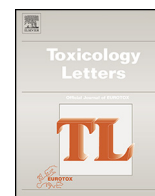




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Mechanism of E-cadherin redistribution in bronchial airway epithelial cells in a TDI-induced asthma model[☆]

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H I G H L I G H T S

- Aberrant distribution of E-cadherin in airway mucus in TDI-induced mouse asthma.
- ERK1/2 was involved in TDI-induced aberrant distribution of E-cadherin.
- Dexamethasone strongly inhibited aberrant distribution of E-cadherin.
- Dexamethasone strongly repressed ERK1/2 activation.

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A B S T R A C T

E-cadherin (epithelial cadherin), a transmembrane protein, provides essential architecture and immunological function to the airway epithelium, a barrier structure that plays an essential role in asthma pathogenesis. Toluene diisocyanate (TDI) is currently one of the leading causes of occupational asthma. However, relatively few studies have been undertaken to determine the biological effects of TDI on the barrier properties of airway epithelium, but it is known that TDI can damage airway epithelial tight junctions in vitro. Here, we hypothesize that TDI can injure E-cadherin both in normal and allergic-induced airway epithelium. To test this, we developed a murine model of TDI-induced asthma characterized by neutrophil-dominated airway inflammation, epithelial shedding, and obvious aberrant distribution of E-cadherin. Pretreatment with dexamethasone (DEX) significantly rescued the immunoreactivity of E-cadherin, accompanied by increased neutrophils in bronchoalveolar lavage fluid (BALF). In vitro, TDI-human serum albumin (HSA)-induced redistribution of E-cadherin was associated with extracellular signal-regulated kinase (ERK)1/2 activation. The inhibition of phospho-ERK (p-ERK)1/2 by DEX can partly reverse this reaction. These results indicate that E-cadherin redistribution may be an important contributor in the generation of TDI-induced asthma.

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1. Background

Epithelial cells play an important role in maintaining physiological homeostasis. They display a highly regulated and impermeable barrier through the formation of tight junctions (TJs), which are composed of zonula occludens (ZO) 1–3, occludin and claudins 1–5, as well as adherens junctions (AJs), which consist of E-cadherin, β -catenin and α -catenin (Nawijn et al., 2011; Xiao et al., 2011). E-cadherin provides critical architectural structure and immunological function of airway epithelium through the regulation of epithelial junctions as well as through proliferation, differentiation, and production of proinflammatory mediators such as thymus and activation-regulated chemokine (TARC) and thymic stromal lymphopoietin (TSLP) (Heijink et al., 2007; Tunggal et al., 2005). Xiao et al. (2011) showed that E-cadherin expression was

Abbreviations: ERK, extracellular signal-regulated kinase; HDM, house dust mite; HBE, human bronchial epithelial; TDI, toluene diisocyanate; TSLP, thymic stromal lymphopoietin; TJs, tight junctions; ZO, zonula occludens.

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significantly lower in the bronchial biopsies of asthmatic subjects compared with nonasthmatics. Additionally, induction of E-cadherin expression can repress NF- κ B activity (Solanas et al., 2008), which is critical for evoking airway inflammation. Thus, E-cadherin could be an epithelial molecular switch that mediates the immunological decision between a tolerogenic and a proinflammatory/immunogenic phenotype (Nawijn et al., 2011).

Toluene diisocyanate (TDI) is currently one of the leading causes of occupational asthma, and accounts for 9–15% of adult asthma in industrialized countries (Balmes et al., 2003; Maestrelli et al., 2009). However, relatively few studies have been undertaken to determine the biological effects of TDI on epithelial barrier properties. In our laboratory, we have demonstrated that TDI can reduce tight junctions and increase the permeability of bronchial epithelial cell in vitro (Zhao et al., 2009). However, we do not yet know whether TDI can influence E-cadherin expression and distribution in both healthy and allergic animals.

Although glucocorticoids are by far the most effective anti-inflammatory therapy for asthma, their potential effects on the distribution and expression of E-cadherin have not been clarified.

Accordingly, our objective was to further define the influence of TDI on the expression and distribution of E-cadherin in a TDI-induced asthma murine model as well as define the mechanism responsible for this in vitro. An additional aim of our study was to evaluate the inhibitory effect of dexamethasone (DEX) in this process.

2. Materials and methods

2.1. Materials

Toluene-2,4-diisocyanate, concanavaline A, DEX 21-phosphate, methacholine and acetone were obtained from Sigma–Aldrich (Shanghai, China). Pentobarbital was obtained from Weijia Biotechnology Company (Guangzhou, China). The vehicle (AOO) used to dissolve TDI consisted of a mixture of 2 vol. of acetone and 3 vol. of olive oil (Selection de Almazara, Carbonell, Madrid, Spain) for dermal sensitization, and 1 vol. of acetone and 4 vol. of olive oil for the challenge. Concentrations of TDI are provided as percent (v/v) in AOO. PD98095 was purchased from Beyotime Institute of Biotechnology (Shanghai, China), 16HBE from Fuxiang Biocompany (Shanghai, China), E-cadherin, phospho-ERK1/2 (p-ERK) and total ERK1/2 (t-ERK) antibody from Santa Cruz Biotech (Santa Cruz, CA, USA), peroxidase conjugated secondary antibody and diaminobenzidine (DAB) peroxidase substrate kit from Zhong Shan Jin Qiao Biotech (Beijing, China).

