

Doxycycline reduces airway inflammation and hyperresponsiveness in a murine model of toluene diisocyanate–induced asthma

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Background: Toluene diisocyanate (TDI) is a leading cause of occupational asthma. Although considerable controversy remains regarding its pathogenesis, TDI-induced asthma is an inflammatory disease of the airways characterized by airway remodeling caused, at least in part, by an excess of extracellular matrix deposition in the airway wall. Matrix metalloproteinases (MMPs) are major proteolytic enzymes that are involved in extracellular matrix turnover because of their ability to cleave all proteins constituting extracellular matrix. Previous studies have reported that MMP-9 might play a role in chronic airway inflammation and remodeling in asthma.

Objective: An aim of the current study was to evaluate the effects of MMP-inhibiting antibiotic, doxycycline, and MMP inhibitors on hyperresponsiveness and inflammation of the airways in TDI-induced asthma.

Methods: We used a murine model for TDI-induced asthma to examine the effect of doxycycline or MMP inhibitors on bronchial inflammation and airway hyperresponsiveness.

Results: The following typical pathophysiologic features are observed in the lungs of the mice: airway inflammation, airway hyperresponsiveness, and increased expression of MMP-9 mRNA and protein. Administration of doxycycline and MMP inhibitors reduced all of these pathophysiologic findings. In addition, the increased phosphorylated Akt but not Akt protein levels in lung tissues after TDI inhalation were significantly reduced by the administration of doxycycline and MMP inhibitors.

Conclusion: These findings suggest that doxycycline may reduce airway inflammation and hyperresponsiveness through phosphatidylinositol 3-kinase pathway in a murine model of TDI-induced asthma. (*J Allergy Clin Immunol* 2004;113: 902-9.)

Key words: Toluene di-isocyanate, matrix metalloproteinase, asthma, doxycycline

Isocyanate chemicals, including toluene diisocyanate (TDI), are currently the most common causes of occupational asthma.^{1,2} TDI-induced asthma is characterized

Abbreviations used

BAL: Bronchoalveolar lavage
DMSO: Dimethyl sulfoxide
ECM: Extracellular matrix
MMP: Matrix metalloproteinase
MMPI-I: Matrix metalloproteinase inhibitor I
MMPI-II: Matrix metalloproteinase 2/matrix metalloproteinase 9 inhibitor II
p-Akt: Phosphorylated Akt
Penh: Enhanced pause
TDI: Toluene diisocyanate

by hyperresponsiveness and inflammation of the airways.^{2,3} This inflammation is associated with infiltration of lymphocytes, eosinophils, and neutrophils into the bronchial lumen.^{3,4} Airway remodeling is caused, at least in part, by an excess of extracellular matrix (ECM) deposition in the airway wall, which leads to subepithelial collagen deposition.⁵ This airway remodeling has been speculated to be irreversible airway obstruction and 1 of the factors that make some patients with asthma difficult to treat.⁶ The matrix metalloproteinases (MMPs) are a large family of Zn²⁺-dependent and Ca²⁺-dependent endopeptidases with distinct substrate specificities that degrade most components of the ECM.⁷ The MMPs play an important role in physiologic and pathologic processes including ECM turnover, tissue degradation and repair, cell migration, and inflammation. Of the MMP family, MMP-9 is 1 of the major proteinases involved in bronchial remodeling in asthma.^{8,9} In addition, MMP-9 induces migration of eosinophils, lymphocytes, and neutrophils across basement membranes.¹⁰⁻¹²

Tetracyclines as antibiotics inhibit bacterial protein synthesis. Recent work has shown that they are pluripotent drugs that affect many cellular functions including proliferation, migration, apoptosis, and matrix remodeling.¹³ Doxycycline and other derivatives of tetracycline are potent MMP inhibitors.¹⁴⁻¹⁸ Inhibition of MMPs by doxycycline occurs independently of antimicrobial activity. The beneficial effect of doxycycline on ECM degradation in animal models and human beings has been attributed to the inhibition of MMPs.¹⁹⁻²¹ On the basis of this anti-MMP activity, they have been used to reduce tissue degradation in aortic aneurysms, periodontal disease, and arthritis and are used to prevent tumor cell invasion and metastasis and tumor angiogenesis.¹⁹⁻²⁸

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MMPs were also reported to play a crucial role in the infiltration of ovalbumin-induced airway inflammatory cells and the induction of airway hyperresponsiveness in a murine model of asthma.²⁹ Recently, we demonstrated that release of MMP-9 was upregulated in association with the accumulation of inflammatory cells to airways in a murine model of TDI-induced asthma and that the inhibition of MMP-9 may be a good therapeutic strategy.³⁰ However, there are little data on the influence of doxycycline on MMPs in TDI-induced asthma, and the mechanisms by which doxycycline and MMP inhibitors affect MMP-9 expression have not been clarified.

