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Blockade of airway hyperresponsiveness and inflammation in a murine model of asthma by a prodrug of cysteine, L-2-oxothiazolidine-4-carboxylic acid

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ABSTRACT

Oxidative stress plays an important role in the pathogenesis of bronchial asthma. An excess production of reactive oxygen species (ROS) and defective endogenous antioxidant defense mechanisms may be present in asthma. Reduced glutathione (GSH) is one of the most important reducing agents against oxidant free radicals. A reducing agent, L-2-oxothiazolidine-4-carboxylic acid (OTC), a prodrug of cysteine, increases intracellular GSH. We have used a mouse model for asthma to determine effects of OTC on allergen-induced bronchial inflammation and airway hyper-responsiveness. The administration of OTC reduced bronchial inflammation and airway hyper-responsiveness. ROS generation in bronchoalveolar lavage fluids was increased by ovalbumin (OVA) inhalation, but this increase was diminished by administration of OTC. The increased IL-4, IL-5, IL-13, and eosinophil cationic protein levels in lungs after OVA inhalation were significantly reduced by the administration of OTC. In addition, the increased expression of ICAM-1, VCAM-1, RANTES, and eotaxin in lungs after OVA inhalation was significantly reduced by the administration of OTC. We also showed that the increased NF- κ B levels in nuclear protein extracts of lung tissues at 72 h after OVA inhalation were decreased by the administration of OTC. These findings suggest that OTC may reduce airway inflammation and hyper-responsiveness through regulation of NF- κ B activity.

Key words: eosinophilia • NF- κ B • oxidative stress • signal transduction

Asthma is a chronic inflammatory disorder of the airways characterized by an associated increase in airway responsiveness (1). Oxidative stress is caused by a large variety of free oxygen radicals (e.g., superoxide and hydroxyl radicals) known as reactive oxygen species (ROS). Accumulating evidence has suggested that ROS play a crucial role in the pathogenesis of airway inflammation (2–4). The inflammatory cells recruited to the asthmatic airways have a capability of producing ROS. Evidence for increase in oxidative stress in asthma

is further provided by the finding of defective endogenous antioxidant capacity in asthmatic patients (4). Therefore, antioxidant treatment of asthma has long been a subject of therapeutic strategy.

Glutathione (GSH) is synthesized from cysteine and is a vital intracellular and extracellular protective antioxidant against oxidative stress. Alterations in alveolar and lung GSH metabolism are widely recognized as a central feature of many inflammatory lung diseases such as asthma. Cysteine derivatives, N-acetylcysteine, and carbocysteine, which may act as antioxidants, have been shown to exhibit anti-inflammatory and antihyperreactivity in animal models of asthma (5, 6). However, benefit of these derivatives in asthma is controversial. A thiazolidine derivative, L-2-oxothiazolidine-4-carboxylic acid (OTC) is a prodrug of cysteine that raises the plasma concentrations of cysteine and GSH (7–9). This compound is readily transportable into cells and converted to L-cysteine by a ubiquitous intracellular enzyme, 5-oxoprolinase (10). Several studies have also demonstrated that OTC repletes cellular glutathione stores when glutathione is acutely depleted (11, 12) and is more effective than N-acetylcysteine in repleting intracellular GSH stores (13, 14). However, there are few data on the influence and the molecular basis of OTC on allergen-induced bronchial inflammation and airway hyper-responsiveness.

In the present study, we used a murine model of asthma to evaluate the effect of OTC on hyper-responsiveness and inflammation of the airways.

METHODS

Animals and experimental protocol

Female BALB/c mice, 8–10 weeks of age and free of murine-specific pathogens, were obtained from the Korean Research Institute of Chemistry Technology (Daejeon, Korea), were housed throughout the experiments in a laminar flow cabinet, and were maintained on standard laboratory chow ad libitum. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of the Chonbuk National University Medical School. Mice were sensitized on days 1 and 14 by intraperitoneal injection of 20 µg ovalbumin (OVA) (Sigma-Aldrich, St. Louis, MO) emulsified in 1 mg of aluminum hydroxide (Pierce Chemical Co., Rockford, IL) in a total volume of 200 µl, as described previously (15) ([Fig. 1](#)). On days 21, 22, and 23 after the initial sensitization, the mice were challenged for 30 min with an aerosol of 3% (w/v) OVA in saline (or with saline as a control) using an ultrasonic nebulizer (NE-U12, Omron, Japan). Bronchoalveolar lavage (BAL) was performed at 72 h after the last challenge. At the time of lavage, the mice (6 mice in each group) were killed with an overdose of sodium pentobarbitone (pentobarbital sodium, 100 mg/kg of body weight, administered intraperitoneally). The chest cavity was exposed to allow for expansion, after which the trachea was carefully intubated and the catheter secured with ligatures. Prewarmed 0.9% NaCl solution was slowly infused into the lungs and withdrawn. The aliquots were pooled and then kept at 4°C. A part of each pool was then centrifuged, and the supernatants were kept at –70°C until use. Total cell numbers were counted with a hemocytometer. Smears of BAL cells were prepared with a cytospin. The smears were stained with Diff-Quik solution (Dade Diagnostics of P. R. Inc. Aguada, PR) in order to examine the cell differentials. Two independent investigators, not associated with this study, counted the cells using a microscope. Approximately 400 cells were counted in each of four different random

locations. Interinvestigator variation was <5%. The mean number from the two investigators was used to estimate the cell differentials.

Administration of OTC

OTC solution (40, 80, or 160 mg/kg body weight/day, Sigma-Aldrich) was freshly prepared as described elsewhere (16) and administered intraperitoneally 4 times at 24 h intervals on days 21–24, beginning 1 h before the first challenge ([Fig. 1](#)).

Measurement of intracellular reactive oxygen species

ROS were measured by a method described previously with modification (17–19). BAL fluids were washed with phosphate-buffered saline (PBS). To measure intracellular ROS, cells were incubated for 10 min at room temperature with PBS containing 3.3 μ M 2',7'-dichlorofluorescein diacetate (DCFDA) (Molecular Probes, Eugene, OR), to label intracellular ROS. The cells were then immediately observed under fluorescence microscope (Carl Zeiss, Inc., Thornwood, NY) and fluorescence-activated cell sorting (FACS) analysis (Partec, Münster, Germany).

Measurement of cytokines and RANTES

Levels of IL-4, IL-5, IL-13, and RANTES were quantified in the supernatants of BAL fluids by enzyme immunoassays according to the manufacturer's protocol (IL-4 and IL-5; Endogen, Inc., Woburn, MA, IL-13, and RANTES; R&D Systems, Inc., Minneapolis, MN). Sensitivities for IL-4, IL-5, IL-13, and RANTES assays were 5, 5, 1.5, and 2 pg/ml, respectively.

Measurement of eosinophil cationic protein (ECP)

ECP was measured in the supernatants of BAL fluids. Levels of ECP were quantified by fluoroenzyme immunoassay, according to the manufacturer's protocol (UniCAP ECP; Pharmacia and Upjohn Diagnostics AB, Uppsala, Sweden). Sensitivity for the assay was 2 ng/ml.

Determination of airway responsiveness to methacholine

Airway responsiveness was measured in mice 3 days after the last challenge in an unrestrained conscious state, as described previously (15, 20, 21). Mice were placed in a barometric plethysmographic chamber (All Medicus Co., Seoul, Korea) and baseline readings were taken and averaged for 3 min. Aerosolized methacholine in increasing concentrations (2.5 to 50 mg/ml) were nebulized through an inlet of the main chamber for 3 min. Readings were taken and averaged for 3 min after each nebulization. Enhanced pause (Penh), calculated as $(\text{expiratory time/relaxation time}-1) \times (\text{peak expiratory flow/peak inspiratory flow})$, according to the manufacturers' protocol, is a dimensionless value that represents a function of the proportion of maximal expiratory to maximal inspiratory box pressure signals and a function of the timing of expiration. Penh was used as a measure of airway responsiveness to methacholine. Results were expressed as the percentage increase of Penh following challenge with each concentration of methacholine, where the baseline Penh (after saline challenge) was expressed as 100%. Penh values averaged for 3 min after each nebulization were evaluated.

Histology

At 72 h after the last challenge, lungs were removed from the mice after sacrifice. Before the lungs were removed, the lungs and trachea were filled intratracheally with a fixative (0.8% formalin, 4% acetic acid) using a ligature around the trachea. Lung tissues were fixed with 10% (v/v) neutral buffered formalin. The specimens were dehydrated and embedded in paraffin. For histological examination, 4 μ m sections of fixed embedded tissues were cut on a Leica model 2165 rotary microtome (Leica, Nussloch, Germany), placed on glass slides, deparaffinized, and stained sequentially with hematoxylin 2 and eosin-Y (Richard-Allan Scientific, Kalamazoo, MI). Inflammation score was graded by three independent investigators who were not associated with this study. The degree of peribronchial and perivascular inflammation was evaluated on a subjective scale of 0 to 3, as described elsewhere (22). A value of 0 was adjudged when no inflammation was detectable, a value of 1 for occasional cuffing with inflammatory cells, a value of 2 for most bronchi or vessels surrounded by thin layer (one to five cells) of inflammatory cells, and a value of 3 when most bronchi or vessels were surrounded by a thick layer (more than five cells) of inflammatory cells.

Western blot analysis

Lung tissues were homogenized in the presence of protease inhibitors to obtain extracts of proteins. Protein concentrations were determined using the Bradford reagent (Bio-Rad, Hercules, CA). Samples (30 μ g of protein per lane) were loaded on a 12% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech, Piscataway, NJ) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% nonfat dry milk in TBST buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 1 h, and the blots were then incubated with antibody against IL-4 (Serotec Ltd., Oxford, United Kingdom), IL-5 (Santa Cruz Biotechnology Inc., Santa Cruz, CA), or IL-13 (R&D Systems, Inc.), overnight at 4°C. Anti-rabbit horseradish peroxidase conjugated IgG was used to detect binding of antibodies. The membranes were stripped and reblotted with anti-actin antibody (Sigma-Aldrich) to verify equal loading of protein in each lane. The binding of the specific antibodies was visualized by exposing to photographic film after treating with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech).