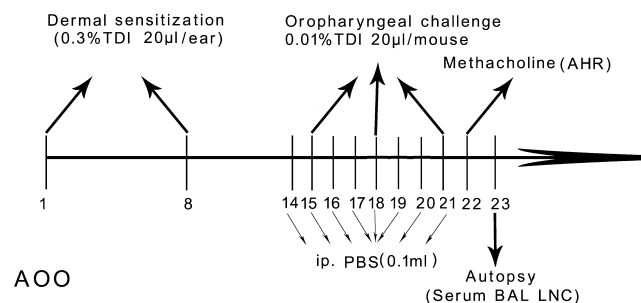
2.2. Intraperitoneal injection of dexamethasone (DEX) in a TDI induced asthma model

Schematic diagram of the experimental protocol are shown in Fig. 1. Six-week-old male BALB/c mice (Southern Medical University) were randomized to the following three groups (20 mice/group): (1) Vehicle (AOO)-sensitized, AOO-challenged, and PBS-treated mice (AOO group); (2) TDI-sensitized, TDI-challenged, and PBS-treated (TDI group); and (3) TDI-sensitized, TDI-challenged, and DEX-treated mice (DEX group). TDI-asthma model was prepared by a modification of De Vooght's method (De Vooght et al., 2009). Briefly, on days 1 and 8, the mice were dermally treated with 0.3% TDI on the dorsum of both ears (20 μ L/ear). On days 15, 18, and 21 the mice underwent an oropharyngeal aspiration (20 μ L) of 0.01% TDI under light pentobarbital anesthesia (50 mg/kg body weight). For a control group, sham mice were sensitized and challenged with a mixture of acetone and olive oil. DEX 21-phosphate dissolved in endotoxin-free PBS (1 mg/kg) was given to the TDI-induced asthma mice via intraperitoneal injection 1 day before the TDI challenge for 8 consecutive days. By comparison, the AOO and TDI groups received injections of 0.1 mL of PBS. All animal experimental protocols were approved by Animal Subjects Committee, Nanfang Hospital (Application No. NFYY-2011-056). The mice were housed in a SPF house with 12-h dark/light cycles.

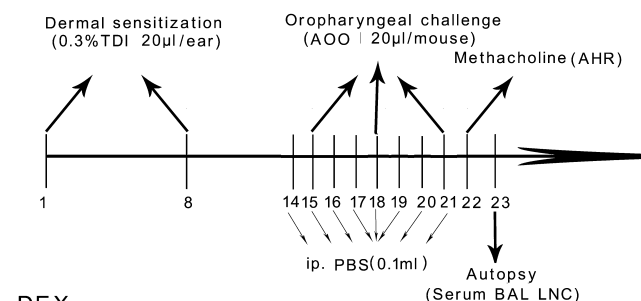
2.3. Measuring airway reactivity to methacholine

Airway parameters were measured 1 day after the last challenge. Mice were placed in a barometric plethysmographic chamber (Buxco Electronics, Troy, NY), and baseline readings were taken for 3 min. Pulmonary assessment of enhanced pause (Penh) was assessed using a protocol by Lomask (2006).

TDI



AOO



DEX

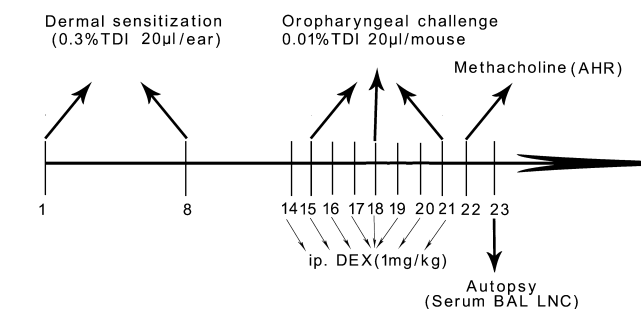


Fig. 1. Schematic diagram of the experimental protocol. Briefly, on day 1 and 8, the animals were dermally treated with 0.3% TDI or vehicle on the dorsum of both ears (20 μ L/ear). On day 15, 18 and 21 the mice underwent an oropharyngeal aspiration (20 μ L) of 0.01% TDI or vehicle. DEX 21-phosphate dissolved in endotoxin-free PBS (1 mg/kg) was given to the TDI-induced asthma mice via intraperitoneal injection 1 day before the TDI challenge for 8 consecutive days. By comparison, the AOO and TDI groups received injections of 0.1 mL of PBS. AHR, airway hyper-reactivity; BAL, broncho-alveolar lavage; LNC, lymph node cells; Penh, enhanced pause; TDI, toluene diisocyanate; vehicle: acetone–olive oil.

2.4. Preparing lymph node cells

Twenty-four hours after measuring airway parameters, the mice were sacrificed with pentobarbital (100 mg/kg, i.p. weight). Blood samples were first taken from the retro-orbital plexus/sinus, then centrifuged (4000 $\times$ g, 20 min) and supernatant was harvested and stored at -80°C .