In the current study, we used a murine model of TDI-induced asthma to examine the effects of doxycycline or MMP inhibitors on hyperresponsiveness and inflammation of the airways.

METHODS

Animals and experimental protocol

Female BALB/c mice, 8 to 10 weeks old and free of murine specific pathogens, were obtained from the Korean Research Institute of Chemistry Technology, Daejeon. All experimental animals used in this study were under a protocol approved by the Institutional Animal Care and Use Committee of Chonbuk National University Medical School. Standard guidelines for laboratory animal care were followed.³¹ Mice were sensitized by intranasal administration of 20 μ L 3% TDI dissolved in ethyl acetate:olive oil (1:4) under light anesthesia with sodium pentobarbitone (30 mg/kg, intraperitoneally) once daily for 5 consecutive days, as previously described^{30,32} (see Fig E1 in the Journal's Online Repository at www.mosby.com/jaci). Animals were kept in a supine position for 10 minutes after each sensitization. After 3 weeks, these animals were further sensitized with the same reagent for 5 consecutive days. On day 7 after the second course of sensitization (day 38), mice were individually placed in a horizontal cylindrical chamber and challenged via the airways with 1% TDI dissolved in ethyl acetate:olive oil (1:4) by ultrasonic nebulization (NE-U12; Omron, Tokyo, Japan). As a control, mice were sensitized and challenged by the same protocol but using only the solvent, ethyl acetate:olive oil (1:4). Six mice in each group were tested.

Bronchoalveolar lavage (BAL) was performed at 72 hours after the TDI challenge. After mice were killed with sodium pentobarbitone (pentobarbital sodium, 100 mg/kg body weight), prewarmed 0.9% NaCl solution was slowly infused into the lungs and withdrawn. Total cell numbers were counted with a hemocytometer. Smears of BAL cells prepared with a Cytospin II (Shandon, Runcorn, United Kingdom) were stained with Diff-Quik solution (Dade Diagnostics of Puerto Rico, Aguada, Puerto Rico) for differential cell counting. Two independent, blind investigators counted the cells by using a microscope. Approximately 400 cells were counted in each of 4 different random locations.

Administration of MMP inhibitors or doxycycline

Inhibitors of MMPs, MMP inhibitor I (MMPI-I; IC₅₀ for MMP-9 = 150 μ mol/L; 4-Abz-Gly-Pro-D-Leu-D-Ala-NHOH; Calbiochem, San Diego, Calif) and MMP-2/MMP-9 inhibitor II (MMPI-II; IC₅₀ for MMP-9 = 30 nmol/L; [2R]-2-[(4-biphenylsulfonyl)amino]-N-hydroxy-3-phenylpropionamide; Calbiochem), were used to inhibit MMP-9 activity. MMPI-I (20 mg/kg body

weight/d) dissolved in distilled water, or MMPI-II (2 mg/kg body weight/d) dissolved in DMSO, was administered intraperitoneally 3 times at 24-hour intervals beginning 30 minutes before the TDI challenge. In the doxycycline study, mice received 20 mg/kg doxycycline by gavage per day. Doxycycline was administered 6 times at 24-hour intervals on days 35 to 40, beginning 3 days before the TDI challenge (see Fig E1 in the Online Repository).