Cytosolic and nuclear protein extractions for analysis of NF- κ B

Lungs were removed and homogenized in 2 volumes of buffer A (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 10% glycerol, 0.5 mM DTT, 5 mM MgCl₂, and 1 mM PMSF) containing protease inhibitor cocktails. These homogenates were centrifuged at 1000 $\times g$ for 15 min at 4°C. The supernatant fraction was incubated on ice for 10 min and centrifuged at 100,000 $\times g$ for 1 h at 4°C to obtain cytosolic protein extracts. The pellets were washed twice in buffer A and resuspended in buffer B (1.3 M sucrose, 1.0 mM MgCl₂, and 10 mM potassium phosphate buffer, pH 6.8) and pelleted at 1000 $\times g$ for 15 min. The pellets were suspended in buffer B with a final sucrose concentration of 2.2 M and centrifuged at 100,000 $\times g$ for 1 h. The resulting nuclear pellets were washed once with a solution containing 0.25 M sucrose, 0.5 mM MgCl₂, and 20 mM Tris-HCl, pH 7.2, and centrifuged at 1000 $\times g$ for 10 min. The pellets were solubilized with a solution containing 50 mM Tris-HCl (pH 7.2), 0.3 M sucrose, 150 mM NaCl, 2 mM EDTA, 20% glycerol, 2% Triton X-100, 2 mM PMSF, and protease inhibitor cocktails. The

mixture was kept on ice for 1 h with gentle stirring and centrifuged at $12,000 \times g$ for 30 min. The resulting supernatant was used as soluble nuclear proteins.

For Western analysis, samples (30 μ g of protein per lane) were loaded on a 10% SDS-PAGE gel. After electrophoresis at 120 V for 90 min, separated proteins were transferred to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech,) by the wet transfer method (250 mA, 90 min). Nonspecific sites were blocked with 5% nonfat dry milk in TBST buffer (25 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 1 h, and the blots were incubated with antibody against NF- κ B p65 (Upstate Biotech, Lake Placid, NY) overnight at 4°C. Anti-rabbit horseradish peroxidase conjugated IgG was used to detect binding of antibodies. The binding of the specific antibodies was visualized by exposing to photographic film after treating with enhanced chemiluminescence system reagents (Amersham Pharmacia Biotech).

Electrophoretic-mobility-shift assay

Electrophoretic-mobility-shift assay was performed as described previously (23). As a probe for the gel retardation assay, an oligonucleotide containing the Ig κ -chain binding site (κ B, 5'-CCGGTTAACAGAGGGG-GCTTTCGAG-3') was synthesized. The two complementary strands were annealed and labeled with [α - ^{32} P]dCTP. Labeled oligonucleotides (10,000 cpm), 10 μ g of nuclear extracts, and binding buffer (10 mM Tris-HCl (pH 7.6), 500 mM KCl, 10 mM EDTA, 50% glycerol, 100 ng of poly (dIdC), and 1 mM dithiothreitol) were incubated for 30 min at room temperature in a final volume of 20 μ l. The reaction mixture was analyzed by electrophoresis on a 5% polyacrylamide gel in Tris-borate/EDTA buffer. Specific binding was controlled by competition with a 50-fold excess of cold κ B or cAMP response element (CRE) oligonucleotide. For supershift assay, 1 μ g of specific supershifting antibodies against p50 or p65 components of NF- κ B (Santa Cruz Biotechnology, Inc.) were incubated with the nuclear extract on ice for 1 h before the addition of labeled oligonucleotide to the binding reaction.

RNA isolation and RT-PCR

Total RNA from lung tissues was isolated using a rapid extraction method (TRI-Reagent) as described previously (24). RNA was quantified by measuring absorption at 260 nm and was stored at -80°C before use. Total RNA (4 μ g) was reverse-transcribed to cDNA in a buffer containing 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 5 mM MgCl₂, 10 mM DTT; 0.5 μ g random hexanucleotide primers, 2.5 mM dNTP, 40 units RNase inhibitor, and 50 units/ μ l SuperScript II RT (Invitrogen, Carlsbad, CA), in a final volume of 20 μ l. This mixture was incubated for 50 min at 42°C and was finally digested with 2 units/ μ l *E. coli* RNase H for 20 min at 37°C . The first-strand cDNAs were used for PCR amplification of ICAM-1, VCAM-1, RANTES, eotaxin, or the housekeeping gene, GAPDH. PCR amplification was performed by mixing 3 μ l of the RT reaction mixture with 47 μ l of buffer containing 2.5 U of Taq DNA polymerase (Promega, Madison, WI) and 30 pmol of specific primer pairs for mouse cDNA of ICAM-1, VCAM-1, RANTES, eotaxin, or GAPDH, designed from published mouse gene sequences. The primers used were as follows: ICAM-1 (predicted length 624 bp) sense: 5'-CATCGGGGTGGTGAAGTCTGT-3', antisense: 5'-TGTGGGGGAAG- TGTGGTC-3'; VCAM-1 (predicted length 518 bp) sense: 5'-CAAGGGTGACCAGC- TCATGAA-3', antisense: 5'-TGTGCAGCCACCTGAGATCC-3'; RANTES (predicted length 294 bp) sense: 5'-GGTACCATGAAGATCTCTGCA-3', antisense: 5'-AAACCC-TCTATCCTAGCTCAT-3'; eotaxin (predicted length 262 bp) sense: 5'-AGAGGCTGAGATCCAAGCAG-3', antisense: 5'-CAGATCTCTTTGCCCAACCT-3', and GAPDH (predicted length

609 bp) sense: 5'-GCCATCAACGACCCCTTCATTGAC-3', antisense: 5'-ACGGAAGGCCATGCCAGTGAGCTT-3'. PCR reactions were performed in a thermocycler (GeneAmp® PCR System 2400, Foster City, CA) using the following reaction conditions; after an initial incubation for 2 min at 95°C, samples were subjected to 35 cycles of 1 min at 94°C, 2 min at 54°C (GAPDH), 55°C (eotaxin), 56°C (RANTES), 60°C (ICAM-1), or 63°C (VCAM-1) and 1 min at 72°C. A final extension step at 72°C for 10 min was performed. The RT-PCR products were electrophoretically fractionated on 2% agarose gels stained with ethidium bromide. DNA bands were visualized after exposure to UV light.

Densitometric analysis and statistics

All immunoreactive and phosphorylation signals were analyzed by densitometric scanning (LAS-3000; Fuji Film, Tokyo, Japan). Data were expressed as mean $\pm$ SEM. Statistical comparisons were performed using one-way ANOVA followed by the Fisher's test. Significant differences between groups were determined using the unpaired Student's *t* test. Statistical significance was set at $P < 0.05$.

RESULTS

Effect of OTC on ROS generation in BAL fluids

ROS generation in BAL fluids was increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 2](#)). The increased ROS generation was significantly reduced by the administration of OTC.

Effect of OTC on cellular changes in BAL fluids

Numbers of total cells, eosinophils, lymphocytes, and neutrophils in BAL fluids were increased significantly at 72 h after OVA inhalation compared with the numbers after saline inhalation ([Fig. 3A](#)). The increased numbers of these cells were significantly reduced by the administration of OTC.

Effect of OTC on pathological changes of OVA-induced asthma

Histological analyses revealed typical pathologic features of asthma in the OVA-exposed mice. Numerous inflammatory cells, including eosinophils infiltrated around the bronchioles ([Fig. 3C](#)) as compared with the control ([Fig. 3B](#)). Mice treated with OTC ([Fig. 3D–F](#)) showed marked reductions in the infiltration of inflammatory cells in the peribronchiolar and perivascular regions. The scores of peribronchial, perivascular, and total lung inflammation were increased significantly at 72 h after OVA inhalation compared with the scores after saline inhalation ([Fig. 3G](#)). The increased peribronchial, perivascular, and total lung inflammation were significantly reduced by the administration of OTC. These results suggest that OTC inhibits antigen-induced inflammation in the lungs, including the influx of eosinophils.

Effect of OTC on NF- κ B expression in lung tissues of OVA-sensitized and OVA-challenged mice

Western blot analysis revealed that levels of NF- κ B p65 protein in nuclear protein extracts from lung tissues were increased at 72 h after OVA inhalation compared with the levels in the control

mice ([Fig. 4A](#)). The increased NF- κ B p65 levels in nuclear protein extracts from lung tissues at 72 h after OVA inhalation were decreased by the administration of OTC. In contrast, levels of NF- κ B p65 protein in cytosolic protein extracts from lung tissues were decreased at 72 h after OVA inhalation compared with the levels in the control mice. The decreased NF- κ B p65 levels in cytosolic protein extracts from lung tissues at 72 h after OVA inhalation were increased by the administration of OTC.

Electrophoretic-mobility-shift assay revealed that NF- κ B-DNA binding in nuclear extracts from lung tissues was increased at 72 h after OVA inhalation compared with the levels in the control mice ([Fig. 4B](#)). The increased NF- κ B-DNA binding in nuclear extracts from lung tissues at 72 h after OVA inhalation was decreased by the administration of OTC. Supershift assay confirmed the presence of p50 and p65 subunits of NF- κ B.

Effect of OTC on levels of IL-4, IL-5, and IL-13 in lung tissues and BAL fluids of OVA-sensitized and -challenged mice

Western blot analysis revealed that levels of IL-4, IL-5, and IL-13 protein in lung tissues were increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 5A](#)). The increased levels of these cytokines were significantly reduced by the administration of OTC. Consistent with the results obtained from the Western blot analysis, enzyme immunoassays revealed that levels of IL-4, IL-5, and IL-13 in BAL fluids were also increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 5B](#)). The increased levels of these cytokines were also significantly reduced by the administration of OTC.

