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Exposure to both formaldehyde and high relative humidity exacerbates allergic asthma by activating the TRPV4-p38 MAPK pathway in Balb/c mice[☆]

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ABSTRACT

Some studies have indicated that formaldehyde, a ubiquitous environmental pollutant, can induce or aggravate allergic asthma. Epidemiological studies have also shown that the relative humidity indoors may be an independent and a key factor associated with the aggravation of allergic asthma. However, the synergy of humidity and formaldehyde on allergic asthma and the mechanism underlying this effect remain largely unknown. In this study, we aim to determine the effect of high relative humidity and/or formaldehyde exposure on allergic asthma and explore the underlying mechanisms. Male Balb/c mice were modeled with ovalbumin (OVA) and exposure to 0.5 mg/m³ formaldehyde and/or different relative humidity (60%/75%/90%). Histopathological changes, pulmonary function, Th1/Th2 balance, the status of mucus hypersecretion and the levels of inflammatory factors were detected to assess the exacerbation of allergic asthma. The levels of the transient receptor potential vanilloid 4 (TRPV4), calcium ion and the activation of p38 mitogen-activated protein kinases (p38 MAPK) were detected to explore the underlying mechanisms. The results showed that exposure to high relative humidity or to 0.5 mg/m³ formaldehyde alone had a slight, but not significant, effect on allergic asthma. However, the pathological response and airway hyperresponsiveness (AHR) were greatly aggravated by simultaneous exposure to 0.5 mg/m³ formaldehyde and 90% relative humidity. Blocking TRPV4 or p38 MAPK using HC-067047 and SB203580 respectively, effectively alleviated the exacerbation of allergic asthma induced by this simultaneous exposure to formaldehyde and high relative humidity. The results show that when formaldehyde and high relative humidity are present this can enhance the activation of the TRPV4 ion channel in the lung leading to the aggravation of the p38 MAPK activation, resulting in the exacerbation of inflammation and hypersecretion of mucus in the airways.

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1. Introduction

The trigger of allergic asthma is varied. Genetic and environmental factors are currently considered to be the two main reasons for asthmatic paroxysms. The environmental factors include temperature, smoking, chemical irritants and humidity.

People used to think that high relative humidity (RH) allowed for an increase in mold and fungi, and that these exacerbate allergic

asthma (Tavernier et al., 2006; van Strien et al., 2015). A large cross-sectional study of school children in Germany indicated that mite antigen levels and damp housing conditions are the main risk factors for bronchial hyperreactivity in adolescence. This study found that bronchial hyperreactivity was still significantly correlated with humidity after adjusting for mite allergen levels, and indicated that relative humidity indoors may contribute to the development of allergic asthma. Further research is needed to confirm this point. (Peden and Reed, 2010).

Formaldehyde is a versatile chemical, widely used in industrial production especially in household products. Because of its preservative and bactericidal properties, formaldehyde is also used in

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textile, food and cosmetics industries (Wen et al., 2016). Previous studies have indicated formaldehyde can induce multiple toxic effects in human body, such as neurotoxicity, immunotoxicity and carcinogenicity (Aydin et al., 2013; Luo et al., 2012). Epidemiological studies have suggested that formaldehyde can induce or aggravate allergic asthma (Gerald McGwin et al., 2010; Kang et al., 2018; Véronique et al., 2007). Previous study has also indicated that exposure to 3 mg/m³ formaldehyde can lead the exacerbation of allergen-induced asthma, which is inhibited by the transient receptor potential ankyrin 1 (TRPA1) channel antagonist HC-030031 and transient receptor potential vanilloid 1 (TRPV1) channel antagonist CPZ (Wu et al., 2013).

Exposure to high relative humidity and formaldehyde is very common in our daily indoor environment. Considering the possible contributions of high relative humidity and formaldehyde to the development of allergic asthma, it is necessary to explore the effect of exposure to high relative humidity together with formaldehyde on allergic asthma, and to investigate the underlying mechanism.

Recent epidemiological investigations have shown that humidity is related to allergic asthma, but the potential mechanisms have not been elucidated. One of the main reasons is that the specific humidity sensor in mammals is not well understood. In insects, the transient receptor potential ion channels, especially TRPV1 and TRPA1, play an important role as a humidity sensor for humidity perception (Liu et al., 2007). In mammals the transient receptor potential ion channels may also play a part in humidity perception (Filingeri, 2015). Studies have indicated that the moisture sensing mechanism of human skin is the result of the interaction of temperature receptors (i.e. transient receptor potential ion channels) with mechanical force receptors (i.e. DEG/ENAC channels), and that TRPV4 is a notable candidate ion channel of the transient receptor potential ion channels (Filingeri and Havenith, 2015; Montell, 2008). A change in humidity may influence TRPV4, while formaldehyde possibly also affects the opening of the TRPV4 ion channel. One study has shown that TRPV4 can be activated by formalin which leads to a calcium influx, resulting in trigeminal irritant pain (Chen et al., 2014).

As a member of the transient receptor potential ion channel family, TRPV4 can regulate the calcium flux between the inside and the outside of a cell. In the respiratory system, TRPV4 has a high expression level in the epithelial cells of the trachea and the lung, and it plays a key role in the development of allergic asthma (Birrell et al., 2016). In the cilia of bronchi, TRPV4 is important to the motility of cilia which regulate the transmission of mucus (Everaerts et al., 2010). Recent studies have shown that TRPV4 can regulate: the thickness of the airway wall; the recruitment of goblet cells; collagen synthesis; fibrosis remodeling; and the expression of transforming growth factor- β (TGF- β) in the airways of allergic asthma model mice. In addition, a cell experiment shows that TRPV4 can regulate the differentiation of TGF- β dependent myofibroblasts. These findings indicate that reducing TRPV4 may be a therapeutic target of allergic asthma (Scheraga et al., 2017). We therefore speculated that if exposure to high relative humidity and/or formaldehyde can aggravate allergic asthma, then the activation of TRPV4 may play a role in this process.

The opening of the TRPV4 ion channel induced calcium influx can regulate different molecular pathways, the p38 mitogen-activated protein kinase (p38 MAPK) pathway being one of these. Activation of TRPV4 can upregulate the p38 MAPK signaling pathway leading to apoptosis in the hippocampus (Jie et al., 2015). Activation of the TRPV4-p38 MAPK pathway is associated with neuropathic pain in rats (Qu et al., 2016). All these studies indicate that there is a close connection between TRPV4 and p38 MAPK. Moreover, studies have shown that the activation of p38 MAPK

signaling plays a key role in ozone and diisodecyl phthalate induced aggravation of allergic asthma, and that inhibiting p38 MAPK signaling significantly attenuates allergen-induced airway inflammation (Bao et al., 2017; Kim et al., 2012; Qin et al., 2018).

