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The IL-31/TRPV1 pathway mediates allergic asthma exacerbated by DINP dermal exposure in OVA-sensitized Balb/c mice

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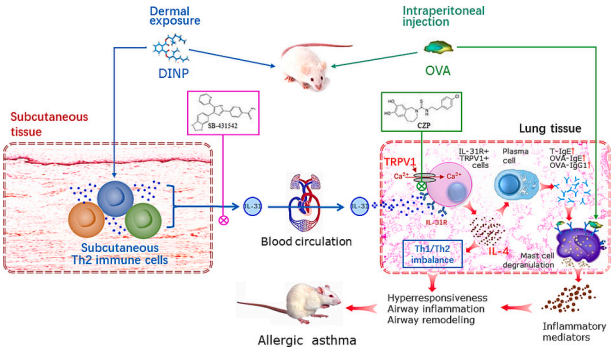
HIGHLIGHTS

- Dermal exposure to DINP aggravates allergic asthma in Balb/c mice.
- IL-31/TRPV1 pathway is involved in allergic asthma exacerbated by DINP.
- Dermal exposure to DINP triggers a Th1/Th2 immune imbalance in Balb/c mice.
- SB-431542 or capsazepine treatment can alleviate allergic asthma exacerbated by DINP.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The potential role of dermal exposure diisononyl phthalate (DINP) as an adjuvant in allergic inflammation and asthma has been suggested. However, the current findings do not provide enough evidence to support this claim.

Objectives: The purpose of this investigation was to examine the impact and mechanisms of allergic asthma exacerbation through the dermal exposure to DINP.

Methods: The study was undertaken using OVA-sensitized mice. Lung histopathology and airway hyperreactivity (AHR) were assessed. Expression levels of immunoglobulins (t-IgE, OVA-IgE and OVA-IgG1), cytokines (IL-31, IL-4, IL-5, IL-6, IL-13 and INF-γ), and TRPV1 were measured. To investigate the mechanism by which allergic asthma worsens due to dermal exposure to DINP, the blockade analysis using the IL-31 antagonist SB-431542 and the TRPV1 antagonist capsazepine (CZP) were performed.

Results: The findings of the study revealed that the simultaneous exposure to DINP and OVA resulted in an increase in inspiratory resistance (Ri) and expiratory resistance (Re), a decrease in the minimum value of lung

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dynamic compliance (Cldyn), and worsened airway remodeling. Additionally, it was found that this exposure led to an increase in the levels of IL-31 and TRPV1, which are biomarkers of Th2 cytokines (IL-4, IL-5, IL-6, and IL-13), as well as immunoglobulins (Total IgE, OVA-lgE, and OVA-IgG1), while decreasing the biomarker of Th1 cytokines (IFN-γ). However, these impairments showed improvement after the administration of SB-431542 or CZP.

Conclusion: The findings of this research indicate that the IL-31/TRPV1 pathway plays a moderating function in OVA-induced allergic asthma worsened by dermal exposure to DINP.

1. Introduction

Asthma is a persistent respiratory condition that affects a significant global population exceeding 339 million individuals (The Global Asthma Report, 2019). Recently, there has been an increasing focus among scientists on investigating the relationship between environmental chemicals and the development of allergic asthma (Cherrie et al., 2021; Hu et al., 2022). One such chemical diisononyl phthalate (DINP), which is commonly used as a plasticizer and is found to be a prevalent contaminant in the environment (Zhang et al., 2022). Epidemiological evidence suggests that both Chinese and Swedish children have a higher susceptibility to asthma due to their exposure to DINP (Preece et al., 2022). In vitro studies have demonstrated that DINP can increase IL-4 concentrations, thereby enhancing the immune response (Lee et al., 2004). Previous animal research has also shown that maternal ingestion of DINP can exacerbate airway inflammation in pups induced by ovalbumin (OVA) (Chen et al., 2015). Additionally, exposure to DINP has been linked to modifications that can disrupt the Th1/Th2 immune balance and potentially trigger allergic asthma (Hwang et al., 2017). Furthermore, in mouse models the oral administration of DINP in combination with allergen exposure has been found to induce airway hyperreactivity (AHR) (Li et al., 2020). Nevertheless, the specific mechanisms by which DINP induces asthma remain uncertain.

Research has indicated that asthmatics have elevated levels of interleukin (IL)-31, a protein that regulates inflammation in the lungs triggered by allergens (Neuper et al., 2021). In mice, IL-31 induces skin inflammation and dermatitis (Dillon et al., 2004). Individuals who suffering from atopic dermatitis show enhanced expression of IL-31 mRNA (Neis et al., 2006). The sensory neurons responsible for T-cell-mediated inflammation express both the IL-31 receptor α and transient receptor potential vanilloid 1 (TRPV1) (Cevikbas et al., 2014). Recent studies indicate that TRPV1 could be a potential target for addressing asthma (Choi Jo et al., 2018; Reyes-García et al., 2022). However, the precise association between DINP, allergic asthma, and the IL-31/TRPV1 pathway has yet to be fully elucidated.

Previous research on environmental asthma has primarily focused on the respiratory and digestive systems. However, recent studies suggest that skin exposure may also play a significant role in the development of immune reactions similar to asthma (Tsui et al., 2020). Dermal absorption is a crucial pathway for the general population's exposure to DINP (Husøy et al., 2019). However, limited research is available on dermal exposure in DINP-induced allergic asthma.

Therefore, we conducted an OVA-sensitized asthma model to investigate whether DINP dermal exposure can aggravate allergic asthma. By using the TGF-β antagonist SB-431542 and the TRPV1 antagonist capsazepine (CZP), we demonstrated the potential involvement of the IL-31/TRPV1 pathway in mediating allergic asthma exacerbated by DINP dermal exposure. These findings will serve as references for conducting respiratory health risk assessments related to dermal exposure to DINP, as well as for developing targeted therapeutic interventions for asthma.

2. Materials and methods

2.1. Reagents and kits

DINP (>99 %), ovalbumin (OVA), and methacholine (MCH) were obtained from Sigma-Aldrich (MO, USA), SB-431542 (CAS: 301836-41-9) and capsazepine (CZP, CAS: 138977-28-3) were purchased from MedChemExpress (NJ, USA). Tween 80 was purchased from Amresco (Ohio, USA). MouseELISA kits for total immunoglobulin E (t-IgE), OVA-specific IgE (OVA-IgE), OVA-specific IgG1 (OVA-IgG1), IL-31, IL-4, IL-5, IL-6, IL-13, and Interferon γ (IFN-γ) were obtained from Enzyme-linked Biotechnology (Shanghai, China). Mouse anti-IL-31 antibodies and anti-TRPV1 antibodies were purchased from Ruiying Biotechnology (Suzhou, China).