Effect of OTC on expression of ICAM-1, VCAM-1, RANTES, and eotaxin mRNA and protein levels in lung tissues and BAL fluids of OVA-sensitized and -challenged mice

RT PCR analysis revealed that expression of ICAM-1, VCAM-1, RANTES, and eotaxin mRNA in lung tissues was increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 6A, B](#); [Fig. 7A, B](#); and [Fig. 8A, B](#)). The increased mRNA expression of these adhesion molecules and chemokines was decreased by the administration of OTC. Western blot analysis revealed that levels of ICAM-1, VCAM-1, and eotaxin protein in the lung tissues were increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 6C, D](#) and [Fig. 8C, D](#)). The increased levels of ICAM-1, VCAM-1, and eotaxin protein were significantly reduced by the administration of OTC. In addition, enzyme immunoassays revealed that levels of RANTES in BAL fluids were also increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 7C](#)). The increased RANTES levels were significantly reduced by the administration of OTC.

Effect of OTC on levels ECP in BAL fluids of OVA-sensitized and -challenged mice

Fluoroenzyme immunoassays revealed that levels of ECP in BAL fluids were increased significantly at 72 h after OVA inhalation compared with the levels after saline inhalation ([Fig. 9](#)). The increased ECP levels were significantly reduced by the administration of OTC.

Effect of OTC on airway hyper-responsiveness

Airway responsiveness was assessed as a percent increase of Penh in response to increasing doses of methacholine. In OVA-sensitized and -challenged mice, the dose-response curve of percent Penh shifted to the left compared with that of control mice ([Fig. 10](#)). In addition, the percent Penh produced by methacholine administration (at doses from 2.5 mg/ml to 50 mg/ml) increased significantly in the OVA-sensitized and -challenged mice compared with the controls. OVA-sensitized and -challenged mice treated with OTC showed a dose-response curve of percent Penh that shifted to the right compared with that of untreated mice. The shift was dose-dependent. These results indicate that OTC treatment reduces OVA-induced airway hyper-responsiveness.

DISCUSSION

Oxidative stress plays an important role in the pathogenesis of bronchial asthma (4). Our present study with the OVA-induced model of asthma has revealed that ROS production in cells from BAL fluids and administration of a prodrug of cysteine, OTC, which is known as a precursor of GSH biosynthesis, reduces the eosinophilic inflammation and airway hyper-responsiveness. Our results also indicate that ROS up-regulate a transcription factor NF- κ B activity and that OTC down-regulates NF- κ B activity.

ROS released by eosinophils and other leukocytes infiltrating the airways play an important role in the airway tissue injury observed in asthma (4). Previous reports have shown that the release of ROS is increased in the airways of OVA-challenged animals (25, 26). In this study, we have found that generation of ROS in BAL fluids is significantly increased after allergen challenge in OVA-induced asthma model and that administration of OTC reduced the generation of ROS. Although the lung has a well-developed antioxidant system (27, 28), overproduction of ROS or depression of the protective system results in epithelial cell damage, cell shedding, and bronchial hyper-reactivity (29, 30). Studies with animal models have indicated that ROS contribute to airway hyper-responsiveness by increasing vagal tone resulting from damage of oxidant sensitive β -adrenergic receptors as well as decreasing mucociliary clearance (31, 32). These findings suggest that ROS induce and maintain pathogenesis of asthma.

The molecular basis of ROS-mediated induction of asthma has not been clearly defined. A recent study has indicated that development of oxidant/antioxidant imbalance in asthma leads to activation of redox-sensitive transcription factor NF- κ B (33). NF- κ B is present in most cell types and is known to play a critical role in immune and inflammatory responses, including asthma (34–39). Consistent with these observations, NF- κ B protein level in nuclear protein extracts from lung tissues is substantially increased in the OVA-induced model of asthma used for the present study. It is known that activation of this transcription factor induces a variety of inflammatory genes that are abnormally expressed in asthma. These genes include cytokines (e.g., IL-4, IL-5, IL-9, IL-15, and TNF- α), chemokines (e.g., RANTES, eotaxin, and monocyte chemoattractant protein-3), and adhesion molecules (e.g., ICAM-1 and VCAM-1) (24, 39–41). We have also assessed whether these genes are up-regulated in the OVA-induced asthma model. As expected, expression of adhesion molecules (ICAM-1 and VCAM-1), chemokines (RANTES and eotaxin), and cytokines (IL-4, IL-5, and IL-13) was increased significantly after allergen challenge in a murine model of asthma. Administration of OTC results in significant reduction of NF- κ B translocation into nucleus and of expression of these adhesion molecules, chemokines, and

cytokines. These results indicate that OTC inhibits NF- κ B activity by preventing translocation of this transcription factor into a nucleus that is induced by ROS. ROS have been directly implicated as second messengers in the activation of NF- κ B, based upon the ability of oxidants to activate NF- κ B by oxidation of its cysteine-SH group or by ubiquitination and proteolysis (42–44). Inhibitory κ B (I κ B) has 5–7 conserved domains known as ankyrin repeats, each consisting of around 30 amino acids, forming a unit that is able to interact with NF- κ B subunits, thus masking the nuclear localization signals and preventing activation of NF- κ B and its translocation to the nucleus (44, 45). Antioxidants have been known to inhibit NF- κ B activation by preventing I κ B degradation in response to various stimuli (46–50). Therefore, we suggest that OTC inhibits NF- κ B signal transduction pathway by decreasing NF- κ B binding activity to the promoter regions of a large variety of genes that are considered to be involved in airway inflammation in asthma.

A significant amount of data showing an increase of oxidative stress in asthma and indicating a potential role of oxidant in pathogenesis of the diseases has been accumulated over years. On the basis of on these observations and to find a potential therapeutic strategy treating asthma, we have administered an antioxidant agent OTC to allergen-challenged mice. Administration of OTC results in a significant reduction of all pathophysiological symptoms examined. Our study provides evidence that oxidative stress is one of the important determinants of asthma and that antioxidant treatment such as administration of OTC, which is transportable into cells, may be a recommendable therapeutic strategy.

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REFERENCES

1. Bousquet, J., Jeffery, P. K., Busse, W. W., Johnson, M., and Vignola, A. M. (2000) Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit. Care Med.* **161**, 1720–1745
2. Rahman, I., Morrison, D., Donaldson, K., and MacNee, W. (1996) Systemic oxidative stress in asthma, COPD, and smokers. *Am. J. Respir. Crit. Care Med.* **154**, 1055–1060
3. MacNee, W. (2001) Oxidative stress and lung inflammation in airways disease. *Eur. J. Pharmacol.* **429**, 195–207
4. Dworski, R. (2000) Oxidant stress in asthma. *Thorax* **55**, S51–S53
5. Blesa, S., Cortijo, J., Martinez-Losa, M., Mata, M., Seda, E., Santangelo, F., and Morcillo, E. J. (2002) Effectiveness of oral N-acetylcysteine in a rat experimental model of asthma. *Pharmacol. Res.* **45**, 135–140
6. Asti, C., Melillo, G., Caselli, G. F., Daffonchio, L., Hernandez, A., Clavenna, G., and Omini, C. (1995) Effectiveness of carbocysteine lysine salt monohydrate on models of airway inflammation and hyperresponsiveness. *Pharmacol. Res.* **31**, 387–392

7. Vita, J. A., Frei, B., Holbrook, M., Gokce, N., Leaf, C., and Keaney, J. F., Jr. (1998) L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J. Clin. Invest.* **101**, 1408–1414
8. Oiry, J., Puy, J. Y., Mialocq, P., Clayette, P., Fretier, P., Jaccard, P., Dereuddre-Bosquet, N., Dormont, D., and Imbach, J. L. (1999) Synthesis and in vitro anti-HIV activity in human monocyte-derived macrophages of 2-oxothiazolidine-4(R)-carboxylic acid derivatives. *J. Med. Chem.* **42**, 4733–4740
9. Porta, P., Aebi, S., Summer, K., and Lauterburg, B. H. (1991) L-2-oxothiazolidine-4-carboxylic acid, a cysteine prodrug: pharmacokinetics and effects on thiols in plasma and lymphocytes in human. *J. Pharmacol. Exp. Ther.* **257**, 331–334
10. Williamson, J. M., and Meister, A. (1981) Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate, a 5-oxo-L-prolinase substrate. *Proc. Natl. Acad. Sci. U.S.A.* **78**, 936–939
11. Moslen, M. T., Whitehead, R. F., Ferguson, A. E., and Kanz, M. F. (1989) Protection by L-2-oxothiazolidine-4-carboxylate, a cysteine prodrug, against 1,1-dichloroethylene hepatotoxicity in rats is associated with decreases in toxin metabolism and cytochrome P-450. *J. Pharmacol. Exp. Ther.* **248**, 157–163
12. Hazelton, G. A., Hjelle, J. J., and Klaassen, C. D. (1986) Effects of cysteine pro-drugs on acetaminophen-induced hepatotoxicity. *J. Pharmacol. Exp. Ther.* **237**, 341–349
13. Williamson, J. M., Boettcher, B., and Meister, A. (1982) Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 6246–6249
14. Mesina, J. E., Page, R. H., Hetzel, F. W., and Chopp, M. (1989) Administration of L-2-oxothiazolidine-4-carboxylate increases glutathione levels in rat brain. *Brain Res.* **478**, 181–183
15. Kwak, Y. G., Song, C. H., Yi, H. K., Hwang, P. H., Kim, J. S., Lee, K. S., and Lee, Y. C. (2003) Involvement of PTEN in airway hyper-responsiveness and inflammation in bronchial asthma. *J. Clin. Invest.* **111**, 1083–1092
16. Han, M. K., Kim, S. J., Park, Y. R., Shin, Y. M., Park, H. J., Park, K. J., Park, K. H., Kim, H. K., Jang, S. I., An, N. H., et al. (2002) Antidiabetic effect of a prodrug of cysteine, L-2-oxothiazolidine-4-carboxylic acid, through CD38 dimerization and internalization. *J. Biol. Chem.* **277**, 5315–5321
17. Sundaresan, M., Yu, Z. X., Ferrans, V. J., Irani, K., and Finkel, T. (1995) Requirement for generation of H₂O₂ for platelet-derived growth factor signal transduction. *Science* **270**, 296–299
18. Cheng, J. J., Chao, Y. J., Wung, B. S., and Wang, D. L. (1996) Cyclic strain-induced plasminogen activator inhibitor-1 (PAI-1) release from endothelial cells involves reactive oxygen species. *Biochem. Biophys. Res. Commun.* **225**, 100–105