Determination of airway responsiveness to methacholine

Airway responsiveness was measured in mice 3 days after the TDI challenge in an unrestrained conscious state, as previously described.^{30,32-34}

Measurements of MMP-9 in BAL fluids

The hydrolytic activity of MMP-9 was measured by gelatin zymography, as previously described.³⁰

Histology, immunohistochemistry, and immunocytochemistry

Sections of fixed embedded tissues were cut at 4 μ m, placed on glass slides, deparaffinized, and stained sequentially with hematoxylin 2 and eosin-Y (Richard-Allan Scientific, Kalamazoo, Mich). The degree of peribronchial and perivascular inflammation was evaluated on a subjective scale of 0 to 3, as described elsewhere.³⁵ For immunohistochemistry and immunocytochemistry of MMP-9, the deparaffinized 4- μ m sections or the cytocentrifuge preparations of BAL cells were incubated sequentially in accordance with the RTU Vectastain Universal Quick kit instructions from Vector Laboratories (Burlingame, Calif). The slides were probed with an affinity-purified rabbit polyclonal anti-MMP-9 antibody (Chemicon International, Temecula, Calif) overnight at 4°C.

Western blot analysis

Western blot analysis was performed as described previously.^{32,33} The blots were incubated with an anti-MMP-9 antibody (Santa Cruz Biotechnology, Santa Cruz, Calif), anti-Akt, or anti-phosphorylated Akt (p-Akt) antibody (Cell Signaling Technology, Beverly, Mass) overnight at 4°C.

RNA isolation and RT-PCR

Total RNA from lung tissues was isolated by using a rapid extraction method (TRI-Reagent, Molecular Research Center, Cincinnati, Ohio) derived from the method of Chomczynski and Sacchi.³⁶ The first-strand cDNAs were used for the PCR amplification of MMP-9 or GAPDH. PCR amplification was performed by mixing 3 μ L of reverse transcriptase reaction with 47 μ L of buffer containing 2.5 U Taq DNA polymerase (Promega, Madison, Wis) and 30 pmol/L specific primer pairs for mouse cDNAs of MMP-9 or GAPDH, designed from published mouse gene sequences.

Densitometric analyses and statistics

All signals were visualized and analyzed by densitometric scanning (LAS-5000; Fuji Film, Tokyo, Japan). Data are expressed as $\pm$ SEMs. Statistical comparisons were performed by using 1-way ANOVA followed by the Fisher test. Significant differences between groups were determined by using the unpaired Student *t* test. Statistical significance was set at *P* < .05.