2.2. Animals

Specific pathogen Free (SPF) Balb/c male mice (6–8 weeks, 18 ± 1 g) were obtained from Changshen Biotechnology (Benxi, China) and housed under standard conditions. Throughout the experiment, mice were maintained at a temperature of 20–25 °C, a humidity of 55–65 %, and a light/dark cycle of 12 h. Measures were taken to prevent contact with allergens. All animal experiments were conducted following the guidelines provided in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). The research ethics for this work have been approved by Hubei University of Science and Technology. A certificate of approval (ID: HBUST-IACUC-2021-010) is available upon request.

2.3. Experimental protocol

Six groups of 120 Balb/c mice were randomly allocated to the study: (1) A group exposed orally to saline (Saline group); (2) A group exposed dermally to DINP at a dosage of 20 mg/kg/day (DINP group); (3) A group exposed to saline orally in combination with OVA (OVA group); (4) A group exposed to DINP at a dosage of 20 mg/kg/day in combination with OVA (DINP+OVA group); (5) A group exposed to DINP at a dosage of 20 mg/kg/day in combination with OVA and the SB-431542 antagonist (DINP + OVA + SB-431542 group); and (6) A group exposed to DINP at a dosage of 20 mg/kg/day in combination with OVA and the CZP antagonist (DINP + OVA + CZP group).

DINP's no-observed-adverse-effect level (NOAEL) is 15 mg/kg/day, according to the European Food Safety Authority (EFSA). (EFSA, 2019). Previous research (Li et al., 2020; EFSA, 2019) has determined that a preferred exposure dose for DINP is 20 mg/kg/day, which is slightly higher than the NOAEL values and indicates a low level of exposure for the occupational population. An epicutaneous application of DINP/Tween-80 (1, 1, v/v) or saline was applied once a day to mice at a smearing volume of 10 ml/kg. According to the National Toxicology Program (1992), mice fed 5 % Tween-80 long-term did not show any toxic effects. After sensitization with 50 g OVA in 300 μl saline, prepared in a ratio of 1:35 with acetone and Al $(OH)_3$, on days 16, 19, 22, 25, and 28, the mice were exposed to 1 % OVA for 30 min/day from days 30 to 36 using an Ultrasonic Nebulizer (model 402AI, Yuyue, China).

The mice in the DINP + OVA + SB-431542 group were injected intraperitoneally with SB-431542 (suspended in 10 % dimethyl

sulfoxide) three times a week (Alyoussef, 2018) Meanwhile, mice in the DINP + OVA + CZP group were intraperitoneally injected once daily with CZP (diluted in 5 % Tween 80). The dose of SB-431542 administered was 1 mg/kg, while the dose of CZP was 1.6 mg/kg, both with an injection volume of 10 ml/kg.

The experimental protocol is illustrated in Fig. 1 and Table 1.

2.4. Histological analysis of the lung tissue

The left lung was fixed in 4 % paraformaldehyde at room temperature, embedded in paraffin, and cut into 4 μm slices for H&E staining, Masson trichrome (MT) staining, and Periodic Acid-Schiff (PAS) staining using standard methods (Langlet et al., 2017). Optical microscope images were taken of the stained slices (DP73, Olympus, Japan). The immunohistochemical staining was carried out as previously described (Yu et al., 2016). The right lung tissue sections were deparaffinized, rehydrated, and subjected to a 10-min heat treatment to improve antigen retrieval. Subsequently, they were incubated with 3 % H_2O_2 for 5 min to deactivate peroxidase. The sections were placed at room temperature (RT) and treated with 5 % goat serum for 10 min. Subsequently, they were incubated overnight at 4 °C with primary antibodies: mouse anti-IL-31 antibody (1100) and mouse anti-TRPV1 antibody (1.50). Following this, the suitable secondary antibody (rabbit lgG SABC-POD, 1200) was administered for a duration of 40 min. Diaminobenzidine (DAB) was utilized as a chromogen. Stained lung tissue was examined under an optical microscope using Image-Pro Plus 6.0 (Rockville, MD). In order to assess the intensity of staining, the optical density analysis involved calculating the average optical density using an unstained region as the reference for background.

2.5. Measurement of airway hyperresponsiveness (AHR)

The AniRes 2005 lung function system (Bestlab, China) was used to measure AHR within 24 h of the last nebulization, in accordance with the provided instructions. The mice were given intraperitoneal administration of 1 % pentobarbital sodium for anesthesia. The pre-established breathing rate was 90 breaths/min and the proportion of exhaling to inhaling was 1.5 to 1. At 5-min intervals, doses of 0.025, 0.05, 0.1, and 0.2 mg/kg body weight of MCH were administered via the jugular vein. AHR was assessed by measuring the inspiratory resistance (Ri), expiratory resistance (Re), and the minimum value of lung dynamic compliance (Cldyn).

2.6. Determination of immune and inflammatory biomarkers

On the 37th day, mice were euthanized, and blood samples were obtained from their hearts. After being kept at room temperature (RT) for half an hour, the blood samples were subsequently spun at 3000 rpm for 15 min at a temperature of 25 °C. The resulting supernatant was stored at −70 °C. After collecting blood from the heart, the right lungs were submerged in phosphate-buffered saline that was chilled with ice, then mixed thoroughly and subsequently spun at a speed of 10,000 rpm