19. Lee, M., You, H. J., Cho, S. H., Woo, C. H., Yoo, M. H., Joe, E. H., and Kim, J. H. (2002) Implication of the small GTPase Rac1 in the generation of reactive oxygen species in response to β -amyloid in C6 astrocytoma cells. *Biochem. J.* **366**, 937–943
20. Hamelmann, E., Schwarze, J., Takeda, K., Oshiba, A., Larsen, G. L., Irvin, C. G., and Gelfand, E. W. (1997) Noninvasive measurement of airway responsiveness in allergic mice using barometric plethysmography. *Am. J. Respir. Crit. Care Med.* **156**, 766–775
21. Lee, Y. C., Kwak, Y. G., and Song, C. H. (2002) Contribution of vascular endothelial growth factor to airway hyper-responsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J. Immunol.* **168**, 3595–3600
22. Tournoy, K. G., Kips, J. C., Schou, C., and Pauwels, R. A. (2000) Airway eosinophilia is not a requirement for allergen-induced airway hyper-responsiveness. *Clin. Exp. Allergy* **30**, 79–85
23. Han, S. J., Ko, H. M., Choi, J. H., Seo, K. H., Lee, H. S., Choi, E. K., Choi, I. W., Lee, H. K., and Im, S. Y. (2002) Molecular mechanisms for lipopolysaccharide-induced biphasic activation of nuclear factor-kappa B (NF-kappa B). *J. Biol. Chem.* **277**, 44,715–44,721
24. Chomczynski, P., and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**, 156–159
25. Nishida, S., Teramoto, K., Kimoto-Kinoshita, S., Tohda, Y., Nakajima, S., Tomura, T. T., and Irimajiri, K. (2002) Change of Cu,Zn-superoxide dismutase activity of guinea pig lung in experimental asthma. *Free Radic. Res.* **36**, 601–606
26. Zhang, M., Nomura, A., Uchida, Y., Iijima, H., Sakamoto, T., Iishii, Y., Morishima, Y., Mochizuki, M., Masuyama, K., Hirano, K., et al. (2002) Ebselen suppresses late airway responses and airway inflammation in guinea pigs. *Free Radic. Biol. Med.* **32**, 454–464
27. Rahman, I., and MacNee, W. (1996) Role of oxidants/antioxidants in smoking-induced lung diseases. *Free Radic. Biol. Med.* **21**, 669–681
28. Rahman, I., Skwarska, E., and MacNee, W. (1997) Attenuation of oxidant/antioxidant imbalance during treatment of exacerbations of chronic obstructive pulmonary disease. *Thorax* **52**, 565–568
29. Hulsmann, A. R., Raatgeep, H. R., den Hollander, J. C., Stijnen, T., Saxena, P. R., Kerrebijn, K. F., and de Jongste, J. C. (1994) Oxidative epithelial damage produces hyper-responsiveness of human peripheral airways. *Am. J. Respir. Crit. Care Med.* **149**, 519–525
30. Cortijo, J., Marti-Cabrera, M., de la Asuncion, J. G., Pallardo, F. V., Esteras, A., Bruseghini, L., Vina, J., and Morcillo, E. J. (1999) Contraction of human airways by oxidative stress protection by N-acetylcysteine. *Free Radic. Biol. Med.* **27**, 392–400
31. Adam, L., Bouvier, M., and Jones, T. L. (1999) Nitric oxide modulates beta(2)-adrenergic receptor palmitoylation and signaling. *J. Biol. Chem.* **274**, 26,337–26,343

32. Owen, S., Pearson, D., Suarez-Mendez, V., O'Driscoll, R., and Woodcock, A. (1991) Evidence of free-radical activity in asthma. *N. Engl. J. Med.* **325**, 586–587
33. Henderson, W. R., Jr., Chi, E. Y., Teo, J. L., Nguyen, C., and Kahn, M. (2002) A small molecule inhibitor of redox-regulated NF-kappa B and activator protein-1 transcription blocks allergic airway inflammation in a mouse asthma model. *J. Immunol.* **169**, 5294–5299
34. Siebenlist, U., Franzoso, G., and Brown, K. (1994) Structure, regulation and function of NF-kappa B. *Annu. Rev. Cell Biol.* **10**, 405–455
35. Baeuerle, P. A., and Baltimore, D. (1996) NF-kappa B: ten years after. *Cell* **87**, 13–20
36. Baldwin, A. S., Jr. (1996) The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu. Rev. Immunol.* **14**, 649–683
37. Gilmore, T. D. (1999) The Rel/NF-kappa B signal transduction pathway: introduction. *Oncogene* **18**, 6842–6844
38. Barnes, P. J., and Karin, M. (1997) Nuclear factor-kappa B: a pivotal transcription factor in chronic inflammatory diseases. *N. Engl. J. Med.* **336**, 1066–1071
39. Barnes, P. J., and Adcock, I. M. (1997) NF-kappa B: a pivotal role in asthma and a new target for therapy. *Trends Pharmacol. Sci.* **18**, 46–50
40. Stutz, A. M., and Woisetschlager, M. (1999) Functional synergism of STAT6 with either NF-kappa B or PU.1 to mediate IL-4-induced activation of IgE germline gene transcription. *J. Immunol.* **163**, 4383–4391
41. Mori, A., Kaminuma, O., Mikami, T., Inoue, S., Okumura, Y., Akiyama, K., and Okudaira, H. (1999) Transcriptional control of the IL-5 gene by human helper T cells: IL-5 synthesis is regulated independently from IL-2 or IL-4 synthesis. *J. Allergy Clin. Immunol.* **103**, S429–S436
42. Sang, F., Gong, X., and Taylor, A. (1997) Activity of ubiquitin-dependent pathway in response to oxidative stress. Ubiquitin-activating enzyme is transiently up-regulated. *J. Biol. Chem.* **272**, 23,086–23,093
43. Ginn-Pease, M. E., and Whisler, R. L. (1996) Optimal NF-κB mediated transcriptional responses in Jurkat T cells exposed to oxidative stress are dependent on intracellular glutathione and costimulatory signals. *Biochem. Biophys. Res. Commun.* **226**, 695–702
44. Rahman, I., and MacNee, W. (1998) Role of transcription factors in inflammatory lung diseases. *Thorax* **53**, 601–612
45. Baldwin, A. S., Jr. (1996) The NF-κB and IκB proteins: new discoveries and insights. *Annu. Rev. Immunol.* **14**, 649–683

46. Schenk, H., Klein, M., Erdbrugger, W., Droge, W., and Schulze-Osthoff, K. (1994) Distinct effects of thioredoxin and antioxidants on the activation of transcription factors NF- κ B and AP-1. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 1672–1676
47. Jun, D. Y., Chae, H. Z., Rhee, S. G., and Jeang, K. T. (1997) Regulatory role for a novel human thioredoxin peroxidase in NF- κ B activation. *J. Biol. Chem.* **272**, 30,952–30,961
48. Lin, Y. L., and Lin, J. K. (1997) (-)-Epigallocatechin-3-gallate blocks the induction of nitric oxide synthase by down-regulating lipopolysaccharide-induced activity of transcription factor nuclear factor- κ B. *Mol. Pharmacol.* **52**, 465–472
49. Verhasselt, V., Vanden Berghe, W., Vanderheyde, N., Willems, F., Haegeman, G., and Goldman, M. (1999) N-acetyl-L-cysteine inhibits primary human T cell responses at the dendritic cell level: association with NF-kappaB inhibition. *J. Immunol.* **162**, 2569–2574
50. Harper, R., Wu, K., Chang, M. M., Yoneda, K., Pan, R., Reddy, S. P., and Wu, R. (2001) Activation of nuclear factor-kappa b transcriptional activity in airway epithelial cells by thioredoxin but not by N-acetyl-cysteine and glutathione. *Am. J. Respir. Cell Mol. Biol.* **25**, 178–185

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Fig. 1

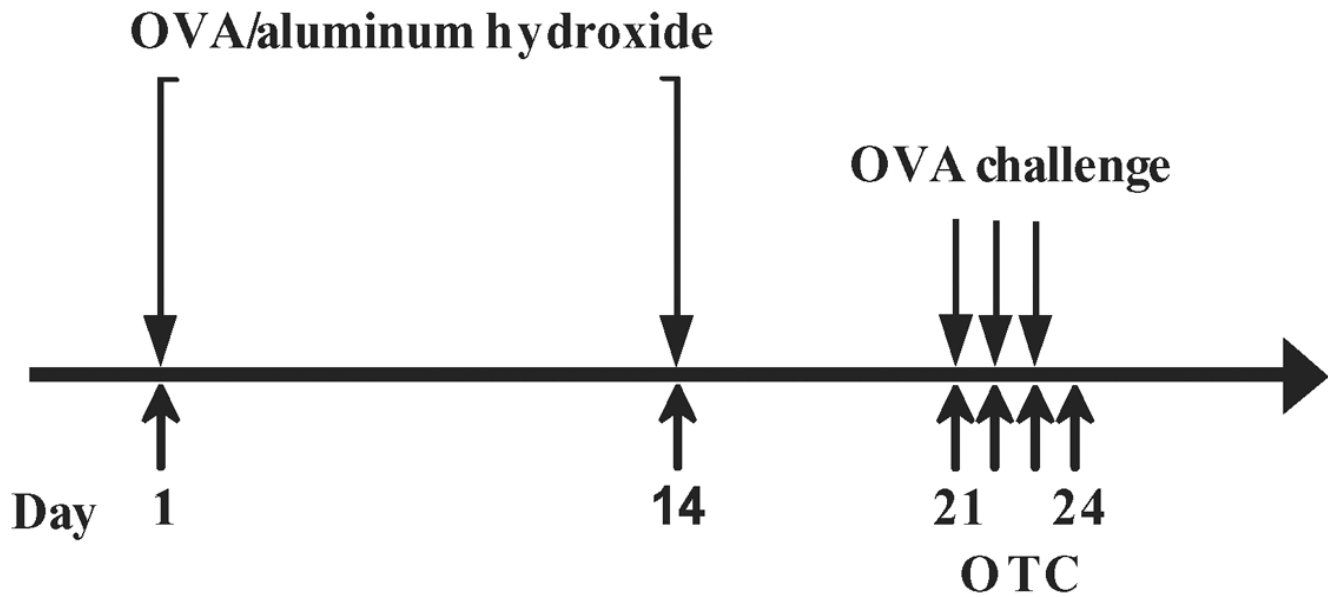


Figure 1. Schematic diagram of the experimental protocol. Mice were sensitized on days 1 and 14 by intraperitoneal injection of OVA emulsified in 1 mg of aluminum hydroxide. On days 21, 22, and 23 after the initial sensitization, the mice were challenged for 30 min with an aerosol of 3% (w/v) OVA in saline (or with saline as a control) using an ultrasonic nebulizer. In the case of treatment with OTC, it was administered intraperitoneally 4 times at 24-h intervals on days 21–24, beginning 1 h before the first challenge.