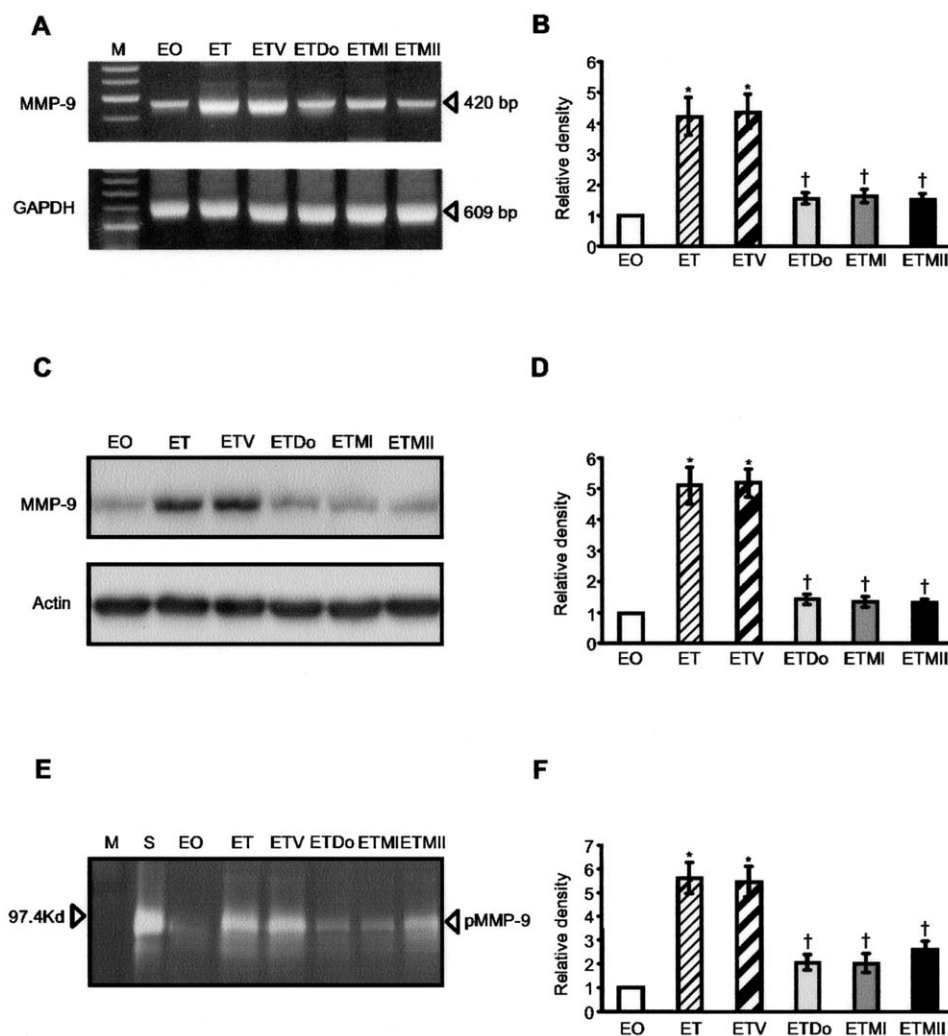


FIG 1. Effect of doxycycline, MMPI-I, or MMPI-II on MMP-9 expression in lung tissues and BAL fluids of TDI-sensitized and TDI-challenged mice. Sampling was performed at 72 hours after the TDI challenge in the mice treated with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETV), EO plus TDI plus doxycycline (ETD0), EO plus TDI plus MMPI-I (ETMI), or EO plus TDI plus MMPI-II (ETMII). **A** and **B**, RT-PCR of MMP-9. Densitometric analyses are presented as the relative ratio of MMP-9 mRNA to GAPDH mRNA. The relative ratio of MMP-9 mRNA in the lung tissues of EO mice is arbitrarily presented as 1. **C** and **D**, Western blotting of MMP-9. Densitometric analyses are presented as the relative ratio of MMP-9 to actin. The relative ratio of MMP-9 in the lung tissues of EO mice is arbitrarily presented as 1. **E** and **F**, Gelatin zymography. Lane S contains standards: the proform of MMP-9 (pMMP-9). Molecular weight markers (open arrowheads on the left) were used to estimate molecular masses. Densitometric analyses are presented as the relative ratio of pMMP-9 levels. The pMMP-9 in the BAL fluids of EO mice (Co) is arbitrarily presented as 1. Data represent the means $\pm$ SEMs from 6 independent experiments. * $P < .05$ versus EO. † $P < .05$ versus EO plus TDI.

RESULTS

MMP inhibitors and doxycycline decreased MMP-9 mRNA and protein levels in BAL fluids and lung tissues of TDI-sensitized and TDI-challenged mice

Matrix metalloproteinase 9 mRNA and protein levels in BAL fluids and lung tissues were significantly increased at 72 hours after TDI inhalation compared with levels in the control group (Fig 1). Administration of doxycycline, MMPI-I, or MMPI-II significantly reduced an increase in

expression of MMP-9 mRNA and protein in the lung tissues at 72 hours after TDI inhalation (Fig 1, A-D). In addition, the administration of these agents substantially reduced the levels of proMMP-9 in BAL fluids at 72 hours after TDI inhalation (Fig 1, E and F).

Localization of immunoreactive MMP-9 in lung tissues and BAL fluids of ovalbumin-induced asthma

Immunohistochemical analyses of lung tissue of TDI-exposed mice revealed immunoreactive MMP-9 on