In this study, we aim to investigate the effects of high relative humidity and/or formaldehyde exposure on allergic asthma, and determine the impact of high relative humidity and formaldehyde exposure on the TRPV4-p38 MAPK pathway. An allergic asthma mouse model was developed and we determined pulmonary function, the pro-inflammatory cytokines, the state of mucus secretion in the airway, and the activation of the TRPV4-p38 MAPK pathway in the lung. In addition, to evaluate the role of TRPV4-p38 MAPK in this study, we used HC-067047 (a specific inhibitor of TRPV4) and SB203580 (a specific inhibitor of p38 MAPK) to block TRPV4 and p38 MAPK.

2. Materials and methods

2.1. Animals

5 week old (approximately 20 g) specific pathogen-free male Balb/c mice were used in this study. They were purchased from the Hubei Experimental Animal Center (Wuhan, China). These mice were housed in pathogen-free cages at 24–26 °C and 60% $\pm$ 5% relative humidity with a 12 h light-dark cycle. Mice were kept 8 mice to a cage, and sterile water and commercial food (Hubei Experimental Animal Center) were given *ad libitum*. Mice were quarantined for 7 days prior to the study. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Central China Normal University on January 24, 2016 (Ratification ID: CCNU-IACUC-2016-003).

2.2. Main reagent and kits

Ovalbumin (>98%, OVA) and formalin solution (10%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HC-067047 and SB203580 were obtained from Medchem Express (Shanghai, China). Mouse enzyme-linked immunosorbent assay (ELISA) kits for IgE, IL-4, IFN- γ , IL-1 β , TNF- α were obtained from eBioscience (San Diego, CA, USA). A Flow 4-AM fluorescent probe was purchased from Beyotime (Shanghai, China). The primary antibodies used in this experiment were: anti-TRPV4 (Proteintech, Wuhan, China, catalog number: 20987-1-AP); anti-phospho-p38 MAPK (Abcam, MA, USA, catalog number: ab4822).

2.3. Experimental design

Fourteen groups of mice were used (8 mice per group): (1) treatment with saline (saline group); (2) sensitization and challenge with OVA (OVA group, RH: 60%); (3) treatment with OVA and formaldehyde (0.5 mg/m³, 6 h/day) exposure (OVA + FA group, RH: 60%); (4) treatment with OVA and moderate relative humidity (RH: 75%) exposure (OVA + 75%RH group); (5) treatment with OVA and high relative humidity (RH: 90%) exposure (OVA + 90%RH group); (6) treated with OVA and formaldehyde plus high relative humidity (RH: 90%) (OVA + FA + 90%RH group); (7)–(10) were the HC-067047 blocking groups (OVA + HC group, OVA + FA + HC group, OVA + 90%RH + HC group, OVA + FA + 90%RH + HC group); (11)–(14) were the SB203580 blocking groups (OVA + SB group, OVA + FA + SB group, OVA + 90%RH + SB group, OVA + FA + 90%RH + SB group).

The allergic asthma model was built by using OVA. Mice were sensitized with 5 mg OVA mixed with 175 mg Al(OH)₃ in 30 mL saline in day 1, day 7 and day 14 via intraperitoneal (*i.p.*) injection. In the final 7 days, mice were challenged by being exposed to 1%

OVA aerosol for 30 min each day. According to the no-observed-adverse-effect level (NOAEL) of formaldehyde for sensory irritation (0.67 mg/m^3) (Nielsen et al., 2013). Mice were exposed to 0.5 mg/m^3 formaldehyde 6 h each day to simulate occupational exposure. For related humidity setting, we referred to a research about indoor humidity environment in 9 Chinese cities' residential buildings. According to the research, in summer day, the indoor relative humidity is between 60% and 90% in 7 cities (Zhang and Yoshino, 2010). Therefore, the mice were exposed to 60%, 75% and 90% relative humidity for 12 h a day. For exposure to gaseous formaldehyde, a standard gaseous formaldehyde dynamic exposure chamber (Yuxin 8.4L WH-2, China) was used to convert the formalin solution to gaseous formaldehyde. The conditions set in the chamber were $60\% \pm 5\%$ relative humidity and $25 \pm 2^\circ\text{C}$. Different relative humidity environments were established by using an artificial climate chamber (BD-PRX150A, China). The conditions set in the chamber were 75% or $90\% \pm 5\%$ relative humidity and 25°C . Mice were exposed to formaldehyde 6 h/day and/or a different relative humidity 12 h/day. The HC-067047 blocking groups received 10 mg/kg bw HC-067047 via *i.p.* weekly. The SB203580 blocking groups received 5 mg/kg bw SB203580 via *i.p.* every 2 days. Mice were treated with HC-067047 or SB203580 in 1 h after exposure to formaldehyde and high relative humidity. All mice were sacrificed on day 28. Lung, serum and bronchoalveolar lavage fluid (BALF) were collected for further biomarker detection and histological analysis. The specific exposure scheme is shown in Fig. 1.

2.4. Pulmonary function tests

Within 24 h after the final OVA aerosol challenge, airway hyperresponsiveness (AHR) measurements were performed using the AniRes 2005 lung function system (Bestlab, version 2.0, China). The specific procedures to measure lung function were done according to the manufacturer's instructions and to our previous study (Kang et al., 2018).

2.5. Histopathological examination

The left lungs from 2 mice in each group were collected and fixed in neutral formalin liquid for 24 h. These tissues were then embedded in paraffin and the lung tissue sections were stained with hematoxylin and eosin (H&E) and Periodic Acid-Schiff (PAS). All pathological sections were examined using a BX53 microscope (OLYMPUS, Japan).

2.6. Enzyme-linked immunosorbent assay

10% tissue homogenate supernatant from 6 mice in each group was collected to determine the levels of total-IgE (T-IgE), IL-4, IFN- γ , IL-1 β , TNF- α using commercial ELISA kits according to the manufacturer's instructions.

2.7. Detection of calcium levels

30 mg of right lung tissue from 5 mice in each group was collected to prepare cell suspensions. Lung tissues were cut into pieces and digested by trypsin. The number of cells in suspension was $1-3 \times 10^6$. Add $100 \mu\text{L}$ the Flow-4AM fluorescent probe ($2 \mu\text{M}$) into $100 \mu\text{L}$ cell suspension, then incubate for 60 min at 37°C . The fluorescence intensity at an excitation wavelength of 494 nm and an emission wavelength of 516 nm was measured using a Fluorescence microplate reader (FLx 800; BioTek Instruments, Winooski, VT, USA).

2.8. Immunohistochemistry for TRPV4 and phosphorylation of p38 MAPK

The levels of TRPV4 and phosphorylation of p38 MAPK were detected using immunohistochemistry, the specific operations performed as described in a previous study (Duan et al., 2018). The average optical density of every sample was analyzed using Image-Pro Plus software (Image-Pro Plus 6.0, Media Cybernetics).

