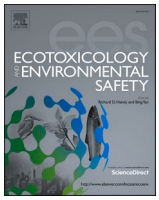
Ecotoxicology and Environmental Safety 266 (2023) 115575



Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv





HMGB1 inhibition reduces TDI-induced occupational asthma through ROS/AMPK/autophagy pathway

Xiangjing Meng [a,1], Sumei Guo [b,1], Xiaoxia Zhang [a], Bo Jiao [c], Xiaohan Yang [a], Ming Li [a], Chao Li [a], Jin He [a], Shangya Chen [a], Cheng Peng [d], Hua Shao [a,*], Qiang Jia [a,*]

[a] Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan, Shandong 250062, China
[b] Erqi District Center for Disease Control and Prevention, Zhengzhou, Henan 450052, China
[c] National Institute of Occupational Health and Poison Control, Chinese Center for Disease Control and Prevention (CDC), Beijing 100050, China
[d] Eusyn Institute of Health Science, Brisbane, Queensland 4108, Australia

ARTICLE INFO

Edited by Professor Bing Yan

Keywords:
Toluene diisocyanate
Asthma
High mobility group box 1
Airway inflammation
Autophagy

ABSTRACT

Exposure to toluene diisocyanate (TDI) can cause pulmonary diseases such as asthma. Inhibition of high mobility group box 1 protein (HMGB1) has been found to be protective against the toxic effects of TDI on human bronchial epithelial (HBE) cells. Here, we evaluated the in vivo positive roles of HMGB1 in the TDI-caused asthma mice and explored its underlying mechanisms in HBE cells. We found that suppression of HMGB1 obviously alleviated airway inflammation, airway hyperresponsiveness, and airway remodeling in the lung tissue of the asthma mice. The in vitro results showed that inhibition of HMGB1 ameliorated TDI-induced reactive oxygen species (ROS) release, inflammatory response, and activation of autophagy in HBE cells. At the molecular level, inhibition of HMGB1 decreased the expressions of HMGB1, Toll-like receptor 4, Vimentin and matrix metalloproteinase-9 proteins, activated NF-κB and NOD-like receptor protein 3 (NLRP3) inflammasome, and increased E-cadherin expression. Importantly, activation of autophagy could lead to the overactivation of NLRP3 inflammasome in TDI-induced asthma. These results suggest that inhibition of HMGB1 can alleviate TDI-induced asthma through ROS/AMPK/autophagy pathways, which may provide valuable evidence for the pathogenesis and therapeutic targets of TDI-induced asthma.

1. Introduction

Occupational asthma (OA) has become a major public health issue owing to its heavy socioeconomic burden and high prevalence (Tiotiu et al., 2020). Toluene diisocyanate (TDI), a major causative agent of OA (Pollaris et al., 2016), has been widely applied in the construct of polyurethane flexible foam, synthetic leather, etc. (Daoud et al., 2022). Workers can be exposed to TDI through inhalation of vapors or aerosols in workplaces where TDI exists (Prueitt et al., 2017). In 2018, the usage of TDI in the North American flat foam marketplace reached up to 428.7 million pounds, of which 142.3 million pounds were from daily supplies (Sayles et al., 2022). With the wide-ranging utilization of TDI in business and daily activities, occupational and environmental exposure to TDI has seriously threatened the health of people.

TDI-induced occupational asthma (TDI-OA) is characterized by airway inflammation, airway hyperresponsiveness (AHR), mucus overproduction and airway remodeling (Pollaris et al., 2016), among which airway inflammation with neutrophil and eosinophil infiltration is regulated by T helper 2 (Th2) cells and its cytokines, including interleukin (IL)− 4, IL-5, and IL-13 (Pollaris et al., 2021; Peters and Wenzel, 2020). Thymic stromal lymphopoietin (TSLP), an epithelial cytokine, plays a central role in regulating immune responses to inhaled environmental insults like allergens and viruses, which can not only promote $CD4^+$ cell differentiation into Th2 cells and generate multiple cytokines, but also reduce the expression of Th1-associated interferons (IFN) (Gauvreau et al., 2020). Additionally, activation of NOD-like receptor protein 3 (NLRP3) inflammasome is also involved in airway inflammation and performs important roles in the pathology of asthma (Wu et al., 2020; Yang et al., 2019). The major regulators of NLRP3 inflammasome include high mobility group box 1 (HMGB1), autophagy, and more.