Superficial parotid lymph nodes were dissected from the mice, which were processed for each mouse separately. The lymph nodes were kept on ice in RPMI-1640 medium, and cell suspensions were obtained by pressing the lymph nodes through a 100- μ m cell strainer and rinsing with 10 mL tissue culture medium (RPMI-1640). Cells were counted using a Bürker hemocytometer. Lymphocytes were then washed three times and suspended (10^7 cells/mL) in complete tissue culture medium (RPMI-1640 supplemented with 10% heat-inactivated FBS). Cells were seeded into 48-well culture plates at a density of 10^6 cells/mL and incubated in complete RPMI-1640 medium for 48 h with 3.5 mg/mL of concanavaline A (ConA), then centrifuged (3000 $\times$ g, 10 min) and the supernatants stored at -80°C .

2.5. Bronchoalveolar lavage fluid (BALF)

After superficial parotid lymph nodes were obtained, PBS was infused slowly into the lungs and withdrawn via a cannula inserted into the trachea to obtain BALF. The cells in the BALF were counted, and a cytospin sample (Shandon Scientific, Runcorn, UK) was prepared and stained using hematoxylin and eosin (H&E) (Jian Chen, Nanjing, China). The remaining BALF was centrifuged at 4000 $\times$ g for 15 min, and the supernatant was collected and stored at -80°C for further analyses.

2.6. Preparing lung tissue for histology, Western blot, and immunohistochemistry

Mice were humanely euthanized with pentobarbital and lungs removed. The left lung was infused with 4% formaldehyde to fully inflate all lobes (judged visually), and the lungs fixed and embedded in paraffin. Lung sections (4 μ m) were cut with a Leica microtome 2030 (Leica Microsystems Nussloch GmbH, Nussloch, Germany). Slides were stained with hematoxylin and eosin (H&E). The right lung was removed and stored at -80°C .

For immunohistochemistry of E-cadherin, lung tissue on slides was treated with 0.3% H_2O_2 for 10 min to block endogenous peroxidase and then incubated 4°C overnight with anti-E-cadherin antibody. After incubation with peroxidase-conjugated secondary antibody, signals were visualized with a DAB peroxidase substrate kit. For Western blot analysis, lung tissue (0.15 g) from animals was homogenized in 4 mL of ice-cold homogenization buffer (250 mM sucrose, 20 mM Tris, 1 mM dithiothreitol). Homogenates were then centrifuged at 4°C , $9000 \times g$ for 30 min. Supernatants were collected and centrifuged at 4°C , $14,000 \times g$ for 30 min, which were then analyzed by Western blotting for p-ERK1/2 and t-ERK1/2, as described below.

2.7. Preparation of TDI-HSA conjugates

TDI-HSA conjugates were prepared by a modification of Son's method (Son et al., 1998) previously described (Zhao et al., 2009).

2.8. Preparation of cells

16HBE cells (Shanghai Fuxiang Biological Technology Co. Ltd., ATCC, Portland, Oregon, USA), a differentiated SV-40 transformed bronchial epithelial cell line, was chosen because of its highly characterized intercellular adhesions (Wan et al., 2000). 16HBE-cells were cultured in DMEM (Ginuo Biopharm Technology Co., Shanghai, China) with 10% fetal calf serum (FCS, Invitrogen, Gibco, CA, USA) and placed in a humidified incubator at 37°C with 5% CO_2 . When reaching 90% confluence, the cells were passaged and seeded to proper culture plates at a density of 10^4 – 10^5 cells/ cm^2 . After 24 h incubation, the culture medium was changed to serum free-DMEM. Different concentrations of TDI-HSA conjugates, DEX (10^{-6} , 10^{-4} M), PD98095 (10^{-7} M) were then added to the culture medium for indicated times. The concentration of DEX and PD98095 used in this experiment followed previous reports (Ogawa et al., 2006; Sekiyama et al., 2012). Cell viability was detected with a MTT colorimetric assay.

2.9. Western blot and immunofluorescence

Cells were harvested and washed twice with ice-cold PBS, then lysed using a cell lysis buffer (KeyGen Biotech, Nanjing, China) containing protease inhibitor, Calceinurin inhibitors, and PMSF for 30 min, and centrifuged at $12,000 \times g$ for 15 min at 4°C . The protein products were normalized and boiled with standard SDS sample buffer, then separated by 10% sodium dodecyl sulfate-polyacrylamide (SDS) gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% skim milk at room temperature for 2 h, and incubated with anti-E-cadherin, anti-p-ERK1/2, anti-t-ERK1/2 respectively at 4°C overnight, then with anti-rabbit IgG at room temperature for 1 h. The immunoreactive bands were detected with the enhanced chemiluminescence ECL system (Weijia Technology Co, Ltd., Guangzhou, China).

In a parallel experiment, collected cells were fixed with 1% paraformaldehyde at room temperature for 20 min, washed with PBS for 30 min, and incubated with 0.2% Triton X-100 in PBS for 10 min, and rinsed again with PBS. Cells were blocked with 5% skim milk in PBS for 2 h. All samples were subsequently incubated with monoclonal anti-E-cadherin and FITC (green)-linked anti-rabbit IgG, cell nuclei were stained with 4',6-diamidino-2-phenylindole dihydrochloride (Sigma-Aldrich, Shanghai, China). A laser-scanning confocal microscope (Olympus, Tokyo, Japan) was used to examine the distribution of E-cadherin in 16HBE cells. Photographs were processed with Photoshop 7.0.