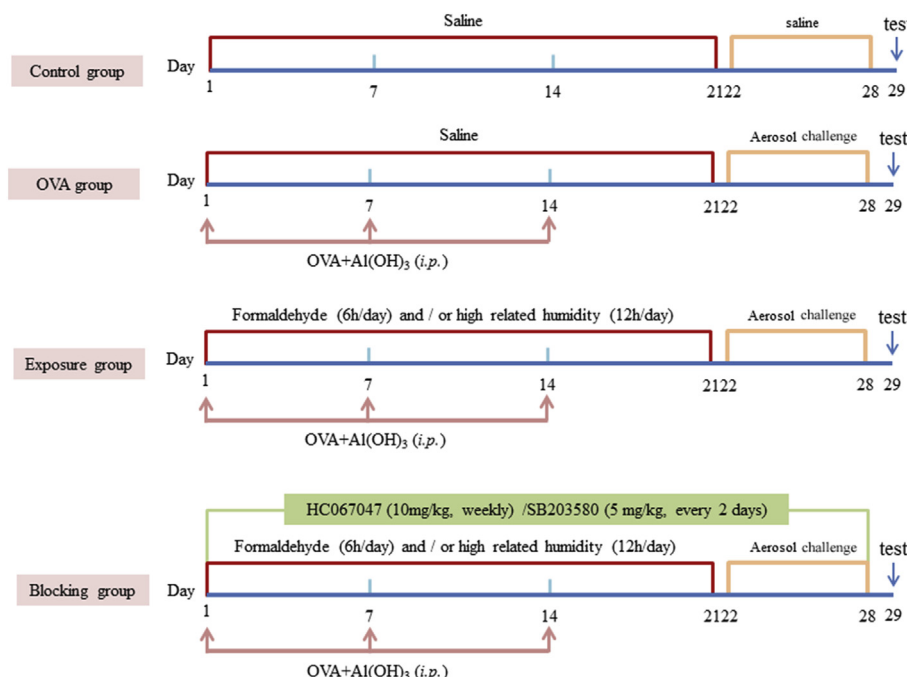


Fig. 1. Sensitization and exposure protocol.

2.9. Statistical analysis

All data analyses were done using IBM SPSS Statistics 20 (Armonk, New York). Data were presented as mean $\pm$ SEM. Statistical graphs were produced by GraphPad 5.0 (San Diego, California). The significance of variation among groups was determined by a one-way ANOVA combined with an LSD multiple comparison test. In our study, $p < 0.05$ was considered significant and $p < 0.01$ was considered extremely significant.

3. Results

3.1. Formaldehyde and high relative humidity aggravated allergic asthma

To examine structural damage to the lung tissue, H&E staining was used to investigate histopathological injury. As Fig. 2A shows, the airway wall is thicker in the OVA group than in the saline group, and infiltration of inflammatory cells was observed around the airway. Compared with the OVA group, the structure of airway wall and the infiltration of inflammation cells only had slight, but not significant exacerbation in the formaldehyde or any of the different relative humidity exposure groups. However, in the formaldehyde and 90% relative humidity co-exposure group, the airway wall is more rougher than the OVA group and the infiltration of inflammatory cells is aggravated.

The changes in AHR are similar to the histopathological injury (Fig. 2 B). Comparing exposure groups with the saline group, the inspiratory and expiratory resistances increased significantly ($p < 0.01$) and dynamic lung compliance decreased significantly

($p < 0.01$). In the formaldehyde or in the different relative humidity exposure groups, the inspiratory resistance (Ri), expiratory resistance (Re) and the dynamic lung compliance (Cldyn) did not result in significant aggravation compared with the OVA group. But in the OVA + FA + 90%RH group, the Ri and Re (increased extremely significantly ($p < 0.01$), and the Cldyn decreased significantly ($p < 0.05$) compared with the OVA group.

3.2. Combined exposure to formaldehyde and high relative humidity exacerbated the predominant Th2 type immunity and production of IgE

To assess the status of Th1/Th2 type immunity balance, we detected the typical Th1 cytokine (IFN- γ) and Th2 cytokine (IL-4) in the lung tissue (Fig. 3A). Compared with the saline group, the level of IL-4 in the OVA group increased sharply ($p < 0.01$), but the level of IFN- γ did not change obviously. In the OVA group, the ratio of IFN- γ /IL-4 decreased extremely significant ($p < 0.01$) compared with the saline group. After being exposed to formaldehyde and 90% relative humidity, the level of IL-4 increased significantly compared with the OVA group ($p < 0.05$). The level of IFN- γ seems to have increased slightly, but the ratio of IFN- γ /IL-4 decreased sharply compared with the OVA group ($p < 0.05$). In the TRPV4 or p38 MAPK blocking groups, the levels of IL-4 decreased and the ratio of IFN- γ /IL-4 increased obviously ($p < 0.01$) compared with corresponding exposure groups.

IgE is a characteristic biomarker of allergic asthma. The level of IgE increased extremely significantly ($p < 0.01$) in the OVA group compared with the saline group (Fig. 3B). Being exposed to formaldehyde or to the different relative humidities seems to not affect