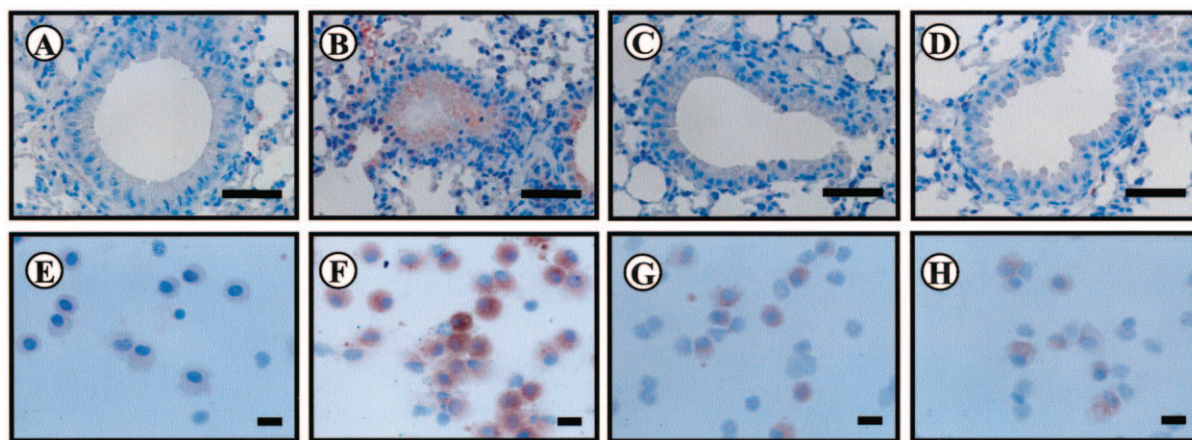


FIG 2. Localization of immunoreactive MMP-9 in lung tissues and BAL fluids of TDI-sensitized and TDI-challenged mice. Sampling was performed at 72 hours after the TDI challenge in the mice treated with vehicle only (**A** and **E**), EO plus TDI (**B** and **F**), EO plus TDI plus doxycycline (**C** and **G**), or EO plus TDI plus MMPI-I (**D** and **H**). **A-D**, Representative light microscopy of MMP-9–positive cells in the bronchioles; the brown color indicates MMP-9–positive cells. **E-H**, Representative light microscopy of MMP-9–positive cells in the BAL fluids; the dark brown color indicates MMP-9–positive cells. Bars indicate scale of 50 (**A-D**) or 10 (**E-H**) μ m.

inflammatory cells and extracellular matrix around bronchioles, and on inflammatory cells and debris in the airway lumen (Fig 2, *B*). In control and TDI-sensitized and TDI-challenged mice treated with doxycycline or MMP inhibitor, MMP-9 positive cells were hardly detected (Fig 2, *A*, *C*, and *D*).

Immunocytologic analysis of BAL fluids showed localization of immunoreactive MMP-9 in the precipitated cells, including cells that resembled macrophages, neutrophils, and eosinophils, from the TDI-induced mice (Fig 2, *F*). However, immunoreactive MMP-9 was markedly reduced in BAL cells from control mice and from TDI-sensitized and TDI-challenged mice treated with doxycycline or MMP inhibitor (Fig 2, *E*, *G*, and *H*).

MMP inhibitors and doxycycline decreased the increased numbers of inflammatory cells in BAL fluids of TDI-sensitized and TDI-challenged mice

The numbers of total cells, lymphocytes, neutrophils, and eosinophils in BAL fluids were significantly increased at 72 hours after TDI inhalation compared with the numbers after inhalation of the control agent, ethyl acetate/olive oil. The administration of doxycycline, MMPI-I, or MMPI-II significantly reduced the increase in total cells, lymphocytes, neutrophils, and eosinophils elicited in the airway lumen 3 days after TDI inhalation (Fig 3). These results indicate that the migration of inflammatory cells into the bronchial lumens of TDI-sensitized and TDI-challenged mice was strongly inhibited by doxycycline and MMP inhibitor.

Effect of doxycycline, MMPI-I, or MMPI-II on pathologic changes of TDI-induced asthma

Histologic analyses revealed typical pathologic features of asthma in the TDI-exposed mice. Numerous inflam-

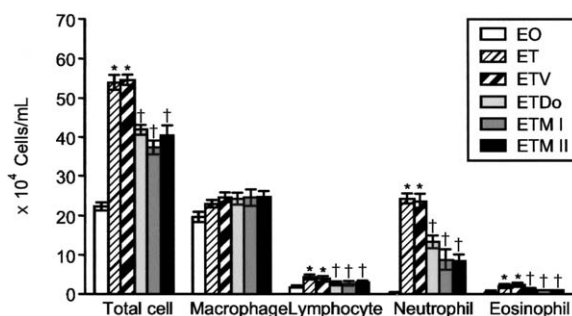


FIG 3. Effect of doxycycline, MMPI-I, or MMPI-II on total and differential cellular components of BAL fluids in the TDI-sensitized and TDI-challenged mice. Sampling was performed after treatment with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETV), EO plus TDI plus doxycycline (ETDo), EO plus TDI plus MMPI-I (ETM I), or EO plus TDI plus MMPI-II (ETM II). Bars represent the means $\pm$ SEMs from 6 independent experiments. * $P < .05$ versus EO. † $P < .05$ versus EO plus TDI.