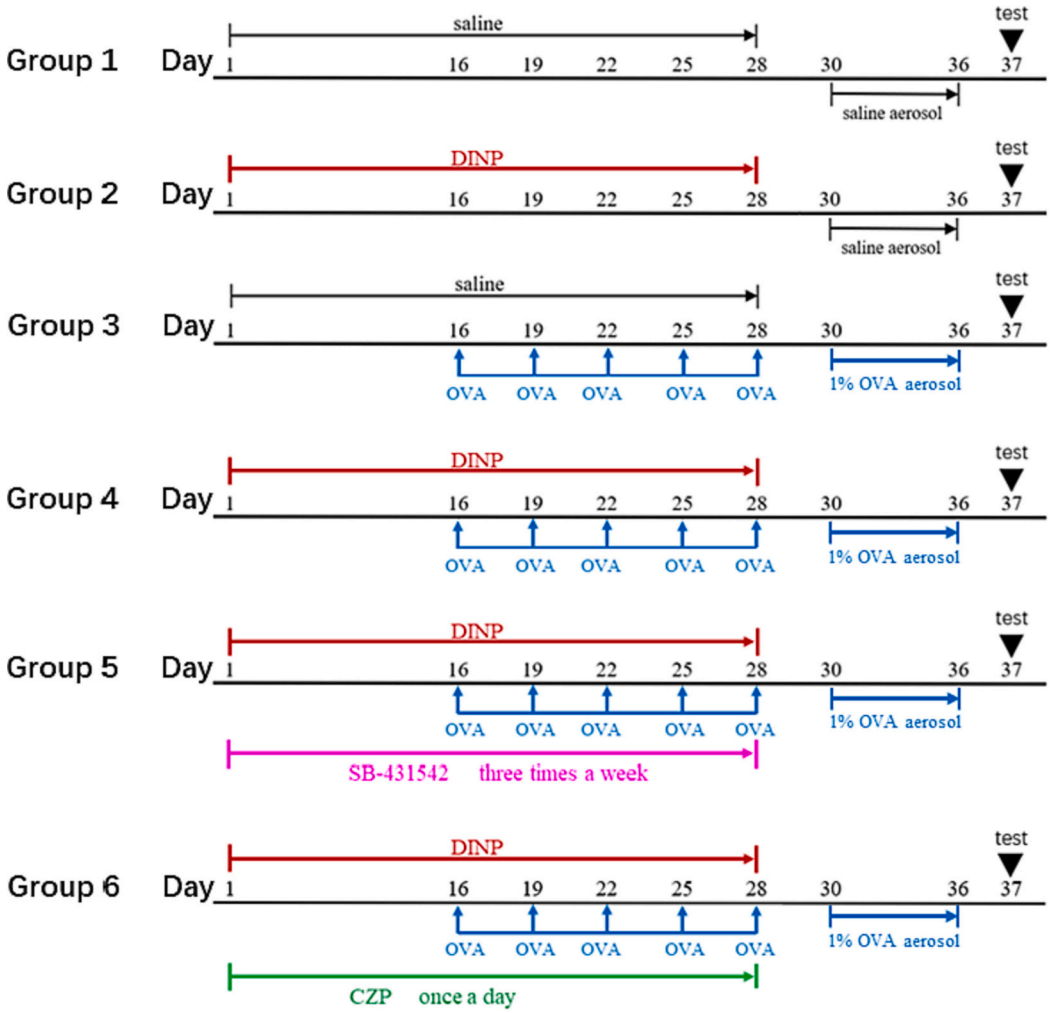

Fig. 1. Sensitization and exposure protocol. DINP represents diisononyl phthalate, OVA stands for ovalbumin, SB-431542 is a TGF-β antagonist used for bloke IL-31 expression and capsazepine (CZP) is a TRPV1 antagonist.

Table 1
Grouping and group treatments.

Group ID	Group names	Treatments for different groups					
		Saline intraperitoneal injection+aerosol	OVA intraperitoneal injection+aerosol	Saline dermal exposure	DINP dermal exposure	SB-431542 intraperitone al injection	CZP intraperitoneal injection
Group 1	Saline group	+	−	+	−	−	−
Group 2	DINP group	+	−	−	+	−	−
Group 3	OVA group	−	+	+	−	−	−
Group 4	OVA + DINP group	−	+	−	+	−	−
Group 5	OVA + DINP + SB-431542 group	−	+	−	+	+	−
Group 6	OVA + DINP + CZP group	−	+	−	+	−	+

for 10 min at a temperature of 4 °C. The supernatant was collected and stored at −70 °C. The levels of T-IgE, OVA-IgE, and OVA-IgG1 in the serum, as well as the levels of IL-31, TRPV1, IFN-γ, IL-4, IL-5, IL-6, and IL-13 in lung tissue, were measured using ELISA kits following the instructions provided in the kit.

2.7. Statistical analysis

GraphPad Prism 7.0 (San Diego, CA) was utilized to generate statistical graphs based on the experimental data. The values were displayed as the mean ± SEM (standard error of the mean). The analysis of data was performed utilizing SPSS Statistics V.18 (Chicago, IL). To evaluate the significance of variations among groups, both ANOVA and Tukey's test were utilized. Significant p-values are represented as $p < 0.05$, $p < 0.01$.

3. Results

3.1. Histopathological changes in lung

Histopathological changes are evident in the groups with OVA and DINP + OVA (Fig. 2). Increasing inflammation around the airways and thickening of the airway walls, as well as deposition of subepithelial collagen and hyperplasia of goblet cells. Compared to the OVA group, there was an increased amount of inflammatory cell infiltration, airway mucus, and goblet cells. Moreover, there was a significant rise in collagen fibers surrounding the airways in the DINP + OVA group. There was a significant reduction in the degree of inflammatory cell infiltration, collagen deposition and fibrosis in the peribronchial cavity, mucus overproduction, and goblet-cell hyperplasia when SB-431542 or CZP was applied.

3.2. Influences on airway hyperresponsiveness

Fig. 3 shows the results of measurements of airway responsiveness. Three parameters of lung function (Ri, Re and Cdyn) were recorded after each injection of MCH (0.025, 0.05, 0.1 and 0.2 mg/kg). In all groups, the expiratory and inspiratory resistance increased with increasing MCH levels, whereas Cdyn decreased. OVA-sensitized groups exhibited a greater airway response to MCH compared with the vehicle control group and DINP exposure significantly enhanced the degree of airway reactivity in OVA-sensitized mice. Treatment with SB-431542 or CZP dramatically reduced Ri, Re and restored Cdyn in DINP + OVA-treated mice in response to MCH, respectively.