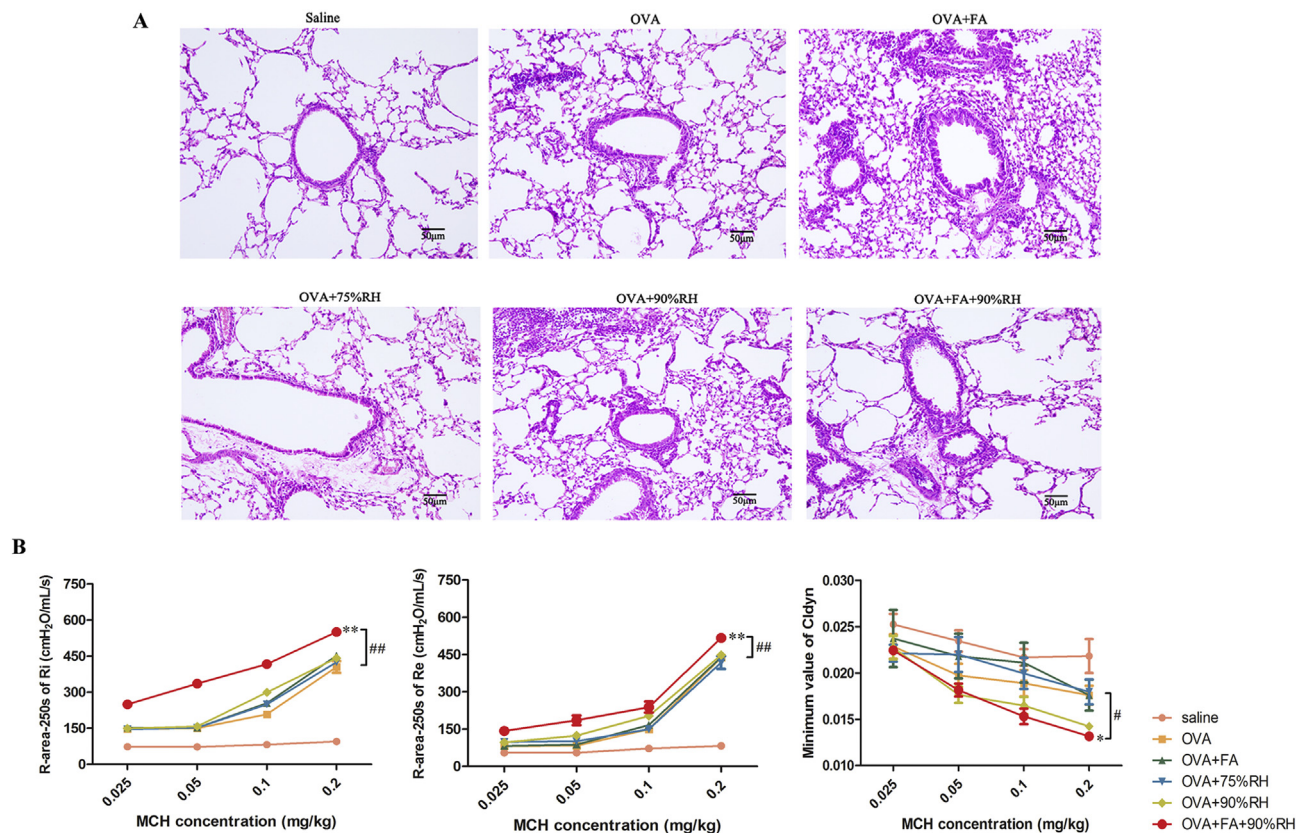


Fig. 2. Pathological injury and AHR changes after exposure to formaldehyde and/or different relative humidities. (A) H&E staining of lung tissue (original magnification, 200 $\times$). (B) Ri, Re and Cldyn values of the different treatment groups. * $p < 0.05$, ** $p < 0.01$, significant difference compared with the saline group. # $p < 0.05$, ## $p < 0.01$, different exposure groups compared with the OVA group. (n = 5).

the IgE level. However, combined exposure to formaldehyde and 90% relative humidity induced a sharp increase compared with the OVA group ($p < 0.05$) while the blocking agents HC-067047 and SB203580 modified the increase of IgE.

3.3. Co-exposure to formaldehyde and high relative humidity aggravate mucus hypersecretion

Mucus hypersecretion is another important indicator of allergic asthma. We examined the extent of mucus hypersecretion through PAS staining and determining the level of mucin 5AC (MUC5AC) in the BALF. As can be seen in Fig. 4A, there is a clear increase in the number of PAS-positive mucus cells in the OVA group compared with the saline group. The number of PAS-positive mucus cells in the OVA + FA + 90%RH group increased visibility compared with the OVA group. The level of MUC5AC in the OVA group increased extremely significantly ($p < 0.01$) compared with the saline group. The level of MUC5AC in the formaldehyde and 90% relative humidity co-exposure group increased sharply ($p < 0.01$) compared with the OVA group. After being treated with inhibitors, both the number of PAS-positive mucus cells and the levels of MUC5AC showed an obvious decrease as compared with the corresponding exposure groups.

3.4. Combined exposure to formaldehyde and high relative humidity exacerbated inflammation in the lung

The levels of IL-1 β and TNF- α were determined to evaluate the status of inflammation in the lung tissue (Fig. 5). A significant elevation in IL-1 β and TNF- α levels were observed in the OVA group. Compared with the OVA group, exposure to formaldehyde or different relative humidity singly did not affect the IL-1 β and TNF- α levels significantly. But in the OVA + FA + 90%RH group, the levels of IL-1 β and TNF- α increased significantly ($p < 0.05$) compared with the OVA group. After blocking with TRPV4 or p38 MAPK, the levels of IL-1 β and TNF- α in all the inhibitor-treated groups decreased to a varying degree.

3.5. Formaldehyde combined with high relative humidity enhanced the activation of the TRPV4 ion channel

To evaluate whether TRPV4 was involved in the formaldehyde and high relative humidity induced aggravation of allergic asthma, we examined the levels of TRPV4 and calcium ions in the lung tissue (Fig. 6). The expression of TRPV4 increased significantly in the OVA group when compared with the saline group ($p < 0.05$). Co-exposure to formaldehyde and 90% relative humidity increased the expression of TRPV4 extremely significantly compared with the OVA group ($p < 0.01$). Similarly, the levels of calcium ions increased

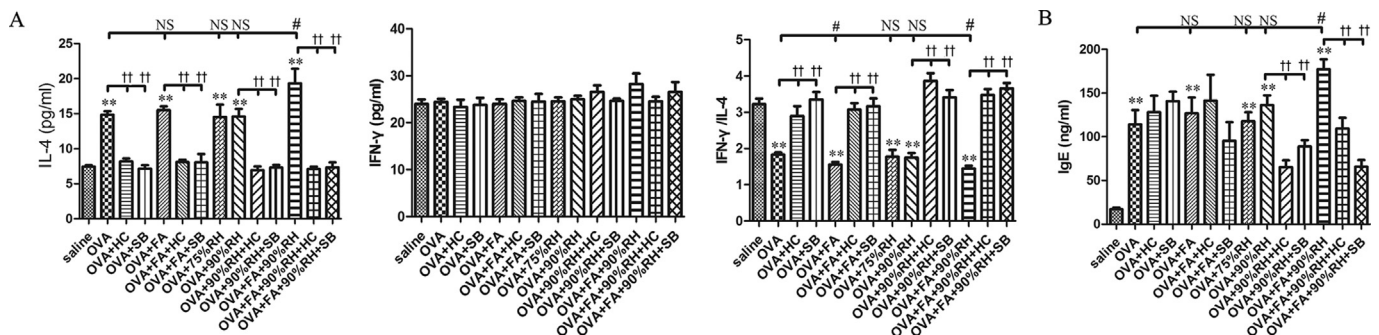


Fig. 3. The impact of exposure to formaldehyde and different relative humidities on the Th2 immune response and IgE. (A) The concentration of IL-4, IFN- γ and the ratio of IFN- γ /IL-4 in the different treatment groups. (B) The level of IgE in the different treatment groups. * $p < 0.05$, ** $p < 0.01$, significant difference compared with the saline group. NS $p > 0.05$, # $p < 0.05$, ## $p < 0.01$, different exposure groups compared with the OVA group. † $p < 0.01$, the blocking groups compared with the corresponding exposure groups ($n = 6$).