matory cells infiltrated around the bronchioles and blood vessels (Fig 4, *B*). Doxycycline-treated (Fig 4, *D*), MMPI-I-treated (Fig 4, *E*), or MMPI-II-treated mice (Fig 4, *F*) showed marked reduction in the infiltration of inflammatory cells in the peribronchiolar and perivascular region. In contrast, no significant changes were observed in vehicle-treated mice (Fig 4, *C*). The scores of peribronchial, perivascular, and total lung inflammation were increased significantly at 72 hours after the TDI inhalation compared with the scores after inhalation of the control agent, ethyl acetate/olive oil (see Fig E2 in the Online Repository at www.mosby.com/jaci). The increased peribronchial, perivascular, and total lung inflammation after TDI inhalation were significantly reduced by the administration of doxycycline, MMPI-I, or MMPI-II. These results suggest that doxycycline, MMPI-I, or MMPI-II inhibits TDI-induced inflammation in the lungs.

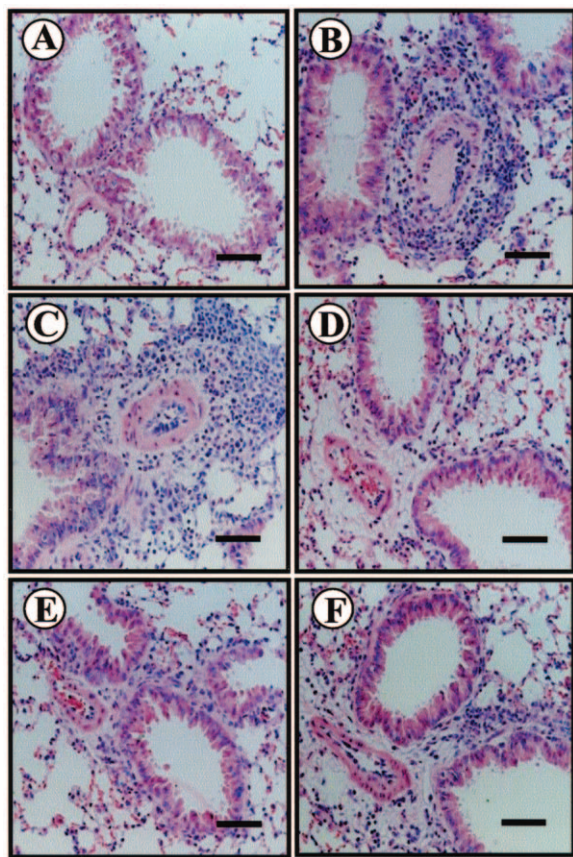


FIG 4. Effect of doxycycline, MMPI-I, or MMPI-II in lung tissues of TDI-sensitized and TDI-challenged mice. Representative hematoxylin and eosin-stained sections of the lungs. Sampling was performed at 72 hours after the TDI challenge in the mice treated with vehicle only (A), EO plus TDI (B), EO plus TDI plus DMSO (C), EO plus TDI plus doxycycline (D), EO plus TDI plus MMPI-I (E), or EO plus TDI plus MMPI-II (F). Bars indicate scale of 50 μ m.

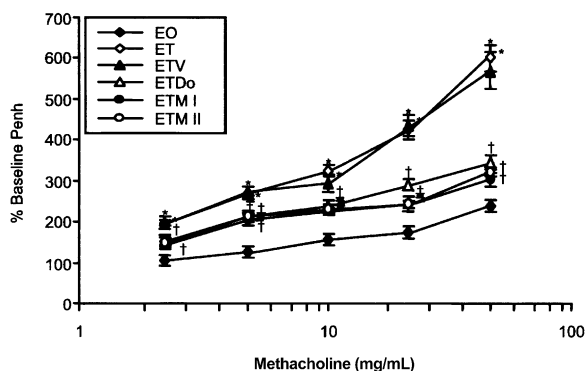


FIG 5. Effect of TDI challenge and doxycycline, MMPI-I, or MMPI-II on airway responsiveness in TDI-sensitized and TDI-challenged mice. Airway responsiveness was measured at 72 hours after the TDI challenge in the mice treated with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETV), EO plus TDI plus doxycycline (ETD), EO plus TDI plus MMPI-I (ETM I), or EO plus TDI plus MMPI-II (ETM II). Data represent the means $\pm$ SEMs from 6 independent experiments. * $P < .05$ versus EO. † $P < .05$ versus EO plus TDI.