Fig. 2

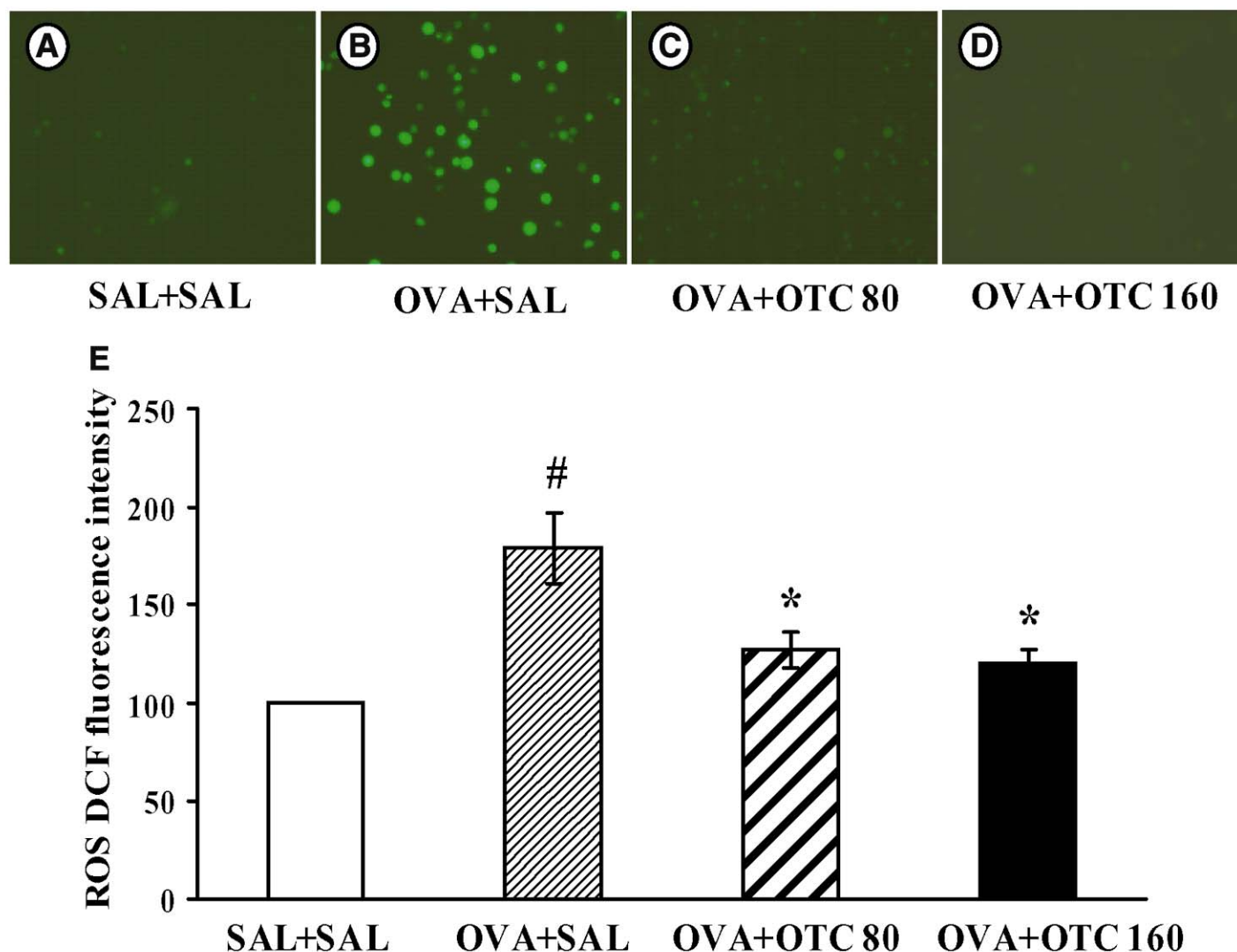


Figure 2. Effect of OTC on ROS levels in BAL fluids of OVA-sensitized and -challenged mice. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). **A–D**) Representative fluorescence microscopy shows ROS-positive cells in the BAL fluids. **E**) DCF fluorescence intensity is presented as the relative ratio of ROS levels in OVA+SAL, OVA+OTC 80, or OVA+OTC 160 to those in SAL+SAL. The relative ratio of ROS levels in the BAL fluids of SAL+SAL is arbitrarily presented as 100. Bars represent mean $\pm$ SEM from 6 independent experiments. $^{\#}P < 0.05$ vs. SAL+SAL; $^{*}P < 0.05$ vs. OVA+SAL.

Fig. 3

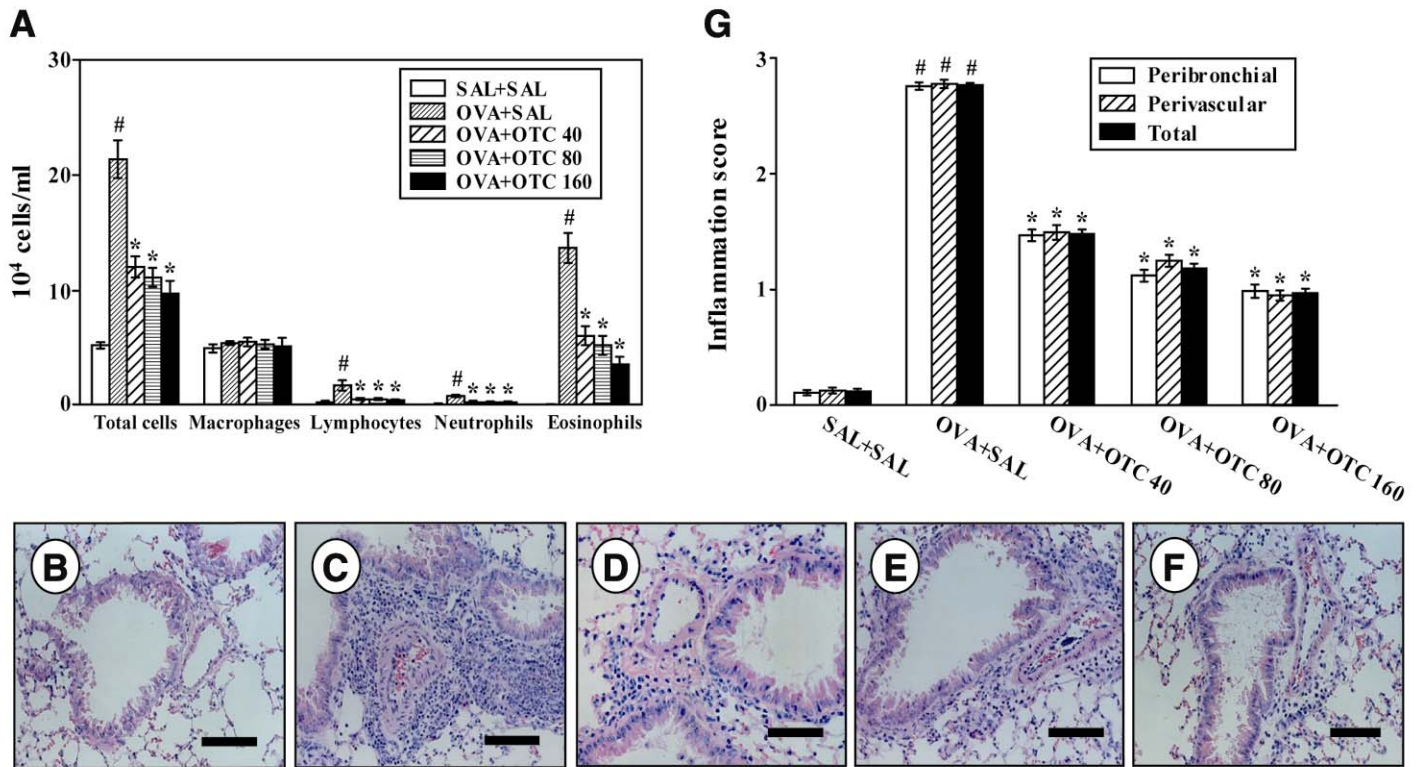


Figure 3. Effect of OTC on total and differential cellular components of BAL and on pathologic changes in lung tissues of OVA-sensitized and -challenged mice. **A)** The numbers of each cellular component of BAL from saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered OTC 40 mg/kg (OVA+OTC 40), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160) were counted at 72 h after the last challenge. **B–F)** Representative hematoxylin and eosin-stained sections of the lungs. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (**B**), OVA-inhaled mice administered saline (**C**), OVA-inhaled mice administered OTC 40 mg/kg (**D**), OVA-inhaled mice administered OTC 80 mg/kg (**E**), and OVA-inhaled mice administered OTC 160 mg/kg (**F**). Bars indicate scale of 50 μ m. **G)** Peribronchial and perivascular lung inflammation were measured at 72 h after the last challenge in SAL+Sal, OVA+Sal, OVA+OTC 40, OVA+OTC 80, and OVA+OTC 160. Bars represent mean $\pm$ SEM from 6 independent experiments. [#] $P < 0.05$ vs. SAL+Sal; ^{*} $P < 0.05$ vs. OVA+Sal.