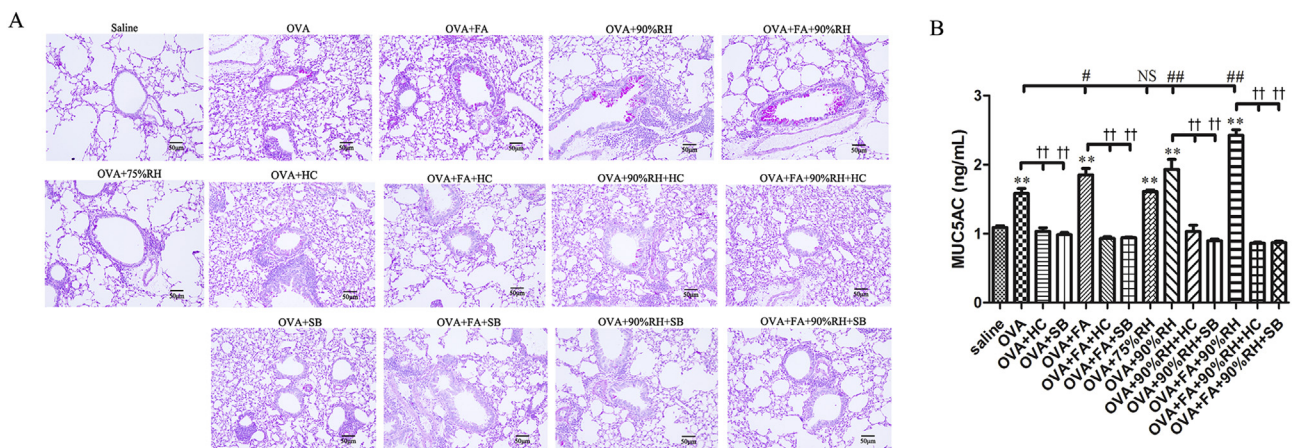


Fig. 4. The impact of exposure to formaldehyde and different relative humidities on airway mucus hypersecretion. (A) PAS staining of lung tissue (the pink part in the figures represent PAS-positive mucus cells, original magnification, 200 $\times$). (B) The level of MUC5AC in the different treatment groups. ** $p < 0.01$, significant difference compared with the saline group. NS $p > 0.05$, # $p < 0.05$, ## $p < 0.01$, different exposure groups compared with the OVA group. † $p < 0.01$, the blocking groups compared with the corresponding exposure groups ($n = 6$).

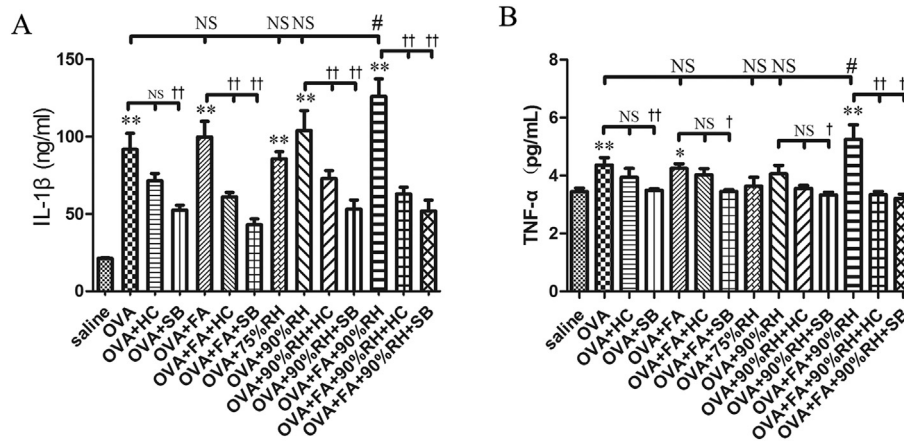


Fig. 5. The impact of exposure to formaldehyde and different relative humidities on inflammation in the lung tissue. (A) The levels of IL-1 β in the lung tissue. (B) The level of TNF- α in the lung tissue. NS $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, significant difference compared with the saline group. NS $p > 0.05$, # $p < 0.05$, ## $p < 0.01$, different exposure groups compared with the OVA group. $\dagger p < 0.05$, $\dagger\dagger p < 0.01$, the blocking groups compared with the corresponding exposure groups ($n = 6$).

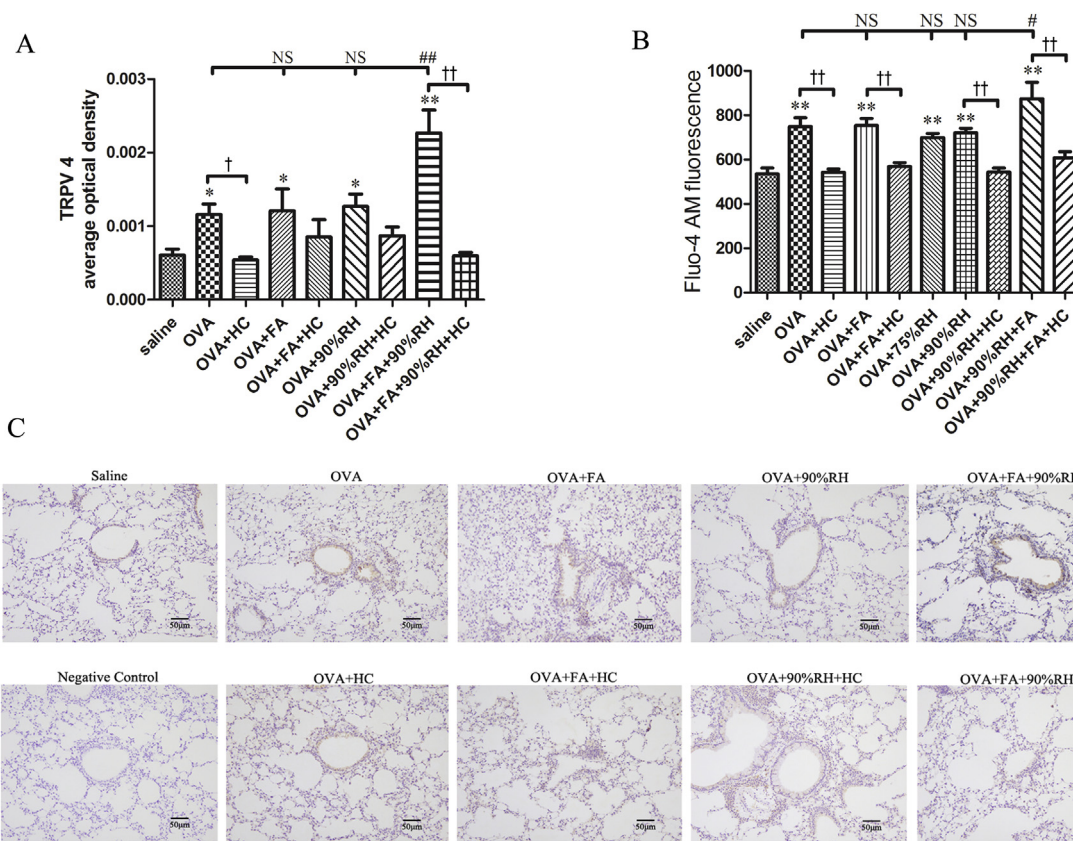


Fig. 6. The impact of exposure to formaldehyde and different relative humidities on the TRPV4 ion channel in the lung tissue. (A) The average optical density of TRPV4 in the lung tissue. (B) The level of calcium ions in the lung tissue. (C) Immunohistochemistry for TRPV4 in the lung tissue (original magnification, 200 $\times$). * $p < 0.05$, ** $p < 0.01$, significant difference compared with the saline group. NS $p > 0.05$, # $p < 0.05$, ## $p < 0.01$, different exposure groups compared with the OVA group. $\dagger p < 0.05$, $\dagger\dagger p < 0.01$, the blocking groups compared with the corresponding exposure groups ($n = 6$).

sharply in the OVA group compared with the saline group ($p < 0.01$), while in the OVA + FA + 90%RH group, the calcium ion levels increased significantly compared with the OVA group ($p < 0.05$). Moreover, after using the specific inhibitors, TRPV4 and HC-067047, the levels of TRPV4 and calcium ion in all the TRPV4 blocking groups decreased to varying degrees.