* Corresponding authors.
E-mail addresses: chinashaohua5888@163.com (H. Shao), jiaqiang5632@163.com (Q. Jia).
[1] Xiangjing Meng and Sumei Guo contributed equally to this paper.

https://doi.org/10.1016/j.ecoenv.2023.115575
Received 30 March 2023; Received in revised form 1 October 2023; Accepted 10 October 2023
Available online 14 October 2023
0147-6513/© 2023 The Authors. Published by Elsevier Inc. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

(Theofani et al., 2019).

Autophagy is a key self-degradative process by which cytoplasmic materials, such as impaired proteins and organelles, are delivered and degraded in the lysosome (Deretic and Klionsky, 2018). It may exert positive or negative effects in the etiology of multiple diseases, including inflammatory disorders, cancer, etc. (Kim and Lee, 2014; Li et al., 2017; Onorati et al., 2018). It is reported that many external factors and interventions could interfere with the response process of autophagy, thereby promoting or aggravating the disease (Klionsky et al., 2021). HMGB1, an immunomodulatory factor in response to airway inflammation and remodeling (Wu et al., 2022), has been confirmed to be a positive governor of autophagy (Le et al., 2020; Zeng et al., 2021). It was highly expressed in the lung tissues of patients with chronic obstructive pulmonary disease (COPD) and involved in the autophagy of lung macrophages (Le et al., 2020). It is demonstrated that inhibition of HMGB1 could aggravate inflammation by blocking epithelial autophagy (Zeng et al., 2021). Although HMGB1 and autophagy have been demonstrated to participate in the pathogenesis of multiple lung diseases, including asthma, the specific mechanisms underlying this association remain unclear (Xia et al., 2018; Yan et al., 2018).

In this work, we evaluated the protective effects of HMGB1 inhibition by establishing a TDI-induced asthma mouse model, and explored its underlying mechanisms in human bronchial epithelial (HBE) cells. The results showed that HMGB1 was involved in TDI-induced asthma through the reactive oxygen species (ROS)/AMP-activated protein kinase (AMPK)/autophagy pathways and influenced the airway inflammation and remodeling.

2. Materials and methods

2.1. Materials

The reagents included enzyme-linked immunosorbent assay (ELISA) kits, bicyclonic acid (BCA) protein detection kits, as well as multiple antibodies. The chemicals incorporated 2,4-toluene diisocyanate (2,4-TDI, 95% pure), glycyrrhizic acid (GL), acetone and olive oil (AOO). More details about reagents and chemicals could be found in our previous study (Jiao et al., 2021a).

2.2. Animal experiments

2.2.1. Establishment of TDI-induced asthma mouse model and GL treatment

We performed in vivo experiments with the male Balb/c mice (SPF, 6–8 weeks). Mice were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. (China, animal batch number: 20190003). This work was approved by the Institutional Review Board of Shandong Academy of Occupational Health and Occupational Medicine, Shandong First Medical University.

