 1995;44:347–58.
- Zhang X, Song Y, Xiong H, Ci X, Li H, Yu L, et al. Inhibitory effects of ivermectin on nitric oxide and prostaglandin E₂ production in LPS-stimulated RAW 264.7 macrophages. *Int J Immunopharmacol*. 2009;9:354–9.
- Zhang X, Song Y, Ci X, An N, Ju Y, Li H, et al. Ivermectin inhibits LPS-induced production of inflammatory cytokines and improves LPS-induced survival in mice. *Inflamm Res*. 2008;57:524–9.
- Van Rijt LS, Kuipers H, Vos N, Hijdra D, Hoogsteden HC, Lambrecht BN. A rapid flow cytometric method for determining the cellular composition of bronchoalveolar lavage fluid cells in mouse models of asthma. *J Immunol Methods*. 2004;288:111–21.
- Zhou J, Kang Z, Xie Q, Liu C, Lou S, Chen Y, et al. Rapid nongenomic effects of glucocorticoids on allergic asthma reaction in the guinea pig. *J Endocrinol*. 2003;177:R1–4.
- Pene J, Rousset F, Briere F, Chretien I, Bonnefoy JY, Spits H, et al. IgE production by normal human lymphocytes is induced by interleukin 4 and suppressed by interferons α , γ and prostaglandin E₂. *Proc Natl Acad Sci USA*. 1988;85:6880–4.
- Umetsu DT, DeKruyff RH. TH1 and TH2 CD4⁺ cells in human allergic diseases. *J Allergy Clin Immunol*. 1997;100:1–6.
- Corry DB, Grunig G, Hadeiba H, Kurup VP, Warnock ML, Sheppard D, et al. Requirements for allergen-induced airway hyperactivity in T and B cell-deficient mice. *Mol Med*. 1998;4:344–55.
- Curtis JL, Byrd PK, Warnock ML, Kaltreider HB. Requirement of CD4-positive T cells for cellular recruitment to the lungs of mice in response to a particulate intratracheal antigen. *J Clin Invest*. 1991;88:1244–54.
- Kips JC. Cytokines in asthma. *Eur Respir J*. 2001;18:24–33.
- Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev*. 2004;202:175–90.
- Ngoc LP, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy, and asthma. *Curr Opin Allergy Clin Immunol*. 2005;5:161–6.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B. Interleukin-13: central mediator of allergic asthma. *Science*. 1998;282:2258–61.
- Purkerson J, Isakson P. A two-signal model for regulation of immunoglobulin isotype switching. *FASEB J*. 1992;6:3245–52.
- Kimber I, Stone S, Dearman RJ. Assessment of the inherent allergenic potential of proteins in mice. *Environ Health Perspect*. 2003;111:227–31.
- Abu-Ghazaleh RI, Kita H, Gleich GJ. Eosinophil activation and function in health and disease. *Immunol Ser*. 1992;57:137–67.
- Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D. Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science*. 1988;245:308–10.
- Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, et al. IL-5-deficient mice have a developmental defect in CD5⁺ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity*. 1996;4:15–24.
- Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, et al. Interleukin-5 expression in the lung epithelium

- of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J Exp Med.* 1997;185:2143–56.
32. Foster PS, Martinez-Moczygemba M, Huston DP, Corry DB. Interleukins-4, -5, and -13: emerging therapeutic targets in allergic disease. *Pharmacol Ther.* 2002;94:253–64.
 33. Toelle BG, Peat JK, Salome CM. Toward a definition of asthma for epidemiology. *Am Rev Respir Dis.* 1992;146:633–7.
 34. Peat J, Toelle B, Salome C. Predictive nature of bronchial responsiveness and respiratory symptoms in a one year cohort study of Sydney schoolchildren. *Eur Respir J.* 1993;6:662–9.
 35. Zhu Z, Homer RJ, Wang Z. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest.* 1999;103:779–88.
 36. Flohé L, Brigelius-Flohé R, Saliou C, Traber MG, Packer L. Redox regulation of NF-kappa B activation. *Free Radic Biol Med.* 1997;22:1115–26.
 37. Vanden Berghe W, Plaisance S, Boone E, De Bosscher K, Schmitz ML, Fiers W. p38 and extracellular signal-regulated kinase mitogen-activated protein kinase pathways are required for nuclear factor-kappaB p65 transactivation mediated by tumor necrosis factor. *J Biol Chem.* 1998;273:3285–90.