Fig. 4

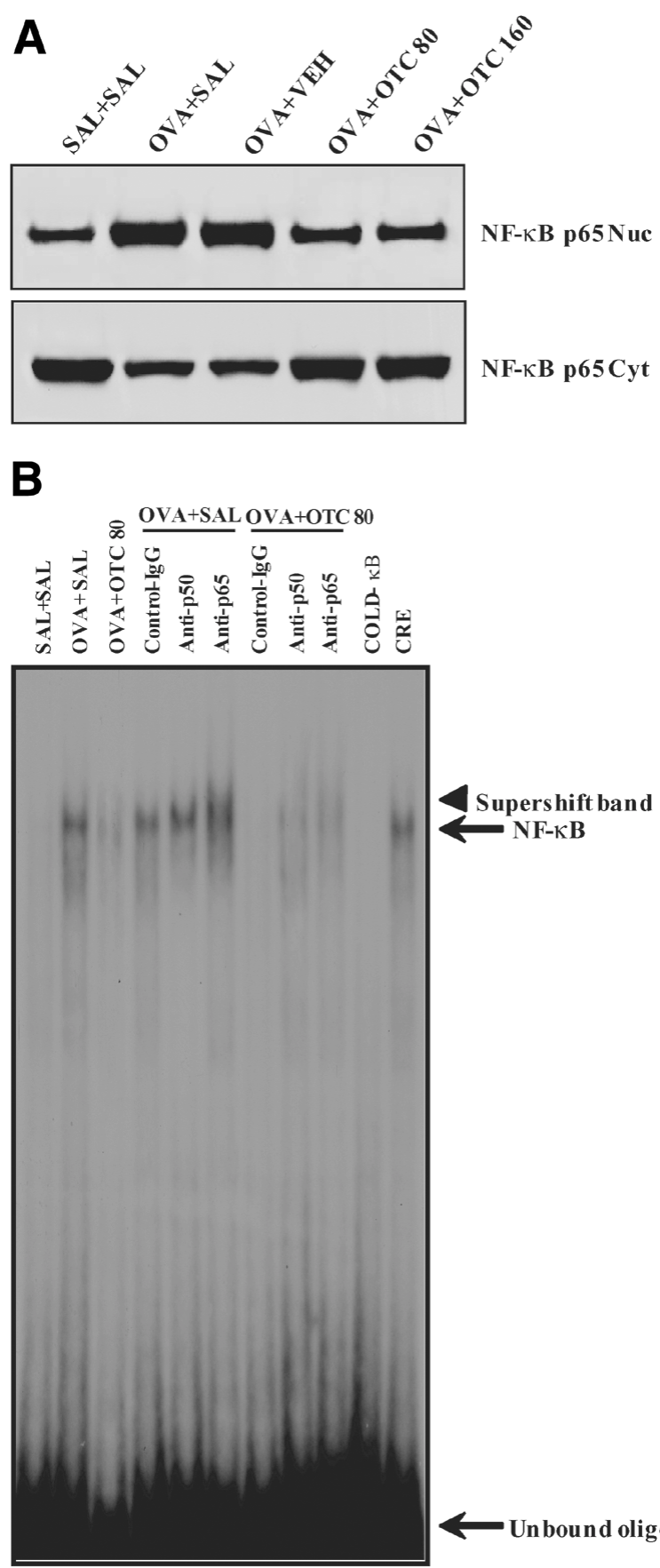


Figure 4. Effect of OTC on NF- κ B expression in nuclear and cytosolic protein extracts from lung tissues of OVA-sensitized and -challenged mice. *A*) NF- κ B p65 expression in nuclear (Nuc) and cytosolic (Cyt) protein extracts from lung tissues. *B*) Representative electrophoretic-mobility-shift assay of nuclear extracts from lung tissues. NF- κ B expression was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). Nuclear extracts were incubated with an α - ^{32} P-labeled κ B oligonucleotide, and the samples were electrophoresed on a 5% polyacrylamide gel. A 50-fold excess of cold κ B or cAMP response element (CRE) oligonucleotide was added as a competitor. Supershift assay was conducted with anti-p50, anti-p65, or control rabbit antibody. Results were similar in six independent experiments.

Fig. 5

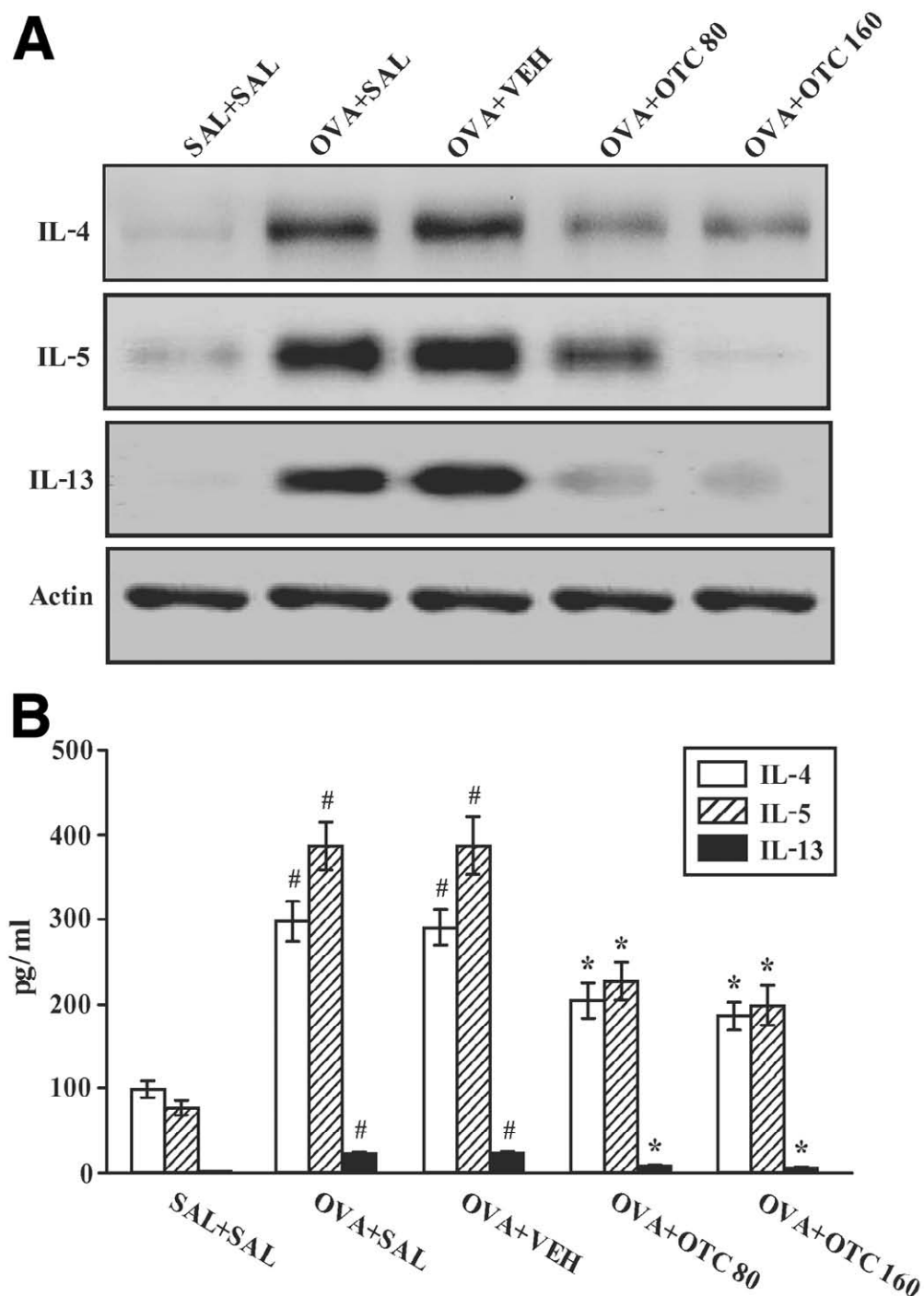


Figure 5. Effect of OTC on IL-4, IL-5, and IL-13 protein expression in lung tissues and BAL fluids of OVA-sensitized and -challenged mice. **A)** Western blotting of IL-4, IL-5, and IL-13. The protein expression of these cytokines was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). **B)** Enzyme immunoassay of IL-4, IL-5, and IL-13. Bars represent mean ± SEM from 6 independent experiments. [#]*P* < 0.05 vs. SAL+SAL; ^{*}*P* < 0.05 vs. OVA+SAL.