2.10. Statistical analysis

Statistical analysis was performed using SPSS version 17.0. Data are expressed as mean $\pm$ standard error (SE) and comparisons among groups were analyzed by one-way analysis of variance (ANOVA) accompanied by Bonferroni post hoc test for multiple comparisons. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of DEX on the airway reactivity and airway inflammation in a TDI induced asthma model

Effects of DEX on airway hyperreactivity (AHR), IL-4 and IFN- γ in auricular lymph node cells and total IgE in the serum are shown in Fig. 2. The differences of Penh values between TDI group and AOO group were significant when stimulated by Ach (6.2, 12.5 and

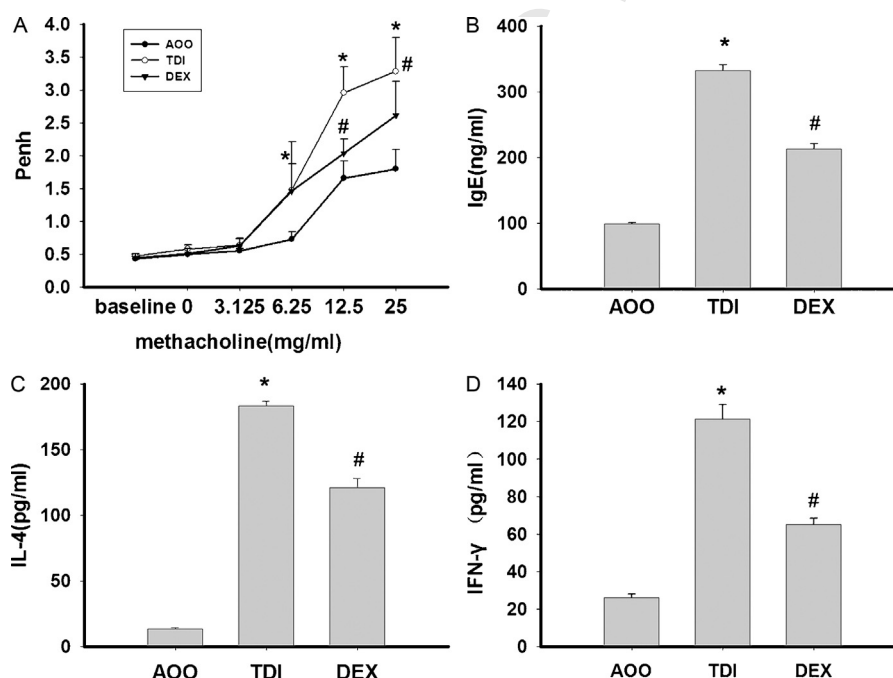


Fig. 2. Dexamethasone (DEX)-reduced, TDI-induced airway hyper-responsiveness (AHR), IL-4 and IFN- γ in auricular lymph node cells and total IgE in serum. At 24 h after the last challenge, AHR to methacholine was assessed (A) on the next day, mice were sacrificed. Blood samples were first taken from the retro-orbital plexus/sinus, and serum total IgE (B) was measured using ELISA. The auricular lymph nodes were processed for each mice separately, and the lymph node cells were cultured with concanavaline A (ConA, 3.5 g/mL) for 48 h. IL-4 (C), and IFN- γ (D) were measured via ELISA in the supernatant. Experimental groups are AOO group: AOO-sensitized, AOO-challenged, and PBS-treated mice; TDI group: TDI-sensitized, TDI-challenged, and PBS-treated, and DEX group: TDI-sensitized, TDI-challenged, and DEX-treated mice. Data are presented as mean $\pm$ SE, $n = 7$ –10 mice per group. * $p < 0.05$ compared with the AOO group. # $p < 0.05$ compared with the TDI group.

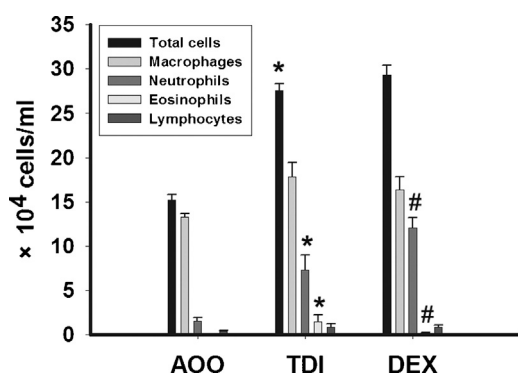


Fig. 3. Cellular profiles of bronchoalveolar lavage fluid (BALF). The number of total cell counts, macrophages, neutrophils, eosinophils and lymphocytes were determined in the BALF. BALF were counted on cytopsin preparation. Data are presented as mean $\pm$ SE, $n = 7$ –10 per group. * $p < 0.05$ compared with the AOO group, # $p < 0.05$ compared with the TDI group.