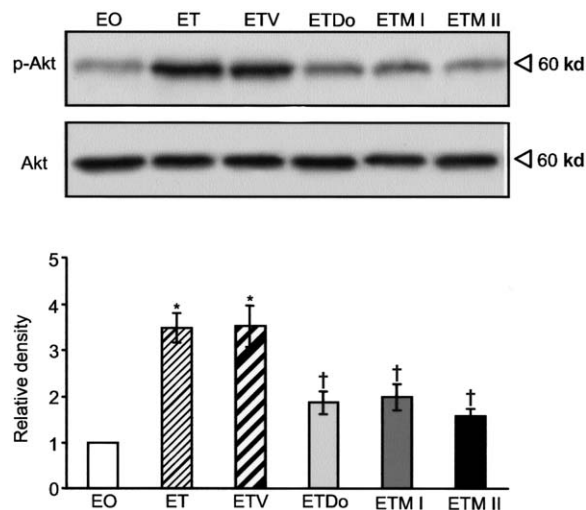


FIG 6. Effect of doxycycline, MMPI-I, or MMPI-II on p-Akt and Akt protein expression in lung tissues of TDI-sensitized and TDI-challenged mice. p-Akt and Akt protein expression were measured at 72 hours after the TDI challenge in the mice treated with vehicle only (EO), EO plus TDI (ET), EO plus TDI plus DMSO (ETV), EO plus TDI plus doxycycline (ETD), EO plus TDI plus MMPI-I (ETM I), or EO plus TDI plus MMPI-II (ETM II). Densitometric analyses are presented as the relative ratio of p-Akt to Akt. The relative ratio of p-Akt in the lung tissues of EO mice is arbitrarily presented as 1. Data represent the means $\pm$ SEMs from 6 independent experiments. * $P < .05$ versus EO. † $P < .05$ versus EO plus TDI.

MMP inhibitors and doxycycline reduced TDI-induced airway hyperresponsiveness

Airway responsiveness was assessed as a percent increase of enhanced pause (Penh) in response to increasing doses of methacholine. In TDI-sensitized and TDI-challenged mice, the dose-response curve of percent Penh shifted to the left side compared with that of control mice (Fig 5). In addition, the percent Penh produced by methacholine (2.5-50 mg/mL) increased significantly in the TDI-sensitized and TDI-challenged mice compared with controls. Sensitized mice treated with doxycycline, MMPI-I, or MMPI-II showed a dose-response curve of percent Penh that shifted to the right compared with untreated mice. Thus, these results indicate that doxycycline, MMPI-I, or MMPI-II treatment reduces TDI-induced airway hyperresponsiveness.

Determination of Akt phosphorylation

To evaluate whether the effects of doxycycline, MMPI-I, or MMPI-II on TDI-induced bronchial inflammation and airway hyperresponsiveness were mediated through the phosphatidylinositol 3-kinase pathway, we used Western blotting to determine Akt phosphorylation. Levels of p-Akt protein in the lung tissues were increased at 72 hours after TDI inhalation compared with levels in the control mice (Fig 6). However, no significant changes in Akt protein levels were observed in any of the groups tested. The increased p-Akt but not Akt protein levels in lung tissues at 72 hours after TDI inhalation were

significantly reduced by the administration of doxycycline, MMPI-I, or MMPI-II.

DISCUSSION

To our knowledge, this report is the first to describe the effect of doxycycline in airway hyperresponsiveness and inflammation in a murine model of TDI-induced asthma. We have found that administration of doxycycline and MMP inhibitors reduced the asthma phenotype.