Fig. 6

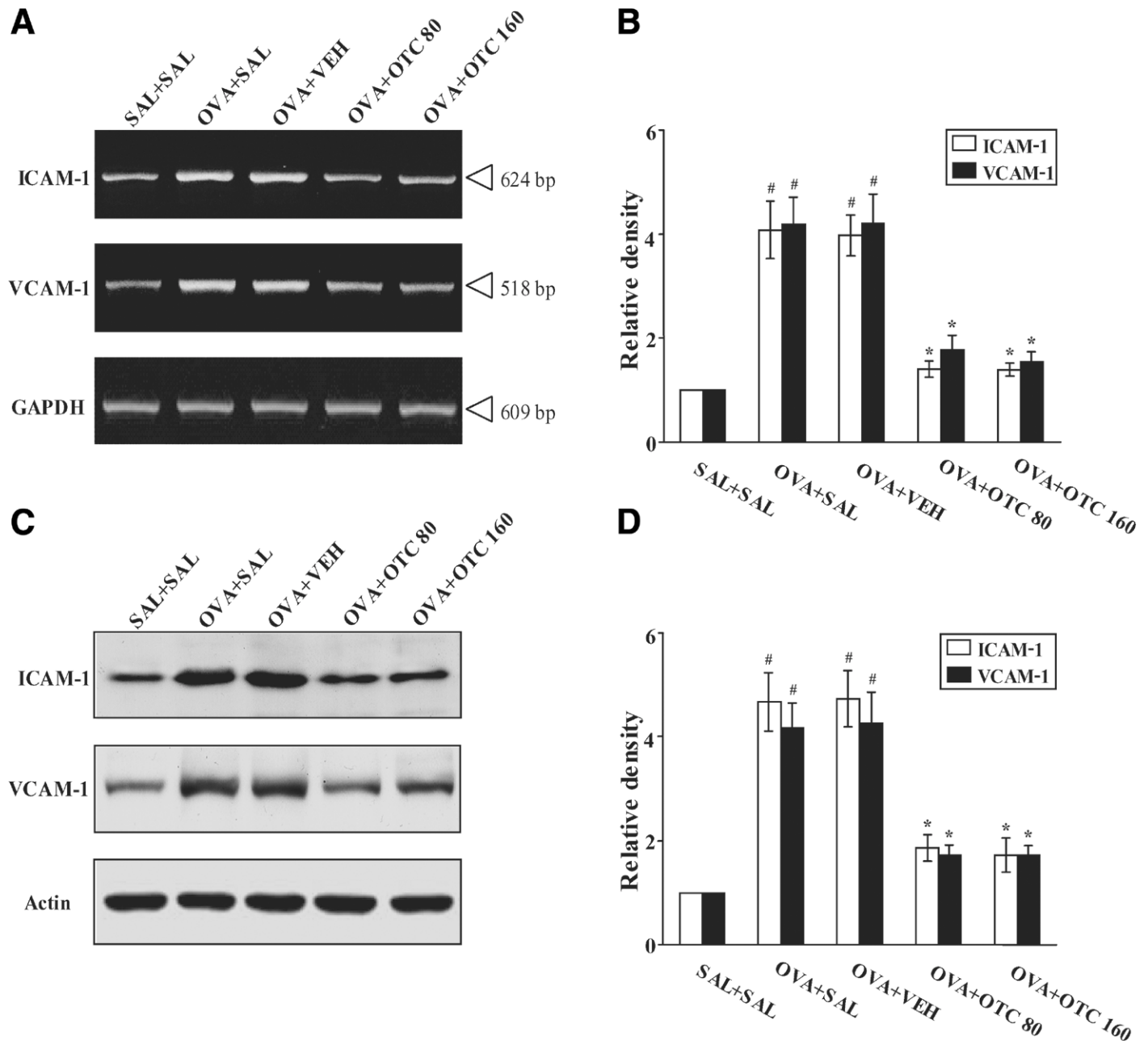


Figure 6. Effect of OTC on ICAM-1 and VCAM-1 expression in lung tissues of OVA-sensitized and -challenged mice. **A)** RT-PCR of ICAM-1 and VCAM-1. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). **B)** Densitometric analyses are presented as the relative ratio of each molecule mRNA to GAPDH mRNA. The relative ratio of each molecule mRNA in the lung tissues of SAL+SAL is arbitrarily presented as 1. **C)** Western blotting of ICAM-1 and VCAM-1. **D)** Densitometric analyses are presented as the relative ratio of each molecule to actin. The relative ratio of each molecule in the lung tissues of SAL+SAL is arbitrarily presented as 1. Data represent mean $\pm$ SEM from 6 independent experiments. $^{\#}P < 0.05$ vs. SAL+SAL; $^{*}P < 0.05$ vs. OVA+SAL.

Fig. 7

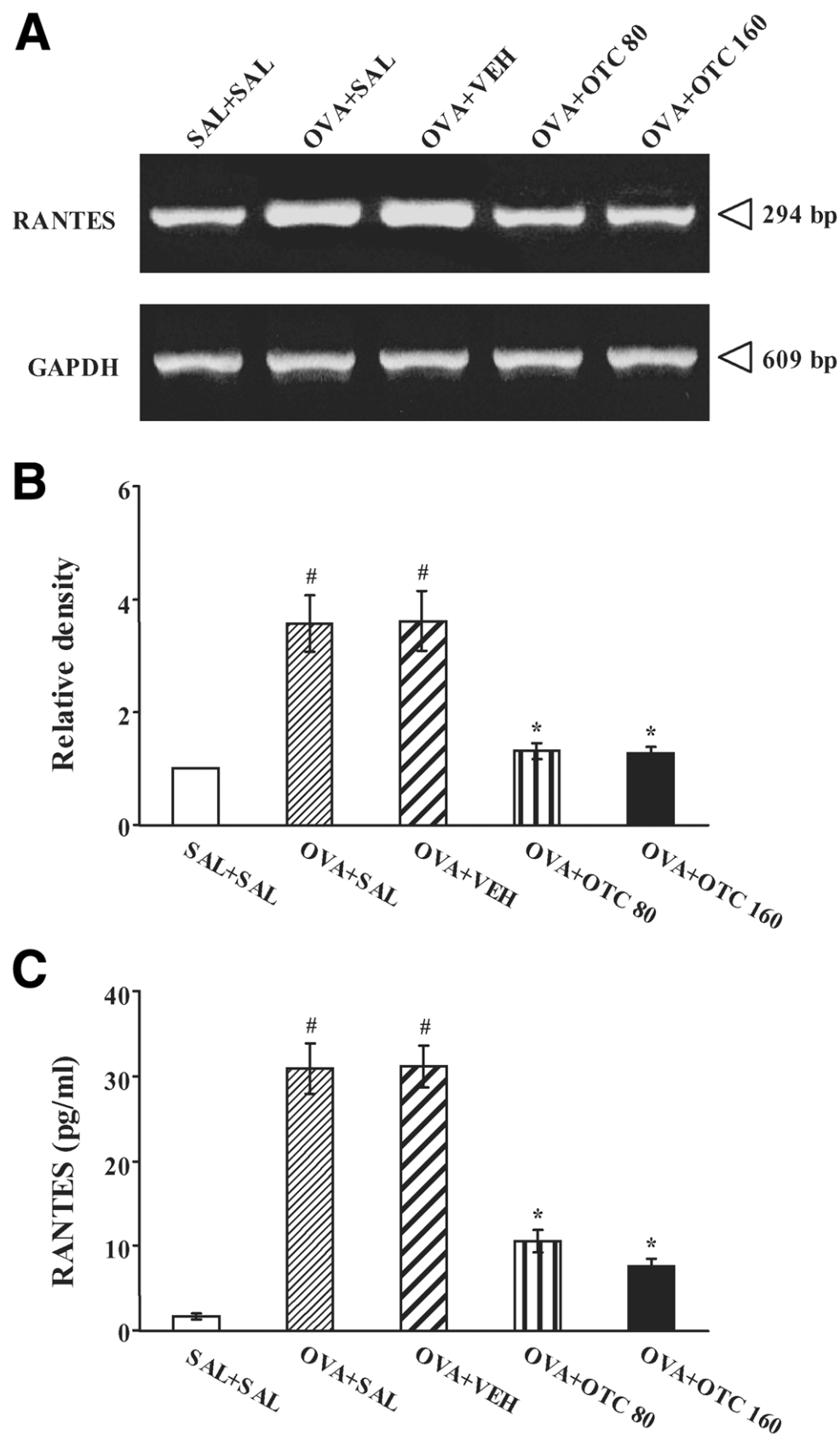


Figure 7. Effect of OTC on RANTES mRNA expression in lung tissues and on RANTES levels in BAL fluids of OVA-sensitized and -challenged mice. **A)** RT-PCR of RANTES. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). **B)** Densitometric analyses are presented as the relative ratio of RANTES mRNA to GAPDH mRNA. The relative ratio of each molecule mRNA in the lung tissues of SAL+SAL is arbitrarily presented as 1. **C)** Enzyme immunoassay of RANTES. Data represent mean $\pm$ SEM from 6 independent experiments. [#]*P* < 0.05 vs. SAL+SAL; **P* < 0.05 vs. OVA+SAL.