25.0 mg/mL ($p < 0.05$). While the AHR level was reduced in mice treated with DEX compared with the TDI group when stimulated by Ach (12.5 and 25.0 mg/mL) ($p < 0.05$), there were notable increases in IL-4, IFN- γ , and total IgE, which were reduced by DEX.

DEX strongly inhibited TDI-induced eosinophil recruitment, but it did not decrease the number of neutrophils and total cells, as expected (Fig. 3). We found a further increased number of neutrophils in the DEX group. On histological examination, TDI-sensitized/challenged mice had a heavy infiltration of inflammatory cells, epithelial cell proliferation and certain degree of epithelial shedding, while DEX displayed extensive capacity of suppressing inflammatory infiltration in the peribronchial and perivascular regions (Fig. 4).

3.2. Effect of DEX on the expression and localization of E-cadherin in airway mucosa

In the AOO group, E-cadherin localized at the lateral side and apicolateral border (Fig. 5A). In the TDI group, the immunoreactivity of E-cadherin was notably decreased at the epithelial cell–cell contact and diffused in the cytoplasm. In the DEX group, the immunoreactivity of E-cadherin was partly rescued.

3.3. Effect of DEX on the expression of ERK1/2 in lung tissue

Western blotting showed a remarkable increase of p-ERK1/2/t-ERK1/2 ratio in TDI group; when pretreated with DEX, this ratio decreased, compared with TDI group (Fig. 5B).

3.4. Preparation and determination of toluene diisocyanate-human serum albumin conjugates

The concentrations of TDI and HSA were 20.444 and 1853.173 mg/mL, respectively. The molar ratio of TDI to HSA was

4.212. Each concentration of TDI-HSA we used had no detectable effect on cell viability (data not shown).

3.5. Changes in the expression and localization of E-cadherin protein induced by TDI-HSA

Confocal microscopy showed strong E-cadherin staining of 16HBE cell–cell contacts in both untreated cells and HSA treated cells, and TDI-HSA (40–100 μ g/mL) exposure for 24 h induced a dose-dependent redistribution of E-cadherin (Fig. 5A). However, no changes in E-cadherin expression were seen by Western blot analysis after treatment with TDI-HSA (Fig. 6B).

3.6. Protective role of DEX and PD98095 on TDI-HSA-induced E-cadherin aberrant distribution of 16HBE

In comparison with mice treated with TDI-HSA, mice pretreated with DEX for 1 h (Fig. 7A) partially prevented the effect of TDI on E-cadherin. A similar change was seen in the PD98095-treated group. Western blot analysis further showed that TDI-HSA exposure increased threonine/tyrosine phosphorylation of ERK1/2, and it was stronger after 10 min of stimulation compared with 30 min of stimulation (Fig. 8A), and DEX partly inhibited ERK1/2 activation (Fig. 8B). This suggested that ERK1/2 pathway might be involved in the aberrant distribution of E-cadherin induced by TDI-HSA and the protective role of DEX in this process.

4. Discussion

To our knowledge, this is the first report to demonstrate that E-cadherin distribution is aberrant in the airway of TDI-induced asthmatic mice and that activation of ERK1/2 by TDI redistributes E-cadherin in vitro.

TDI-induced asthma is characterized by AHR and Th2-dominated airway inflammation (Ban et al., 2006), which we verified in our study. Also in line with previous reports (De Vooght et al., 2009), we found increased numbers of total cells, eosinophils, and neutrophils in BALF; raised levels of IL-4 and IFN- γ in auricular lymph nodes; and higher total IgE serum concentrations and more infiltration of inflammatory cells in the peribronchial and perivascular regions of TDI-treated mice. Taken together, these findings suggest that a successful TDI-induced asthma model was developed in our study. In this model, we know that using penh to measure airway reactivity is controversial; however, Lomask (2006) reported that it did have a relationship to airway resistance under strict testing conditions. Furthermore, Tarkowski et al. (2007) demonstrated that AHR indicated by penh does agree with resistance obtained by FlexiVent in a TDI-induced asthma mouse model.

Also, in those mice pretreated with DEX, the luminal neutrophil influx was enhanced while tissue inflammation was reduced. The reason for this may be that glucocorticoid treatment inhibits apoptosis of neutrophils (Cox, 1995). Consistent with our results, Ito et al. (2008) found that DEX almost completely suppressed the

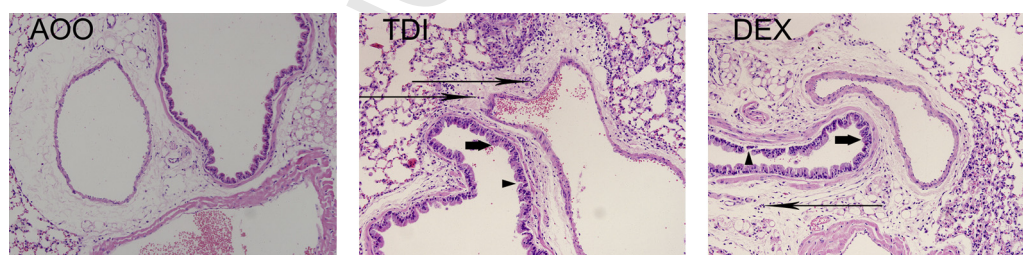


Fig. 4. Histology of lung tissue after oropharyngeal aspiration. H&E staining was performed on lung slices 48 h after challenging with the vehicle or TDI. Experimental groups are AOO, TDI and DEX, $n = 7$ –10 per group ($\rightarrow$) inflammation, ($\blacktriangle$) epithelial shedding and ($\blacksquare$) epithelial proliferation. Original magnification 200 $\times$.