3.6. Formaldehyde combined with high relative humidity aggravated the activation of p38 MAPK

Calcium ion level increase induced by the activation of TRPV4 can lead to a series of signaling molecule activations. In this study, we determined the activation status of the p38 MAPK pathway

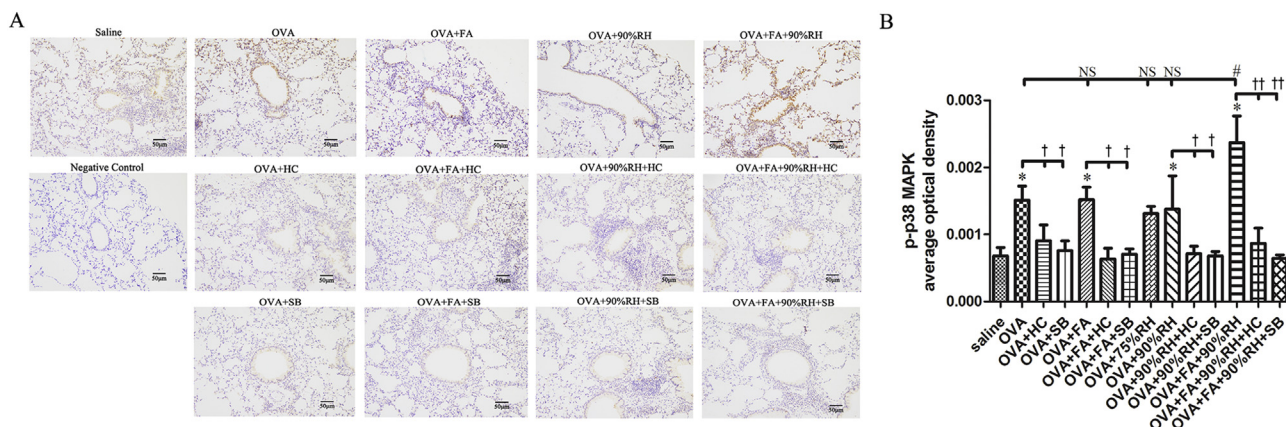


Fig. 7. The impact of exposure to formaldehyde and different relative humidities on p38 MAPK. (A) Immunohistochemistry for phospho-p38 MAPK in the lung tissue (original magnification, 200 ×). (B) The average optical density of phospho-p38 MAPK in the lung tissue. * $p < 0.05$, significant difference compared with the saline group. NS $p > 0.05$, # $p < 0.05$, different exposure groups compared with the OVA group. † $p < 0.05$, †† $p < 0.01$, the blocking groups compared with the corresponding exposure groups ($n = 6$).

(Fig. 7), which plays an important role in the inflammation response and in airway remodeling (Qin et al., 2018). In the OVA group, the level of phospho-p38 MAPK increased significantly ($p < 0.05$) compared with the saline group. Combined exposure to formaldehyde and 90% relative humidity enhanced the activation of p38 MAPK. In the OVA + FA + 90%RH group, the level of p38 MAPK phosphorylation increased significantly ($p < 0.05$) compared with the OVA group. In the SB203580 blocking groups the level of phospho-p38 MAPK decreased obviously indicating that, in this study, SB203580 inhibited the phosphorylation of p38 MAPK induced by exposure to formaldehyde and/or high relative humidity. After treatment with HC-203580, the levels of phospho-p38 MAPK also decreased reminding us that in this study, the TRPV4-p38 MAPK pathway was activated in the allergic asthma model, and that combined exposure to formaldehyde and 90% relative humidity can enhance the activation of this pathway.

4. Discussion

Formaldehyde is a high-profile environmental pollutant. Many studies have shown how formaldehyde influences the occurrence, or the exacerbation, of some diseases such as allergic rhinitis, allergic asthma, atopic dermatitis and Alzheimer-like changes (Han et al., 2017; Liu et al., 2018; Yon et al., 2019). Relative humidity has also been linked to several diseases, especially respiratory diseases, but most of these studies are epidemiological or clinical investigations, and these sorts of study do not aim to uncover the underlying causal mechanisms.

In this study, we observed that a combined exposure to 0.5 mg/m³ formaldehyde and high relative humidity (RH: 90%) leads to an aggravation of allergic asthma. As shown in our results, in the presence of OVA sensitization, exposure to formaldehyde or to different relative humidities alone does not seem to affect airway remodeling nor inflammatory cell infiltration. However, we did find that co-exposure to formaldehyde and a high relative humidity can aggravate airway wall remodeling and increase the infiltration of inflammatory cells.

To evaluate any changes in pulmonary function, we determined the levels of AHR, which is a typical characteristic of allergic asthma, and is a feature of chronic airway inflammation (Grundström et al., 2014; Kim et al., 2013). The results for Ri, Re and Cldyn suggest that combined exposure to formaldehyde and 90% relative humidity leads to a great exacerbation of AHR.

The Th2 cell-mediated immune response plays a very important

role in the progress of allergic asthma. We looked at levels of IFN- γ and IL-4 which reflect any imbalance of the Th1/Th2 immune response. The data showed that there is a sharp increase in IL-4 levels in the lung tissue in the OVA + FA + 90%RH group, but that there was only a slight increase in the level of IFN- γ . The ratio of IFN- γ /IL-4 indicated that the Th2 cell-mediated immune response gained the upper hand in the balance of the Th1/Th2 immune response after exposure to formaldehyde and 90% relative humidity. IgE plays a critical role in the pathogenesis of allergic asthma, and IgE-mediated immunoregulatory effects can drive the Th2 inflammatory response (Djukanovic et al., 2016; Geha et al., 2003). The results showed that combined exposure to formaldehyde and 90% relative humidity lead to a significant increase in the level of IgE in the lung tissue.

Based on the above results, we conclude that exposure to 0.5 mg/m³ formaldehyde or 90% relative humidity alone leads to only a slight and not significant aggravation of allergic asthma. But combined exposure to formaldehyde and 90% relative humidity can greatly aggravate allergic asthma like-pathology.

We also determined the status of mucus hypersecretion and pro-inflammatory cytokines in the lung to clarify whether exposure to formaldehyde and/or high relative humidity can induce other symptoms in the allergic asthma model mice. We used PAS staining to count the number of mucus cells and examined the level of MUC5AC, the primary macromolecular component of airway mucus, to evaluate any change in airway mucus hypersecretion (Wang et al., 2012). We observed that exposure to either a low-dose of formaldehyde or to 90% relative humidity alone can aggravate airway mucus hypersecretion, but that co-exposure to formaldehyde and 90% relative humidity enhances this aggravation.

We also measured the levels of IL-1 β and TNF- α to assess inflammation in the lung tissue. Co-exposure to formaldehyde and 90% relative humidity lead to a significant increase of IL-1 β and TNF- α , indicating that there is an exacerbation of inflammation in the lung.