3.3. IL-31/TRPV1 pathway

Groups exposed to OVA showed elevated levels of lung IL-31 and

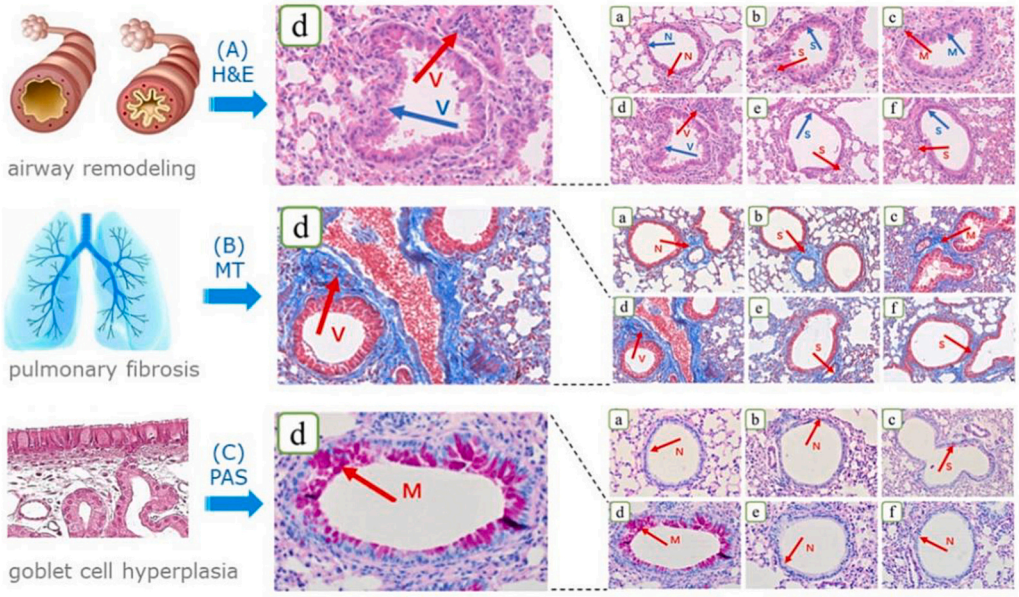

Fig. 2. Lung histology. N: normal tissue; S: slight change; M: moderate change; V: severe changes. (A) H&E staining: shows infiltration of inflammatory cells (red arrow) and airway remodeling (blue arrow). (B) MT staining: shows peribronchial deposition of collagen (blue color stain, red arrow). (C) PAS staining: shows goblet cell proliferation and mucus hypersecretion (purple color stain, red arrow). Panel: (a) Saline group; (b) DINP group; (c) OVA group; (d) OVA + DINP group; (e) OVA + DINP + SB431542 group; (f) OVA + DINP + CZP group. (n = 8 per group).

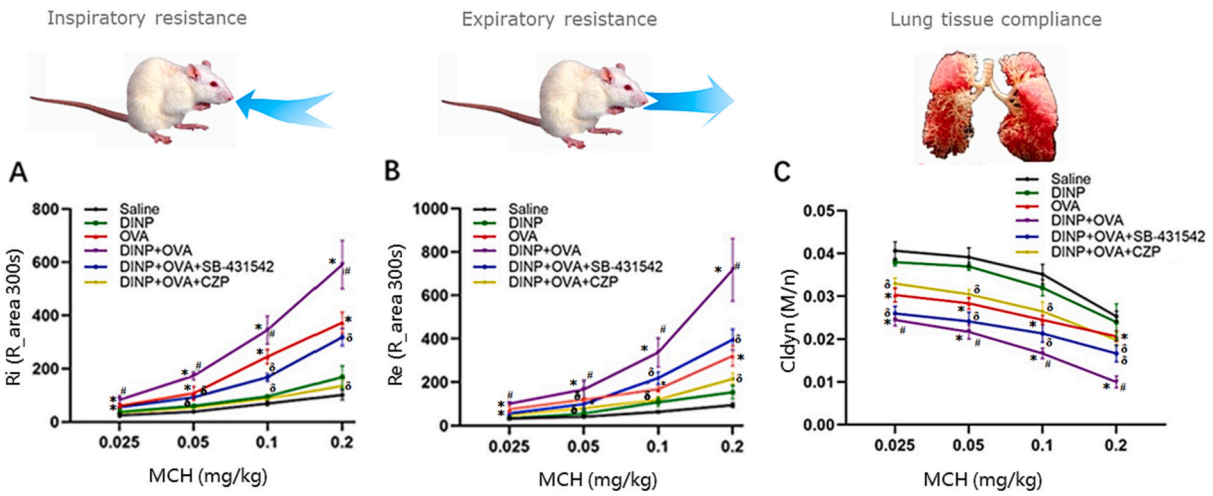

Fig. 3. Analysis of airway hyperresponsiveness. A, B, and C represent Ri, Re, and the Cldyn for the different treatment groups. *: $p < 0.05$, **: $p < 0.01$, compared with the control group; #: $p < 0.05$, ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, compared with the OVA + DINP group. (n = 8 per group).

TRPV1 (Fig. 4B (L) and C (L)). Exposure to DINP + OVA induced the highest levels. Treatment with SB-431542 or CZP significantly reduced the levels of IL-31 or TRPV1 compared with DINP + OVA group. To identify IL-31 and TRPV1 expression in lung tissue, immunohistochemical analyses were conducted. The expression of IL-31 and TRPV1 was primarily observed in the epithelial layers surrounding the bronchioles (Fig. 4B (R) and C (R)).

3.4. Th1/Th2 imbalance and related cytokines

Lung tissue samples were assessed for levels of the Th1 cytokine: IFN-γ and Th2 cytokines: IL-4, IL-5, IL-6, IL-13 (Fig. 5). Compared to the OVA group, the DINP + OVA group exhibited elevated levels of IL-4, IL-5, IL-6, and IL-13, while displaying decreased levels of IFN-γ. Treatment with SB-431542 or CZP treatment alleviated the Th1/Th2 immune imbalance