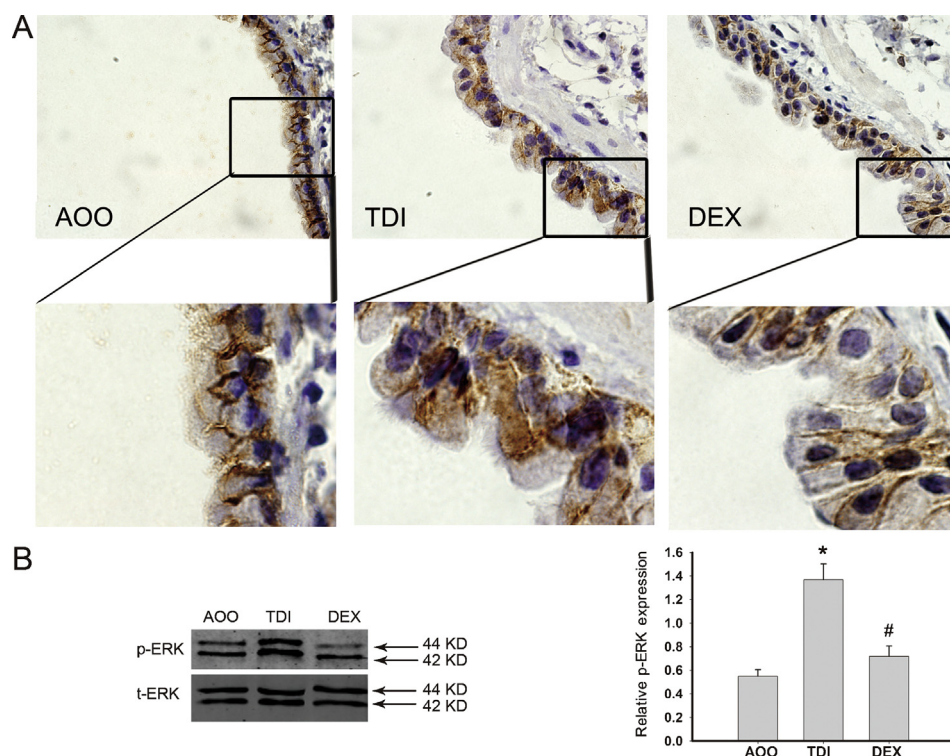


Fig. 5. Immunohistochemical staining for E-cadherin in bronchial epithelium of airway mucosa and the phosphorylation of ERK1/2 in lung tissue. Experimental groups are AOO, TDI and DEX, $n = 7-10$ mice per group. In the AOO group, E-cadherin localized especially at the lateral side and apicolateral border. In the TDI group, the immunoreactivity of E-cadherin significantly decreased at the epithelial cell-cell contact and diffused into the cytoplasm; in the DEX group, the immunoreactivity of E-cadherin was partly rescued (A). Western blotting showed p-ERK1/2/t-ERK1/2 ratio was increased in TDI group; when pretreated with DEX, this ratio decreased, compared with TDI group (B). Original magnification 1000 $\times$. * $p < 0.05$ compared with the AOO group. # $p < 0.05$ compared with the TDI group.

increase in eosinophils and T lymphocytes but had little effect on neutrophil count in BALF. They also further found increased neutrophils and decreased eosinophils and T cells in lung tissues, which needs to be further clarified in our study.

Previous studies (Lange et al., 1999; Zhao et al., 2009) have shown that TDI could increase airway epithelial cell permeability and impair TJs, indicating a direct role for TDI in the dysfunction of TJs and epithelial barrier. Using a TDI-induced model, we found that

TDI could injure airway epithelium, inflict some epithelial shedding and induce E-cadherin redistribution, as expected.

However, the mechanisms responsible for this remain uncertain. In a previous study (Evans et al., 2002), it was reported that E-cadherin extracellular domain can be cleaved by neutrophils during epithelial penetration in an OVA-induced mouse model, suggesting a potential role of neutrophils in the dislocation of E-cadherin in TDI-induced asthma. In this study, we found that mice