The mice were divided into four groups ($n = 8$), including AOO, TDI, TDI+GL (10 mg/kg) and TDI+GL (60 mg/kg) groups. The mouse asthma model was established according to previous studies with some modifications (Yu et al., 2019; Liang et al., 2015). Briefly, 2:3 mixture of AOO was used to dissolve TDI for sensitization, and 1:4 mixture for the challenge. In the AOO group, the mice were sensitized with AOO through the dorsa of two ears on d1 and d8. Then, on d15, d18 and d21, they were challenged with 1:4 mixture of AOO for 40 min through the mouth-nose exposed system (Beijing Huironghe Technology Co., Ltd., China). In the TDI group, the mice were sensitized using 0.3% TDI via the dorsa of two ears (20 μL/ear) on d1 and d8. Then, on d15, d18 and d21, they were challenged with 3% TDI that was dissolved in 1:4 mixture of AOO for 40 min through the exposed system. The mice in the TDI+GL (10 mg/kg) and TDI+GL (60 mg/kg) groups were exposed as described in the TDI group. Importantly, the mice took 10 and 60 mg/kg of GL orally at 24 h and 1 h before the challenge, respectively. For the AOO group, the mice were treated with saline. The experimental protocol is illustrated in Fig. 1A.

2.2.2. AHR detection

AHR to methacholine (Mch) was detected at 24 h after finishing three times of TDI challenges (Fig. 1A). As described in the previous literature (Lundblad et al., 2002; Ye et al., 2012), the mice were exposed to different concentrations of Mch (0, 3.12, 6.25, 12.5, and 25 mg/mL). After atomization, they were monitored for 3 min using a plysmograph chamber (PLY 310, EMMS, Hants, UK). Penh referred to the airway recovery resistance index.

2.2.3. Analysis of peripheral blood cells

Mouse tail vein blood was collected and mixed with 20 μL buffer containing EDTA-K2 anticoagulants after the last challenge. Immune cells including neutrophils, eosinophils and lymphocytes were assessed by Idexx ProCyte Dx® Hematology Analyzer (IDEXX Laboratories, Inc., USA).

2.2.4. Cytokine detection

The lung tissue was homogenized and centrifuged, and the supernatant was taken. The levels of IFNγ, IL-4, IL-18 and IL-1β in the lung homogenate were measured using commercially available ELISA kits based on the manufacturer's instructions. Then, the expression of these cytokines was analyzed using SpectraMax (450 nm).

2.2.5. Histopathological analysis

The left lungs and bronchi were collected at 24 h after finishing three times of TDI challenges, and inflammatory scores were performed. The left lungs and bronchi were fixed and embedded with 4% paraformaldehyde and paraffin, respectively. Then, the sections with 5 μm thickness were stained with H&E and Alcian blue-periodic acid Schiff (AB-PAS) for histopathological assessment.

2.2.6. Immunohistochemistry and western blotting

Immunohistochemistry was used to analyze the HMGB1 expression. The expressions of HMGB1, AMPK pathway, autophagy-related proteins, NF-κB and NLRP3 inflammasome proteins were detected through Western blotting, including HMGB1, TLR4, p65, P-p65, NLRP3, ASC, Caspase-1, LC3, TSLP, MMP-9, E-cadherin, Vimentin, β-actin and GAPDH. The concentration of the separation gel for most proteins was 10%, except for LC3 (12%).

2.3. Cell experiments

2.3.1. Preparation for TDI-HSA conjugates

We used TDI-HSA conjugates in the in vitro assays. As described previously (Jiao et al., 2021b), TDI-HSA conjugates were prepared. Based on our cell viability assay, the highest concentration of TDI-HSA was 120 μg/mL.

2.3.2. HBE exposure

The HBE cells were cultivated in Pneuma Cult™-Ex Plus Medium. The details of cell culture and medium information were shown in our previous study (Jiao et al., 2021a). Simply, HBE cells were pre-treated with compound C (CC, 1 μM, AMPK inhibitor) for 2 h, N-acetylcysteine (NAC, 5 mM, antioxidant) for 4 h, HMGB1 inhibitor (GL, 150 μM) for 24 h, RAPA (100 nM) for 1 h and 3-MA (2 mM) for 4 h, and then incubated with TDI-HSA (120 μg/mL) for 12 h.

2.3.3. ROS analysis

ROS kits were used to analyze the expression of ROS. Under the fluorescence microscope, the mean fluorescence intensity was analyzed using Image-J software. More details could be found in our previous study (Jiao et al., 2021a).