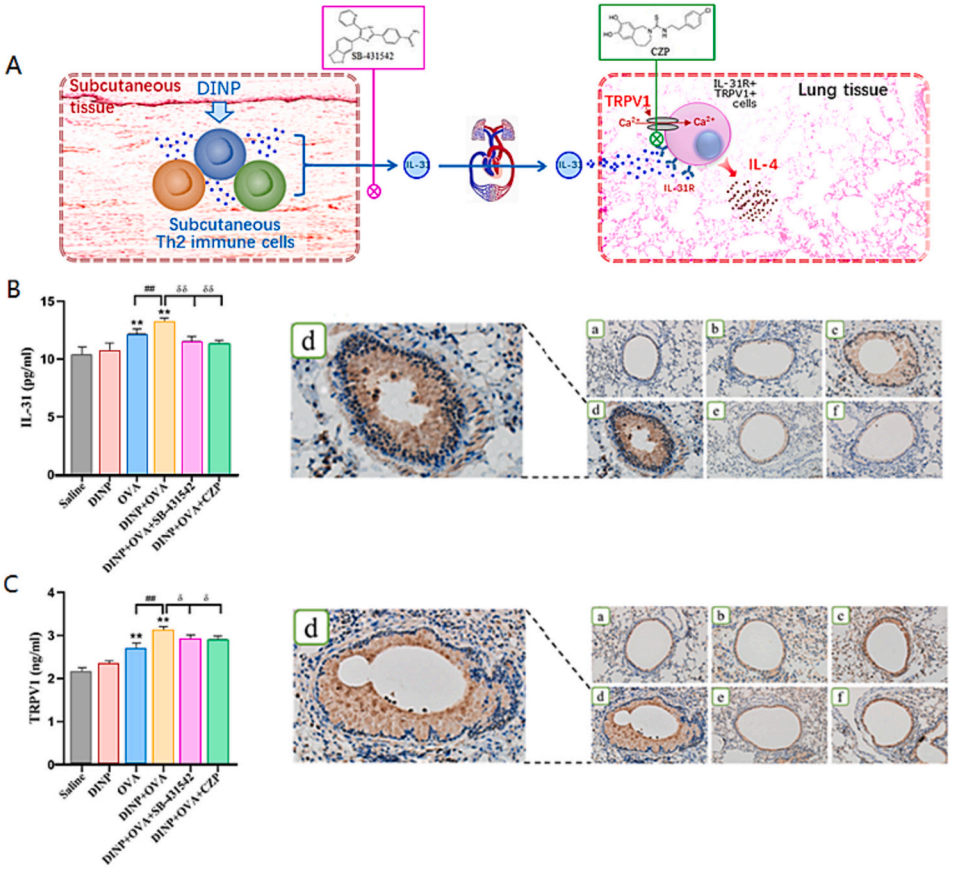

Fig. 4. IL-31 and TRPV1 levels in lung tissue. (A) IL-31/TRPV1 pathway illustration. (B) IL-31 expression. (C) TRPV1 expression. Analysis of expression levels was conducted based on the average optical density. The images were magnified at ×20. Panels: (a) Group treated with saline; (b) Group treated with DINP; (c) Group treated with OVA; (d) Group treated with OVA + DINP; (e) Group treated with OVA + DINP + SB-431542; (f) Group treated with OVA + DINP + CZP. **: $p < 0.01$, compared to the saline group; ##: $p < 0.01$, compared to the OVA group; δδ: $p < 0.01$, compared to the OVA + DINP group, the total number of samples in each group was 8.

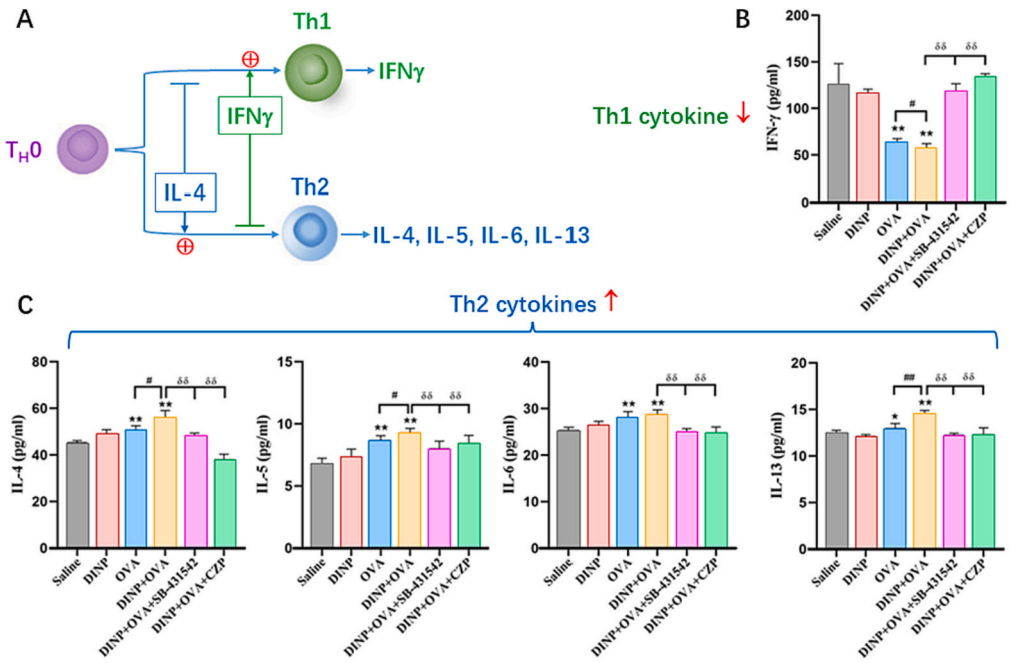

Fig. 5. Th1/Th2 imbalance and related cytokines. (A) Th1/Th2 balance illustration. (B) Th1 cytokine: IFN-γ. (C) Th2 cytokines: IL-4, IL-5, IL-6 and IL-13 levels. The figures were amplified at 20 times. Panels: (a) Group treated with saline; (b) Group treated with DINP; (c) Group treated with OVA; (d) Group treated with OVA + DINP; (e) Group treated with OVA + DINP + SB-431542; (f) Group treated with OVA + DINP + CZP. **: $p < 0.01$, compared to the saline group; ##: $p < 0.01$, compared to the OVA group; δδ: $p < 0.01$, compared to the OVA + DINP group, with a sample size of 8 per group.