Tetracyclines possess antimicrobial properties as well as inhibitory effects on MMP activity.¹³⁻¹⁸ The mechanisms by which doxycycline inhibits the MMPs are not completely understood. Doxycycline binds directly to Zn²⁺ or Ca²⁺ associated with the enzyme, resulting in blocking the active site or inducing conformational changes that render the proenzyme susceptible to fragmentation during activation.^{14,37} In this study, doxycycline and MMP inhibitors (MMPI-I and MMPI-II) decreased MMP-9 mRNA and protein levels in BAL fluids and lung tissues of TDI-sensitized and TDI-challenged mice. Doxycycline and MMP inhibitors inhibit MMP-9 activity, although they are not specific inhibitors of MMP-9. On the basis of the marked increase in MMP-9 in the lungs by TDI inhalation, we administered doxycycline or MMP inhibitors to examine the therapeutic effect. Doxycycline and MMP inhibitors were dramatically effective on all pathophysiologic features examined. One possible mechanism for the effectiveness of doxycycline and MMP inhibitors is that MMP-9 alters the ECM to allow the migration of inflammatory cells, including neutrophils and eosinophils, to the airways. In this study, we found that administration of doxycycline and MMP inhibitors inhibits not only the MMP-9 activity in the lungs but also the migration of inflammatory cells through the endothelial and epithelial basement membrane. Without these inflammatory cells, the other pathologic changes do not occur. Thus, doxycycline and MMP inhibitors abolished airway hyperresponsiveness. Our results suggest that doxycycline and MMP inhibitors may be good therapeutic agents for TDI-induced asthma.

A balance between expression of proteases and antiproteases, particularly MMPs and their inhibitors, maintains homeostasis between synthesis and degradation of ECM components under normal conditions. The expression of these proteases and antiproteases is critical in tissue repair and remodeling in some pulmonary inflammatory diseases.³⁸⁻⁴¹ Previous studies have demonstrated that MMP-9 may play a role in chronic airway inflammation, including induction of migration of eosinophils, lymphocytes, and neutrophils across basement membranes, and remodeling in asthma.⁸⁻¹² Recently, we demonstrated that MMP-9 plays a crucial role in the infiltration of airway inflammatory cells and the induction of airway hyperresponsiveness in TDI-induced asthma.³⁰ In this study, we also found that the release of MMP-9 was upregulated in association with

the accumulation of inflammatory cells to airways. Moreover, administration of doxycycline or MMP inhibitors prevented migration of inflammatory cells to the airways. Therefore, these findings indicate that MMP-9 may have an important role in inducing and maintaining TDI-induced asthma.

Several studies have provided evidence of involvement of neutrophils in the pathogenesis of TDI-induced asthma.^{3,4,30,32} Neutrophils could be an important source of proinflammatory cytokines and proteolytic enzymes.^{42,43} Sustained release of these inflammatory mediators enhances neutrophil-induced inflammation in the airway and subsequent airway hyperreactivity.^{44,45} Moreover, the number of eosinophils in the BAL fluid increased in our TDI-induced asthma model. Eosinophils release toxic granule proteins during their activation. These products can cause airway hyperresponsiveness.⁴⁶ These findings suggest that inflammatory mediators of neutrophils and eosinophils may play critical roles in development of airway hyperresponsiveness in TDI-induced asthma.

Many inflammatory mediators attract and activate inflammatory cells via signal transduction pathways involving the enzyme phosphatidylinositol 3-kinase.⁴⁷⁻⁵⁰ Tigani et al⁵¹ reported that phosphatidylinositol 3-kinase inhibitor reduced the increased number of eosinophils and eosinophil peroxidase activity in the BAL fluid of allergen-challenged animals. We previously found that phosphatidylinositol 3-kinase activity was increased significantly after allergen challenge in a murine model of asthma.³³ To support the contention that the effects of doxycycline or MMP inhibitors on TDI-induced bronchial inflammation and airway hyperresponsiveness were mediated through the phosphatidylinositol 3-kinase pathway in this study, we used Western blotting to determine Akt phosphorylation. Levels of p-Akt protein in the lung tissues were increased after TDI inhalation compared with levels in the control mice. The increased p-Akt but not Akt protein levels in lung tissues after TDI inhalation were significantly reduced by the administration of doxycycline or MMP inhibitors. Our data are consistent with the results obtained in previous studies^{52,53} showing that the phosphatidylinositol 3-kinase pathway regulates the production of MMP-9. Esteve et al⁵² reported that phosphatidylinositol 3-kinase is likely involved in the signaling cascade that inhibits transcription of MMP-9 gene.

In conclusion, our results strongly indicate that doxycycline and MMP inhibitors may reduce airway inflammation and hyperresponsiveness through phosphatidylinositol 3-kinase pathway/Akt in a murine model of TDI-induced asthma.

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