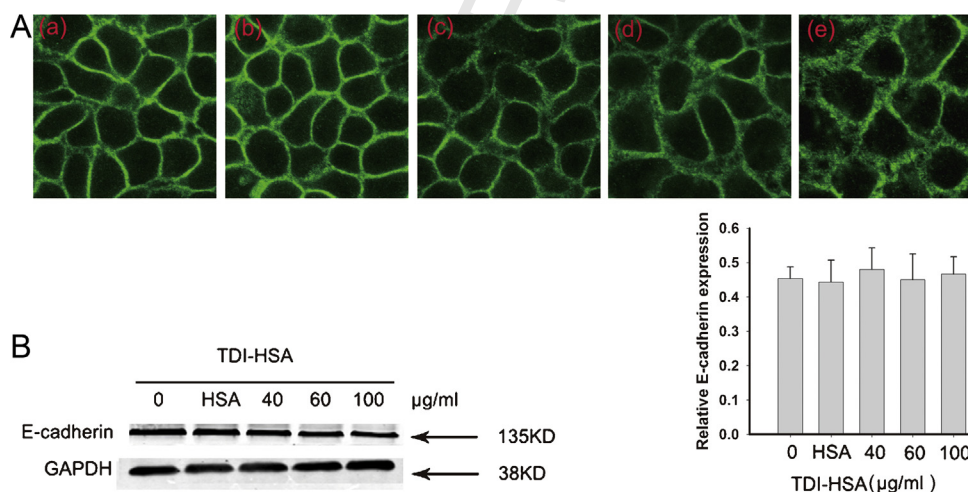


Fig. 6. Aberrant distribution of E-cadherin induced by TDI-HSA. 16HBE cells were treated with different concentrations of TDI-HSA conjugates for 24 h, Western blot and immunofluorescence were used to evaluate the expression and distribution of E-Cadherin. (A) Representative photomicrographs obtained by Laser-scanning confocal microscope of the does-dependent changes in E-cadherin assembly induced by TDI-HSA conjugates. 16HBE cells were cultured in medium alone (a) or in the presence of HSA (b) or TDI-HSA conjugates at 40 ($\mu\text{g/ml}$) (c), 60 $\mu\text{g/ml}$ (d), and 100 ($\mu\text{g/ml}$) (e). (B) The expression of E-Cadherin in 16HBE cells treated with different concentrations of TDI-HSA was not different by Western blot. The results shown are representative of three independent experiments.

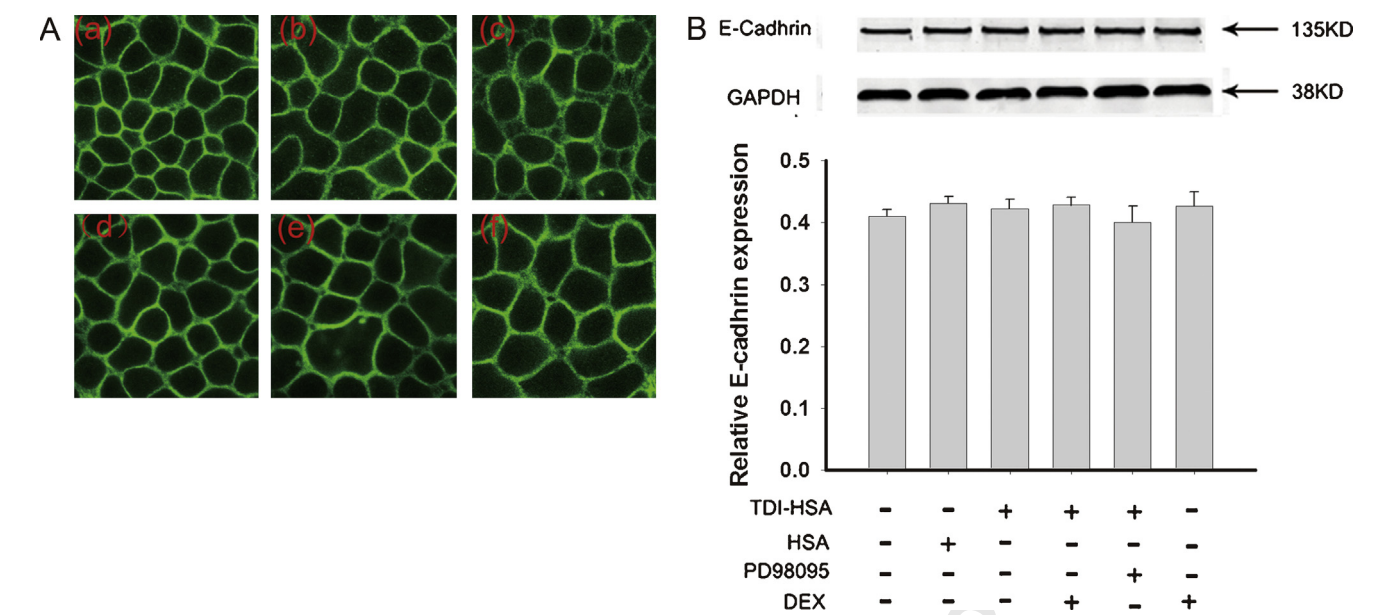


Fig. 7. The ERK1/2 inhibitor PD98095 and DEX significantly inhibited TDI-HSA induced E-cadherin redistribution. (A) Representative photomicrographs obtained by Laser-scanning confocal microscope. 16HBE cells were treated with medium alone (a) or TDI-HSA at 100 ($\mu\text{g}/\text{mL}$) (c) for 24 h, PD98095 (10^{-7} M) or DEX (10^{-4} M) were preincubated 1 h before TDI-HSA treatment (d and e), HSA (100 $\mu\text{g}/\text{mL}$) incubated cells were used as control (b). (B) Expression of E-cadherin in 16HBE cells. Cells were preincubated for 1 h with culture medium, HSA (100 $\mu\text{g}/\text{mL}$), TDI-HSA (100 $\mu\text{g}/\text{mL}$) alone or with PD98095 (10^{-7} M) or DEX (10^{-6} M). The results shown are representative of three independent experiments.