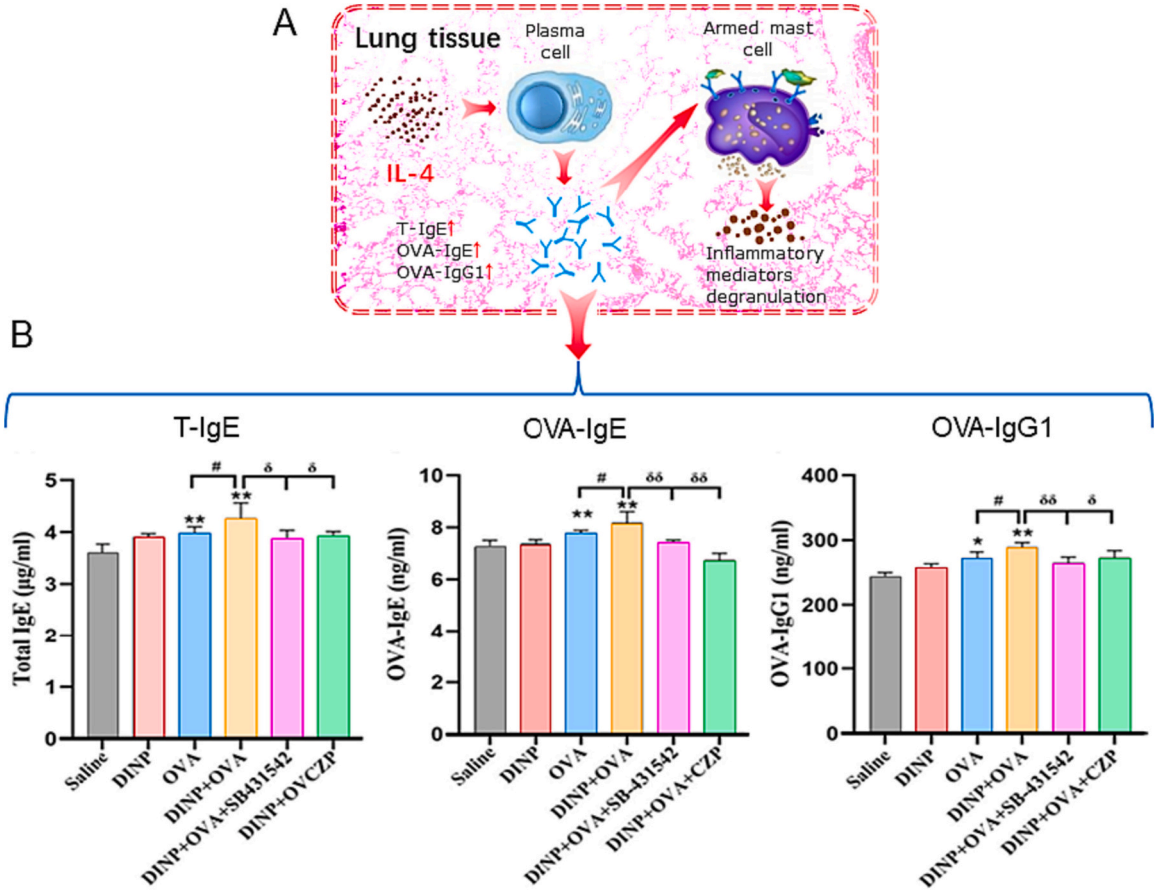

Fig. 6. Immunoglobulin levels in lung tissue. (A) Illustration of type I hypersensitivity in lung tissue. (B) Total IgE, OVA-lgE and OVA-IgG1 levels. *: $p < 0.05$, **: $p < 0.01$ compared with the saline group; ##: $p < 0.01$, compared with the OVA group; δ: $p < 0.05$, δδ: $p < 0.01$, compared with the OVA + DINP group. The study included eight participants in each group.

seen in the DINP + OVA group.

3.5. Immunoglobulins in lung tissue

Immunoglobulin levels were measured by assessing lung tissue samples (Fig. 6). In comparison to the saline group, the OVA groups exhibited notably elevated levels of T-IgE, OVA-IgE, and OVA-IgG1. Furthermore, all three immunoglobulin levels were higher in the DINP + OVA group compared to the OVA group. The administration of SB-431542 or CZP resulted in decreased immunoglobulin levels in comparison to the DINP + OVA group.

4. Discussion

4.1. Background of this study

The role and mechanism of exposure to man-made dangerous chemicals in inducing human allergic diseases is a significant scientific issue in the field of environment and health. One specific area of focus is the relationship between phthalate exposure and allergic asthma, which has garnered considerable attention. Diisononyl phthalate (DINP) is a commonly used plasticizer derived from phthalates, comprising approximately 30 % of the market. Human exposure to DINP is inevitable. Although DINP was previously believed to be environmentally friendly, recent research indicates otherwise. It has recently been revealed that several toxicological studies have been conducted on DINP oral exposure and its adjuvant effect on allergic asthma. However, the potential mediating effect of DINP dermal exposure on allergic asthma remains unclear. Therefore, we conducted this study to address this research gap.

4.2. Scientific hypothesis of this study

Fig. 7 illustrates our scientific hypothesis for the potential mechanism by which dermal exposure to DINP promotes allergic asthma.

As seen in Fig. 7, our scientific hypothesis involves the following three processes: (1) information about DINP dermal exposure is transmitted to lung tissue via IL-31; (2) in lung tissue via the IL-31/TRPV1 pathway, there is an increased release of IL-4 secretion; and (3) IL-4 further contributes to Th1/Th2 imbalance, and exacerbates type I hypersensitivity and allergic asthma induced by OVA.

4.3. The principles of SB-431542 blocking IL-31 expression

SB-431542 is an inhibitor of the TGF-β signaling pathway (Sun et al., 2016). TGF-β signaling pathway functions as a major regulator of cell proliferation, differentiation, apoptosis, organ formation, immune function, and inflammation (Wu et al., 2020), and one function of TGF-βpathway, in allergic diseases, is to induce Th2 immune cells express IL-31 (Xu et al., 2020). The expression of IL-31 by Th2 immune cells depends on the activation of the TGF-βpathway. Therefore, we used the TGF-β antagonist, SB-431542, to block the overexpression of IL-31, and our study result demonstrated that SB-431542 treatment can effectively reduce the levels of IL-31 (Fig. 4B) and other Th2 cytokines (Fig. 5).