To confirm whether the TRPV4 ion channel plays a role in formaldehyde and high relative humidity induced allergic asthma, we looked at the protein level of TRPV4 and at the level of calcium ions in the lung. The data showed that co-exposure to formaldehyde and 90% relative humidity can lead to an increase in TRPV4 and the opening of the ion channel. To determine the role of TRPV4 in formaldehyde and high relative humidity induced aggravation of allergic asthma, we treated the mice with TRPV4, a selective antagonist of HC-067047 (Wu et al., 2017). After treatment with

HC-067047, the levels of TRPV4 and calcium ions decreased significantly suggesting that the antagonist worked. In addition, the exacerbation of the biomarkers of allergic asthma that we noted in this study, including the Th2 cell-mediated immune response, the level of IgE, the mucus hypersecretion and lung inflammation were significantly alleviated. We think that the TRPV4 ion channel plays an important role in the aggravation of allergic asthma induced by co-exposure to formaldehyde and 90% relative humidity.

Activation of the TRPV4 ion channel can regulate a series of signaling molecules. Previous studies have indicated that p38 MAPK is associated with pro-inflammation effects in allergic asthma, and that suppressing activation of p38 MAPK can effectively inhibit mucus hypersecretion (Duan et al., 2005; Wijerathne et al., 2017). In this study, we determined the status of p38 MAPK phosphorylation to assess the activation of p38 MAPK. The data showed that OVA sensitization resulted in activation of p38 MAPK in the lung, and that co-exposure to formaldehyde and 90% relative humidity greatly aggravated this activation. SB203580 is a specific inhibitor of p38 MAPK (Wu et al., 2019). We therefore treated mice with SB203580 to verify whether p38 MAPK plays an important role. As we speculated, after treatment with SB203580 the phosphorylation of p38 MAPK receded. Similarly, treatment with HC-067047 significantly alleviated the aggravation of the allergic asthma biomarkers. Moreover, the levels of phospho-p38 MAPK decreased significantly in all the HC-067047 treatment groups. These data indicate that the aggravation of opening of TRPV4 ion channel that was induced by exposure to formaldehyde and/or high relative humidity can lead to the enhance of the activation of p38 MAPK, resulting in an exacerbation of the airway inflammation and mucus hypersecretion.

In this study, we have verified that exposure to 0.5 mg/m³ formaldehyde or 90% relative humidity can lead to a slight but not significant aggravation of allergic asthma, while combined exposure to formaldehyde and high relative humidity has the effect of greatly exacerbating allergic asthma in mice. Our data have indicated that co-exposure to formaldehyde and high relative humidity can strengthen the opening of the TRPV4 ion channel leading to increased levels of calcium ions, and the enhanced activation of p38 MAPK resulting in increased levels of airway inflammation and mucus hypersecretion. There are still some inadequacies and limitations in this study. The enhance of activation of the TRPV4-p38 MAPK pathway plays a critical role in aggravation of allergic asthma, but exactly how the activation of TRPV4 ion channel is aggravated by exposure to formaldehyde and high relative humidity still needs to be clarified. The question of whether there are other signaling molecules that can be activated by TRPV4 also warrants further study.

5. Conclusion

In summary, our work demonstrated that combined exposure to formaldehyde and high relative humidity (90%) can exacerbate allergic asthma in mice. In OVA-induced allergic asthma mice model the TRPV4-p38 MAPK was activated, and co-exposure to formaldehyde and high relative humidity enhanced the activation of the TRPV4-p38 MAPK pathway, leading to an aggravation of airway inflammation and mucus hypersecretion. Eventually this results in the exacerbation of allergic asthma.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

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References

- Aydin, S., Canpinar, H., Undeger, U., Guc, D., Colakoglu, M., Kars, A., Basaran, N., 2013. Assessment of immunotoxicity and genotoxicity in workers exposed to low concentrations of formaldehyde. *Arch. Toxicol.* 87, 145–153.
- Bao, A., Yang, H., Ji, J., Chen, Y., Bao, W., Li, F., Zhang, M., Zhou, X., Li, Q., Ben, S., 2017. Involvements of p38 MAPK and oxidative stress in the ozone-induced enhancement of AHR and pulmonary inflammation in an allergic asthma model. *Respir. Res.* 18.
- Birrell, M., Bonvini, S.J., Baker, K.E., Dekkak, B., Miralpeix, M., Tarrason, G., Belvisi, M.G., 2016. The TRPV4 ion channel plays a key role in allergic asthma. *Eur. Respir. J.* 48, 2.
- Chen, Y., Kanju, P., Fang, Q., Lee, S.H., Parekh, P.K., Lee, W., Moore, C., Brenner, D., Gereau, R.W.t., Wang, F., Liedtke, W., 2014. TRPV4 is necessary for trigeminal irritant pain and functions as a cellular formalin receptor. *Pain* 155, 2662–2672.
- Djukanovic, R., Hanania, N., Busse, W., Price, D., 2016. IgE-mediated asthma: New revelations and future insights. *Respir. Med.* 112, 128–129.
- Duan, J., Kang, J., Qin, W., Deng, T., Liu, H., Li, B., Yu, W., Gong, S., Yang, X., Chen, M., 2018. Exposure to formaldehyde and diisobutyl phthalate exacerbate neuro-inflammation through NF-kappaB activation in a mouse asthma model. *Eco-toxicol. Environ. Saf.* 163, 356–364.
- Duan, W., Chan, J.H.P., McKay, K., Crosby, J.R., Choo, H.H., Leung, B.P., Karras, J.G., Wong, W.S.F., 2005. Inhaled p38 mitogen-activated protein kinase antisense oligonucleotide attenuates asthma in mice. *Am. J. Respir. Crit. Care Med.* 171, 571–578.
- Everaerts, W., Nilius, B., Owsianik, G., 2010. The vanilloid transient receptor potential channel TRPV4: from structure to disease. *Prog. Biophys. Mol. Biol.* 103, 2–17.
- Filingieri, D., 2015. Humidity sensation, cockroaches, worms, and humans: are common sensory mechanisms for hygrosensation shared across species? *J. Neurophysiol.* 114, 763–767.
- Filingieri, D., Havenith, G., 2015. Human skin wetness perception: psychophysical and neurophysiological bases. *Temp. (Austin)* 2, 86–104.
- Geha, R.S., Jabara, H.H., Brodeur, S.R., 2003. The regulation of immunoglobulin E class-switch recombination. *Nat. Rev. Immunol.* 3, 721.
- Gerald McGwin, J., Lienert, J., John, L., Kennedy, J., 2010. Formaldehyde exposure and asthma in children: a systematic review. *Environ. Health Perspect.* 118, 313–317.
- Grundström, J., Saarne, T., Kemi, C., Gregory, J.A., Wadén, K., Pils, M.C., Adner, M., Gafvelin, G., van Hage, M., 2014. Development of a mouse model for chronic cat allergen-induced asthma. *Int. Arch. Allergy Immunol.* 165, 195–205.
- Han, R.T., Back, S.K., Lee, J., Kim, H.Y., Kim, H.J., Na, H.S., 2017. Effects of exposure of formaldehyde to a rat model of atopic dermatitis induced by neonatal capsaicin treatment. *J. Vis. Exp.* 6.
- Jie, P., Hong, Z., Tian, Y., Li, Y., Lin, L., Zhou, L., Du, Y., Chen, L., Chen, L., 2015. Activation of transient receptor potential vanilloid 4 induces apoptosis in hippocampus through downregulating PI3K/Akt and upregulating p38 MAPK signaling pathways. *Cell Death Dis.* 6 e1775–e1775.
- Kang, J., Duan, J., Song, J., Luo, C., Liu, H., Li, B., Yang, X., Yu, W., Chen, M., 2018. Exposure to a combination of formaldehyde and DINP aggravated asthma-like pathology through oxidative stress and NF-kappa B activation. *Toxicology* 404, 49–58.
- Kim, K.-H., Jahan, S.A., Kabir, E., 2013. A review on human health perspective of air pollution with respect to allergies and asthma. *Environ. Int.* 59, 41–52.
- Kim, S.R., Lee, K.S., Park, S.J., Jeon, M.S., Lee, Y.C., 2012. Inhibition of p38 MAPK reduces expression of vascular endothelial growth factor in allergic airway disease. *J. Clin. Immunol.* 32, 574–586.
- Liu, L., Li, Y., Wang, R., Yin, C., Dong, Q., Hing, H., Kim, C., Welsh, M.J., 2007. Drosophila hygrosensation requires the TRP channels water witch and nanchung. *Nature* 450, 294–298.
- Liu, X., Zhang, Y., Wu, R., Ye, M., Zhao, Y., Kang, J., Ma, P., Li, J., Yang, X., 2018. Acute formaldehyde exposure induced early Alzheimer-like changes in mouse brain. *Toxicol. Mech. Methods* 28, 95–104.
- Luo, F.C., Zhou, J., Lv, T., Qi, L., Wang, S.D., Nakamura, H., Yodoi, J., Bai, J., 2012. Induction of endoplasmic reticulum stress and the modulation of thioredoxin-1 in formaldehyde-induced neurotoxicity. *Neurotoxicology* 33, 290–298.
- Montell, C., 2008. TRP channels: it's not the heat, it's the humidity. *Curr. Biol.* 18, R123–R126.
- Nielsen, G.D., Larsen, S.T., Wolkoff, P., 2013. Recent trend in risk assessment of formaldehyde exposures from indoor air. *Arch. Toxicol.* 87, 73–98.
- Peden, D., Reed, C.E., 2010. Environmental and occupational allergies. *J. Allergy Clin.*