Fig. 8

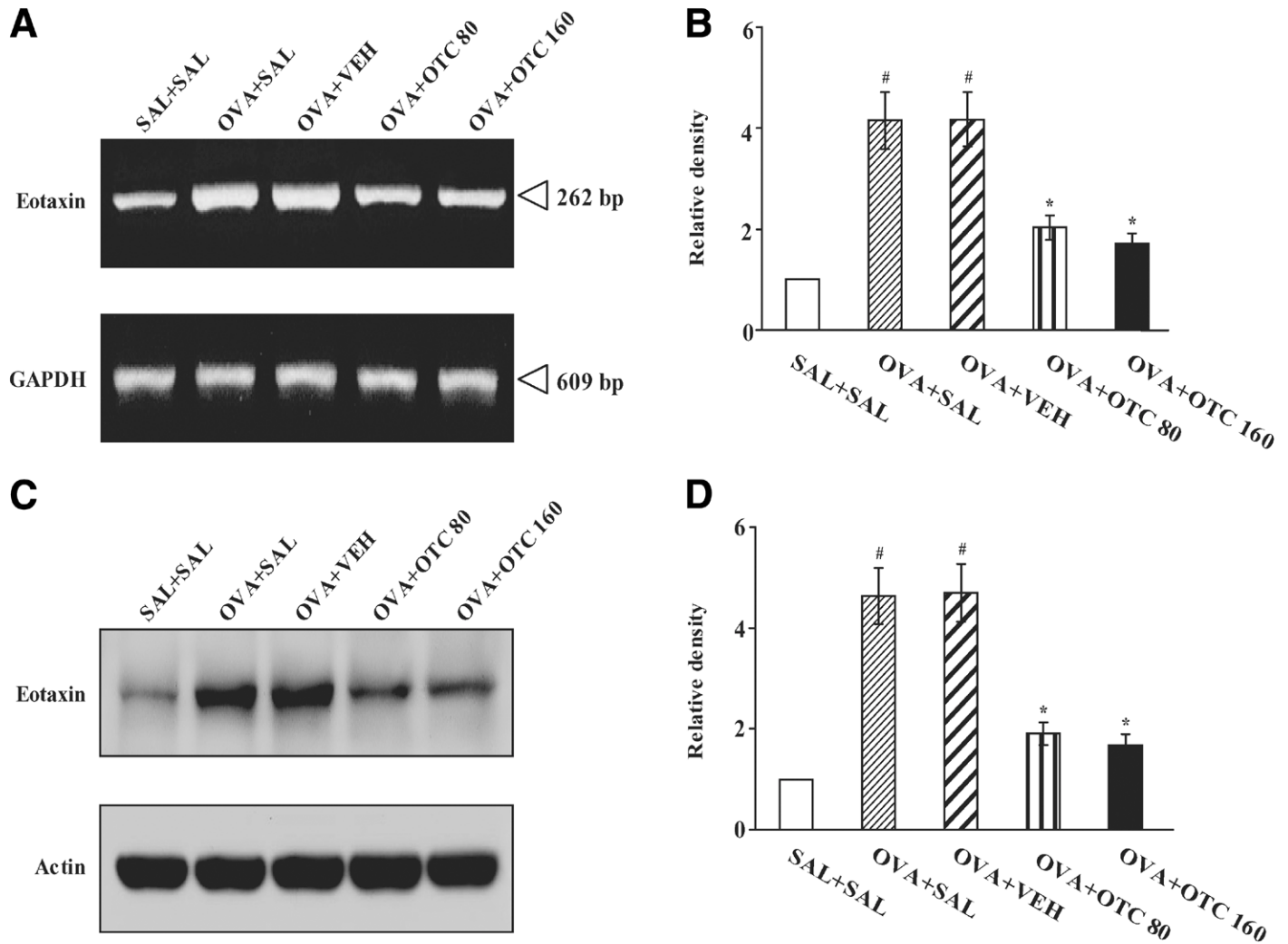


Figure 8. Effect of OTC on eotaxin expression in lung tissues of OVA-sensitized and -challenged mice. **A)** RT-PCR of eotaxin. Sampling was performed at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+Sal), OVA-inhaled mice administered saline (OVA+Sal), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). **B)** Densitometric analyses are presented as the relative ratio of eotaxin mRNA to GAPDH mRNA. The relative ratio of eotaxin mRNA in the lung tissues of SAL+Sal is arbitrarily presented as 1. **C)** Western blotting of eotaxin. **D)** Densitometric analyses are presented as the relative ratio of eotaxin to actin. The relative ratio of eotaxin in the lung tissues of SAL+Sal is arbitrarily presented as 1. Data represent mean $\pm$ SEM from 6 independent experiments. [#] $P < 0.05$ vs. SAL+Sal; ^{*} $P < 0.05$ vs. OVA+Sal.

Fig. 9

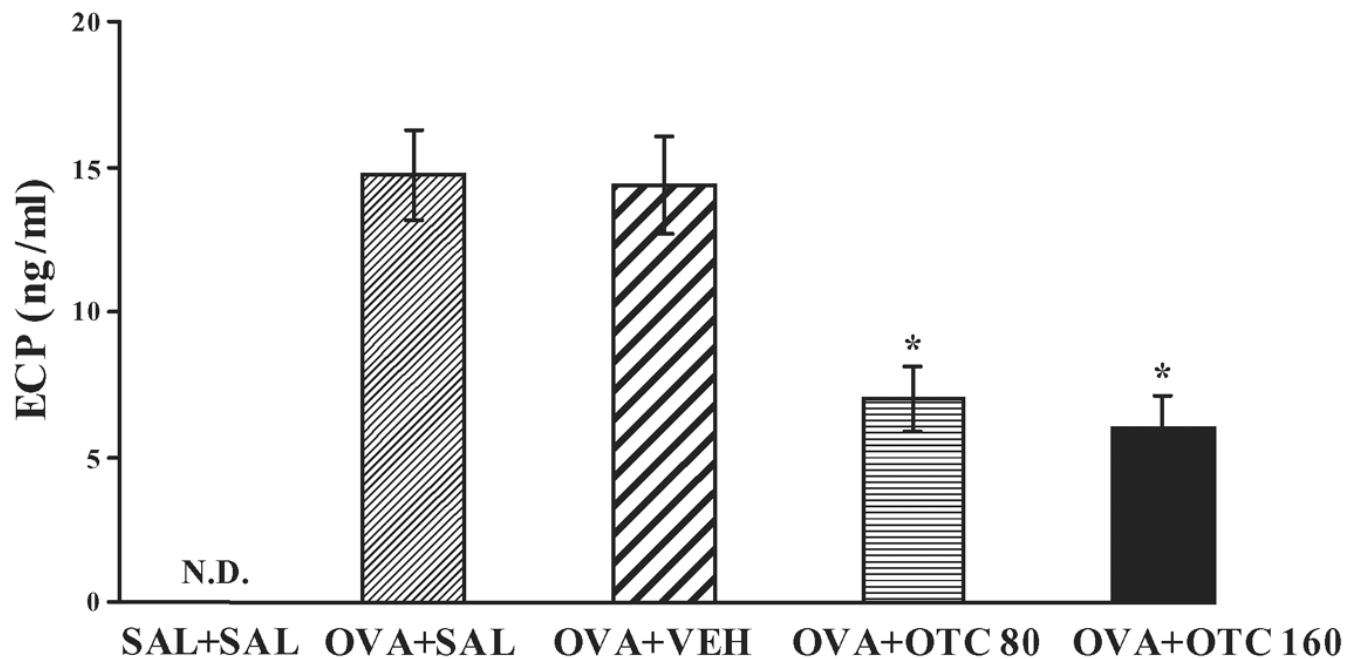


Figure 9. Effect of OTC on ECP levels in BAL fluids of OVA-sensitized and -challenged mice. Fluoroenzyme immunoassay of ECP. Sampling was performed at 72 h after the past challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered drug vehicle (OVA+VEH), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). Bars represent mean $\pm$ SEM from 6 independent experiments. N.D., not detected. * $P < 0.05$ vs. OVA+SAL.

Fig. 10

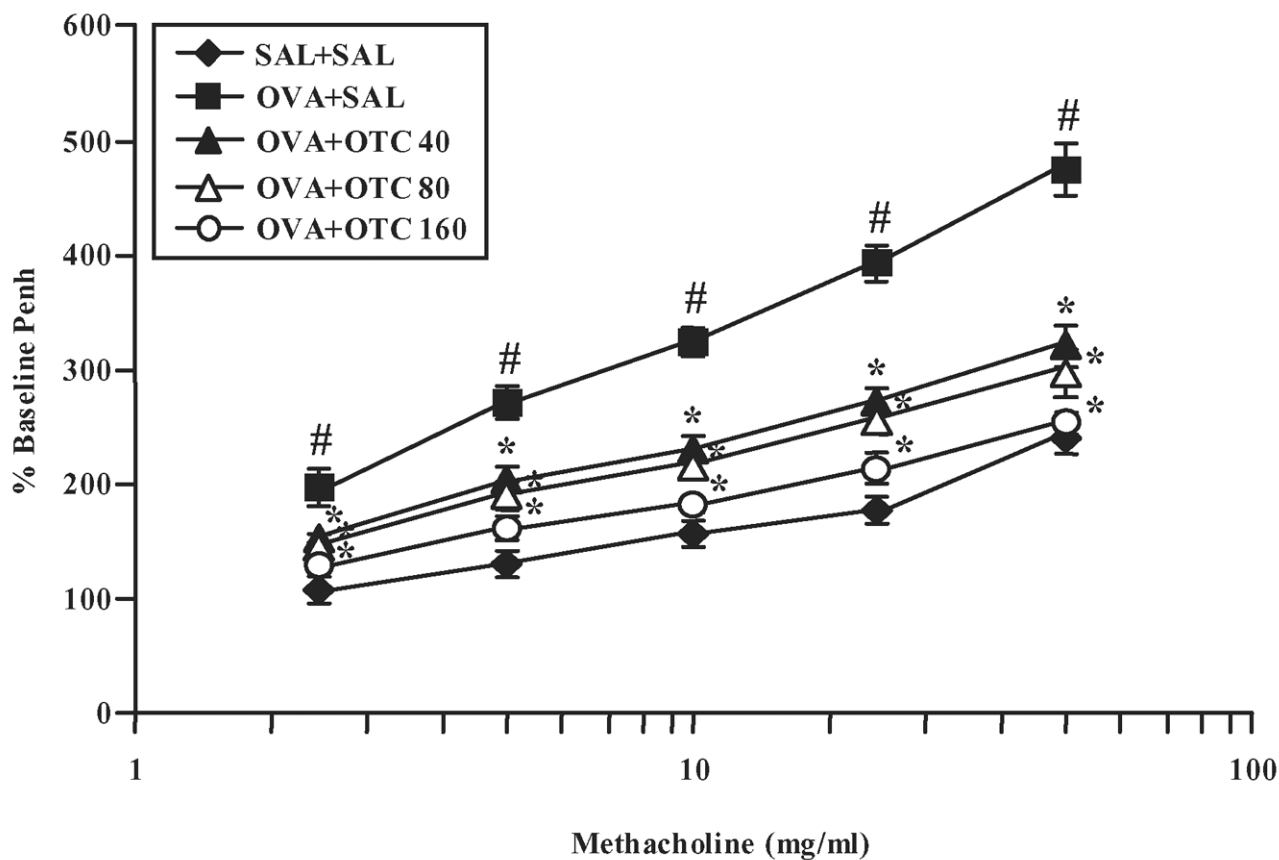


Figure 10. Effect of OTC on airway responsiveness in OVA-sensitized and -challenged mice. Airway responsiveness was measured at 72 h after the last challenge in saline-inhaled mice administered saline (SAL+SAL), OVA-inhaled mice administered saline (OVA+SAL), OVA-inhaled mice administered OTC 40 mg/kg (OVA+OTC 40), OVA-inhaled mice administered OTC 80 mg/kg (OVA+OTC 80), and OVA-inhaled mice administered OTC 160 mg/kg (OVA+OTC 160). Airway responsiveness to aerosolized methacholine was measured in unrestrained, conscious mice. Mice were placed into the main chamber and were nebulized first with saline, then with increasing doses (2.5 to 50 mg/ml) of methacholine for 3 min for each nebulization. Readings of breathing parameters were taken for 3 min after each nebulization during which Penh values were determined. Data represent mean $\pm$ SEM from 6 independent experiments. [#] $P < 0.05$ vs. SAL+SAL; ^{*} $P < 0.05$ vs. OVA+SAL.