4.4. Molecular and cellular basis of the IL-31/TRPV1 pathway

IL-31, a cytokine of the IL-6 family, is mainly produced by CD4+ Th2 cells (Kim et al., 2018). The IL-31 receptor (IL-31R) consists of IL-31RA and Oncostatin M receptor β, and it is highly expressed in skin and bronchial tissues (Kang et al., 2019). After attaching to its receptor, IL-31 triggers multiple signal pathways, such as JAK/STAT1, and PI3K/Akt. Its role in regulating cell proliferation and involvement in allergic inflammation has been extensively documented (Bağci and Ruzicka, 2018). On the other hand, TRPV1 is an ion channel primarily found in afferent neurons, responsible for sensing temperature, pain, and taste (Lawton et al., 2017). TRPV1 experiences a conformational alteration in its central pore when activated by physical, chemical, or endogenous stimuli. (Yang et al., 2015). The result of this process is the production of intracellular depolarization currents and a rise in Ca^{2+} levels, which in turn mediates immune responses by triggering immunogenic inflammation (Reyes-García et al., 2022; Wu et al., 2013).

IL-31R and TRPV1 can be co-expressed in DRG neurons (Xu et al., 2020) as well as some other cells (Dillon et al., 2004; Güzel and Akpınar, 2021; Jo et al., 2018), forming the IL-31/IL-31R/TRPV1 pathway (in short, IL-31/TRPV1 pathway). The study conducted by Xu et al. (2020) has confirmed that IL-31, through IL-31R, promotes allergic reactions by increasing calcium influx into cells, this, in turn, leads to increased expression of TRPV1 in DRG neurons. It means that these DRG neurons contain both IL-31R and TRPV1 ion channels, when TRPV1 is activated, it results in the release of substance P, CGRP and tachykinin from the neurons, which can induce surrounding macrophages, mast cells, or certain Th2 cells to release IL-4 (Lu et al., 2005). Moreover, to my knowledge, no studies (human or animal) have evaluated the

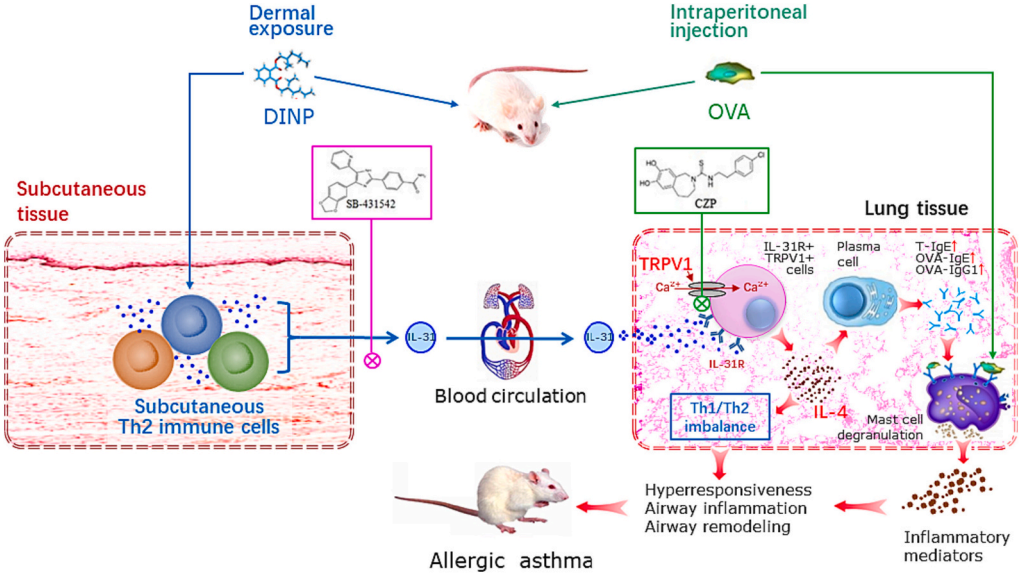

Fig. 7. Scientific hypothesis of this study.

relationship of DINP and IL-31/TRPV1 pathway in allergic asthma. Based on our findings (refer Fig. 5C and Fig. 7), IL-4 increased to the highest level in DINP + OVA group, we conclude that the IL-31/TRPV1 pathway plays a vital role in the worsening of allergic asthma triggered by DINP dermal exposure in OVA-sensitized Balb/c mice, primarily through the release of IL-4.

4.5. The pivotal role of IL-4

IL-4 is a globular protein composed of four short α-helices and is classified as a Th2 cytokine (Kim et al., 2018). It serves as a multifunctional cytokine, promoting Th2 immune responses and stimulating B-cells. In this process, IL-4 plays a crucial role in inducing isotype-switching in B cells into plasma cells, leading to the production of IgG1 and IgE antibodies (Zhang et al., 2018). In the context of the IL-31/TRPV1 pathway, which is implicated in allergic asthma exacerbated by DINP dermal exposure, IL-4 assumes a pivotal role. The function of IL-4 includes several aspects. Firstly, IL-4 has a positive feedback effect on IL-31, an upstream molecule, through autocrine and paracrine secretion. This resulted in an increase in IL-31 expression (Kim et al., 2018). Secondly, IL-4 plays a crucial role in promoting Th1/Th2 immune imbalance. As a result, it inhibits the function of Th1 cells and reduces the expression level of Th1 cytokines, while activating Th2 cells and promoting the expression and release of Th2 cytokines (refer to Fig. 5; Zhang Jo et al., 2018). Thirdly, as a result of IL-4 stimulation, the B2 cells proliferate and transform into plasma cells, which synthesize and secrete IgE and IgG1. This process mediates the occurrence of type I hypersensitivity reaction (Abe et al., 2020). Lastly, in addition to increasing Th2 cells and self molecules, IL-4 affects the Th2 cell system through autocrine and paracrine secretion (King and Mohrs, 2009). The evidence shown by all of these events supports the idea that IL-4 plays an important role in allergic asthma development and progression.