- Immunol. 125, S150–S160.
- Qin, W., Deng, T., Cui, H., Zhang, Q., Liu, X., Yang, X., Chen, M., 2018. Exposure to diisodecyl phthalate exacerbated Th2 and Th17-mediated asthma through aggravating oxidative stress and the activation of p38 MAPK. *Food Chem. Toxicol.* 114, 78–87.
- Qu, Y.-J., Zhang, X., Fan, Z.-Z., Huai, J., Teng, Y.-B., Zhang, Y., Yue, S.-W., 2016. Effect of TRPV4-p38 MAPK pathway on neuropathic pain in rats with chronic compression of the dorsal root ganglion. *BioMed Res. Int.* 2016, 1–12.
- Scheraga, R.G., Southern, B.D., Grove, L.M., Olman, M.A., 2017. The role of transient receptor potential vanilloid 4 in pulmonary inflammatory diseases. *Front. Immunol.* 8, 503.
- Tavernier, G., Fletcher, G., Gee, I., Watson, A., Blacklock, G., Francis, H., Fletcher, A., Frank, T., Frank, P., Pickering, C.A., 2006. IPEADAM study: indoor endotoxin exposure, family status, and some housing characteristics in English children. *J. Allergy Clin. Immunol.* 117, 656–662.
- Véronique, E., Marcel, B., Catherine, N., Gaëlle, O.G., Monique, D., Serge, K., Pierre-André, C., Jacques, L., Michel, A., 2007. Effect of formaldehyde on asthmatic response to inhaled allergen challenge. *Environ. Health Perspect.* 115, 210–214.
- van Strien, R.T., Gehring, U., Belanger, K., Triche, E., Gent, J., Bracken, M.B., Leaderer, B.P., 2015. The influence of air conditioning, humidity, temperature and other household characteristics on mite allergen concentrations in the northeastern United States. *Allergy* 59, 645–652.
- Wang, G., Xu, Z., Wang, R., Al-Hijji, M., Salit, J., Strulovici-Barel, Y., Tilley, A.E., Mezey, J.G., Crystal, R.G., 2012. Genes associated with MUC5AC expression in small airway epithelium of human smokers and non-smokers. *BMC Med. Genomics* 5, 1(2012-06-07) 5, 21.
- Wen, H., Yuan, L., Wei, C., Zhao, Y., Qian, Y., Ma, P., Ding, S., Yang, X., Wang, X., 2016. Effects of combined exposure to formaldehyde and benzene on immune cells in the blood and spleen in Balb/c mice. *Environ. Toxicol. Pharmacol.* 45, 265–273.
- Wijerathne, C.U.B., Seo, C.-S., Song, J.-W., Park, H.-S., Moon, O.-S., Won, Y.-S., Kwon, H.-J., Son, H.-Y., 2017. Isoimperatorin attenuates airway inflammation and mucus hypersecretion in an ovalbumin-induced murine model of asthma. *Int. Immunopharmacol.* 49, 67–76.
- Wu, Q.-F., Qian, C., Zhao, N., Dong, Q., Li, J., Wang, B.-B., Chen, L., Yu, L., Han, B., Du, Y.-M., Liao, Y.-H., 2017. Activation of transient receptor potential vanilloid 4 involves in hypoxia/reoxygenation injury in cardiomyocytes. *Cell Death Dis.* 8, e2828.
- Wu, Y., You, H., Ma, P., Li, L., Yuan, Y., Li, J., Ye, X., Liu, X., Yao, H., Chen, R., Lai, K., Yang, X., 2013. Role of transient receptor potential ion channels and evoked levels of neuropeptides in a formaldehyde-induced model of asthma in BALB/c mice. *PLoS One* 8, e62827.
- Wu, Z.H., He, D., Zhao, S.Y., Wang, H.J., 2019. IL-17A/IL-17RA promotes invasion and activates MMP-2 and MMP-9 expression via p38 MAPK signaling pathway in non-small cell lung cancer. *Mol. Cell. Biochem.* 455, 195–206.
- Yon, D.K., Hwang, S., Lee, S.W., Jee, H.M., Sheen, Y.H., Kim, J.H., Lim, D.H., Han, M.Y., 2019. Indoor exposure and sensitization to formaldehyde among inner-city children with increased risk of asthma and rhinitis. *Am. J. Respir. Crit. Care Med.* 200(3), 388–393.
- Zhang, H., Yoshino, H., 2010. Analysis of indoor humidity environment in Chinese residential buildings. *Build. Environ.* 45, 2132–2140.