pretreated with DEX showed significant increases in neutrophils in BALF, while the distribution of E-cadherin was partly reversed; and in our pilot study (data not shown), we found that the distribution of E-cadherin was altered, however no obvious airway inflammation was observed when mice only received TDI airway challenge in

the absence of sensitization. These suggest that other mechanisms may play important roles in this process. Heijink et al. (2010) demonstrated that ERK1/2 activation is involved in the aberrant distribution of E-cadherin induced by house dust mites in bronchial epithelial lines. We assumed that

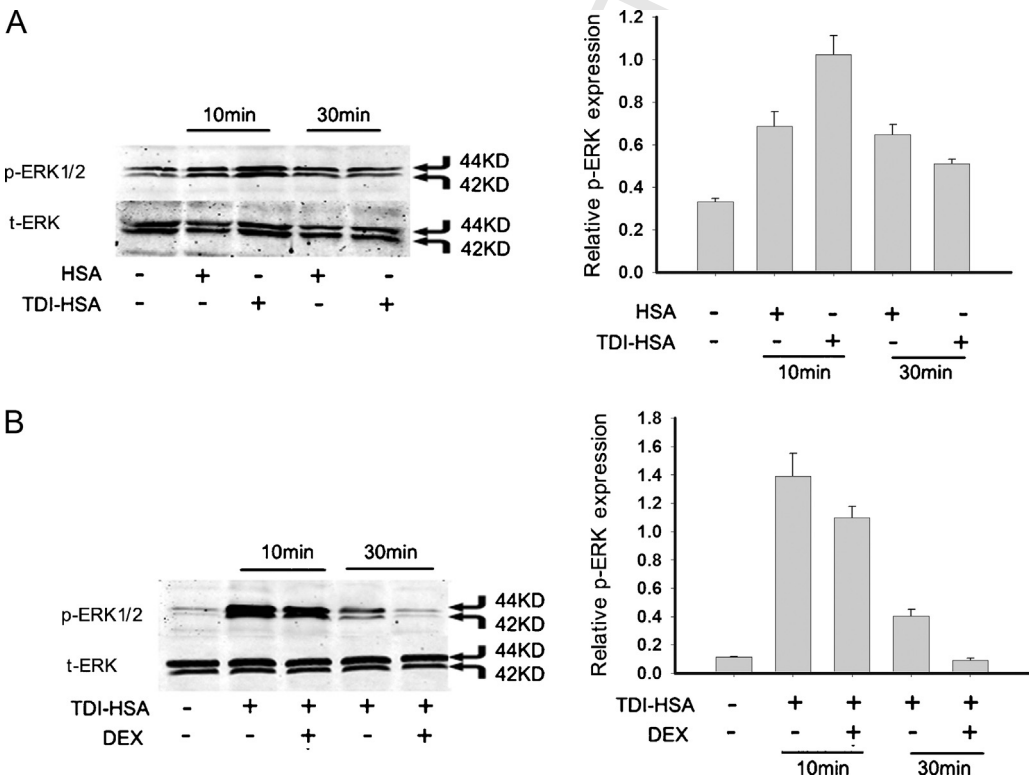


Fig. 8. DEX (10^{-6} M) reduced TDI-HSA induced phosphorylation of ERK1/2 in 16HBE cells. (A) 16HBE cells were treated with serum-free medium, HSA (100 $\mu\text{g}/\text{mL}$) or TDI-HSA (100 $\mu\text{g}/\text{mL}$) for 10 or 30 min. (B) 16HBE cells were treated with TDI-HSA (100 $\mu\text{g}/\text{mL}$) alone or pretreated for 1 h with DEX (10^{-6} M) for 10 and 30 min. The results shown are representative of three independent experiments.

ERK1/2 activation by TDI in epithelial cells may be another reason for the alternation of E-cadherin in the mouse asthma model. By evaluating ERK1/2 levels in lung tissues of mice, we confirmed this. To further explore if ERK1/2 activation could take part in aberrant distribution of E-cadherin in bronchial mucous, 16HBE cells were stimulated with TDI-HSA using specific concentrations of TDI-HSA based on a study by Lee et al. (2003), and the distribution of E-cadherin was observed by immunohistochemistry. Surprisingly, we found that the distribution was also aberrant, yet no change of E-cadherin expression was detected after treatment with TDI-HSA when analyzed by Western blot, consistent with one report (Chun and Prince, 2009). Chun and Prince also identified an increased level of cleavage products of E-cadherin, which needs to be clarified in our model.

Furthermore, we discovered that the activation of ERK1/2 was involved in TDI-induced redistribution of E-cadherin by pretreatment with PD98095. Similar results were obtained with DEX, suggesting that DEX inhibits the redistribution of E-cadherin by interfering with the activation of ERK1/2.

5. Conclusions

In conclusion, we confirmed that there is an obvious aberrant distribution of E-cadherin in our mouse model and that TDI-induced ERK1/2 activation in airway epithelium may participate in this abnormality, which can be partly inhibited by DEX. Our data provide new insights into the mechanisms of TDI-induced asthma and suggest novel therapeutic targets for the treatment of asthma.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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