4.6. Th1/Th2 imbalance and related cytokines

It is commonly believed that asthma arises when Th1 and Th2 mechanisms are imbalanced (Gans and Gavrilova, 2020). This imbalance in cytokine secretion is characterized by reduced levels of the Th1 cytokine IFN-γ, and elevated secretion of the Th2 cytokines IL-4, IL-5, IL-6, and IL-13. This imbalance is often referred to as Th2 dominance. Among the common serological techniques, enzyme-linked immunosorbent assay (ELISA) can be easily automated to evaluate multiple samples at low cost. Our findings effectively demonstrate and describe this phenomenon (see Fig. 5). There is a close link between this imbalance and airway inflammation (Gans and Gavrilova, 2020). IL-4 promotes Th2 differentiation and activates B cells to be transformed into plasma cells, resulting in the production of IgE and IgG1. Furthermore, it stimulates mast cell degranulation and releases inflammatory factors, including heparin, prostaglandin, and leukotrienes. These responses lead to bronchoconstriction and the aggregation of inflammatory cells (Oeser et al., 2015). IL-5 activates eosinophils and recruits them to inflammation sites. Studies have shown that IL-5 is significantly elevated in asthmatic patients, and the level of IL-5 is inextricably linked to the disease (Flood-Page et al., 2007; Haldar et al., 2009). The IL-6 receptor facilitates the production of IL-4 and IL-5 by B cells and Th2 cells. Additionally, IL-13 stimulates goblet cells to produce mucus and induces airway remodeling by acting on eosinophils and airway smooth muscle cells (Licona-Limón et al., 2013; Zhang et al., 2015). Research findings have demonstrated that the presence of airway hyper-responsiveness, pulmonary eosinophil infiltration, and pulmonary fibrosis serve as indicators of the significant involvement of IL-13 in the pathogenesis of allergic asthma (Kasaian and Miller, 2008). IFN-γ is the predominant cytokine produced by Th1 and its level is reduced in individuals with asthma (Kianmehr et al., 2017). The findings of our research indicate that the exposure of mice to a concentration of 20 mg/kg/day DINP resulted in a significant increase in levels of IL-4, IL-5, and IL-13, accompanied by a significant decrease in IFN-γ levels. These changes suggest that the immune response in OVA-sensitized mice followed a Th2 type reaction, ultimately exacerbating allergic asthma in the mice.

4.7. Type I hypersensitivity and related immunoglobulins

Allergic asthma is a type I hypersensitivity response triggered by mediators released by multiple cells (Kim et al., 2018). The role of IL-4 and Th2 cells in type I hypersensitivity has long been established. Allergen-specific IgE causes type I hypersensitivity (Koning et al., 2019). In rodents, such as mice, type I hypersensitivity can also be mediated by IgG1 (Mall et al., 2016). The gold standard for diagnosing type I hypersensitivity also involves the detection of total IgE (t-IgE) to assess the overall level of type I hypersensitivity in the body. There is no significant difference in serum levels of total IgE, OVA-IgE, and OVA-IgG1 between the DINP and control groups when mice are exposed to DINP alone. However, when exposed to dermal DINP and OVA, the levels of these immunoglobulins reached their highest points (see Fig. 6). These findings are consistent with previously reported results (Larsen et al., 2007) and can be attributed to the immune adjuvant effect.

4.8. Characteristics of allergic asthma

Airway hyperresponsiveness and airway hyperreactivity are hallmarks of asthma.

4.8.1. Airway remodeling

Airway remodeling is the primary abnormal response to persistent airway inflammation (Jiang et al., 2020). There are various changes in the airway structure involved in this remodeling, including thickening of the airway wall, epithelial cell fibrosis, and hyperplasia of goblet cell mucous glands (Koopmans et al., 2016). Our observations using H&E staining (Fig. 3A) and Masson staining (Fig. 3B) revealed significant airway damage in the OVA group, characterized by inflammatory cell infiltration, airway wall thickening, airway cavity narrowing, and collagen fibrosis. Furthermore, we observed that exposure to both OVA and DINP resulted in more severe airway remodeling in asthmatic mice. PAS staining (Fig. 3C) demonstrated an increase in mucous cell proliferation in the group exposed to both DINP and OVA. These findings suggest that dermal exposure to DINP does indeed exacerbate airway remodeling in mice with asthma.

4.8.2. Airway hyperreactivity (AHR)

Airway hyperresponsiveness (AHR) is a well-established characteristic of allergic asthma, hypothesized to arise from inflammation of the airway mucosa, damage to the epithelial lining, and hypertrophy of smooth muscle, accompanied by an increase in goblet cell proliferation (Harb et al., 2020). A significant aspect of asthma involves an amplified bronchoconstrictor reaction to diverse stimuli, clinically evident through symptoms such as chest tightness and cough. The extent of AHR typically aligns with the extent of airway inflammation (Zasłona et al., 2020). For our study, the MCH provocation test as described by Brusasco and Crimi (2001) was used to document the phenotype. In comparison with techniques that require the removal of an animal's lungs or airways, this has obvious advantages. Chronic airway inflammation, caused by various cells and inflammatory factors, is responsible for asthma. The severity of the condition can be objectively measured by the level of airway responsiveness, i.e., lung function (Beasley et al., 2023; Hsu et al., 2020; Højen et al., 2019; Ham et al., 2012). According to clinical studies, activated airway inflammatory cells can change the morphology of airway tissue and lead to AHR (Mims, 2015; Padem and Saltoun, 2019). The Ri and Re explain the variation in the large airways, whereas the change in Cdyn represents the state of the small airways or the parenchyma (Drazen et al., 1999). Since airway resistance explains the state of large airways and airway compliance reflects the state of small airways, apositive correlation exists between Ri and Re and

abnormal airway changes, and a negative correlation between Cldyn and small airway obstruction (Poli et al., 2022). There are several mechanisms that contribute to AHR, such as inflammation, dysfunctional neuroregulation, and structural changes. Among these mechanisms, inflammation seems to play a significant role in determining the extent of AHR. Therefore, therapeutic interventions aimed at reducing inflammation have the potential to alleviate airway hyperresponsiveness and enhance asthma management. According to our research findings, the group exposed to both OVA and DINP exhibited a higher degree of AHR compared to the group solely exposed to OVA. This implies that the use of DINP topically could exacerbate allergic asthma in mice.

4.9. Limitation of this study

While our study has identified a novel mechanism in the aggravation of allergic asthma through the activation of the IL-31/TRPV1 pathway by dermal exposure to DINP, we should acknowledge the limitations of our current research. In this study, our focus was solely on investigating the effects of DINP exposure on the skin during a limited time frame. Unfortunately, we were unable to explore the possible consequences of sub-chronic exposure to DINP on asthma. Further research is necessary to ascertain the potential impact of prolonged exposure to DINP on individuals with asthma.

5. Conclusions

It is the first study of its kind to investigate the association between IL-31/TRPV1 pathway activation and dermal DINP exposure in OVA-sensitized mice with asthma. Our findings suggest that DINP aggravates the symptoms of allergic asthma, including hyperresponsive airways, remodeling of the airways, and destruction of lung tissue. Additionally, we examined the risk of DINP by assessing IL-31 and TRPV1 activation and the Th1/Th2 imbalance response. Our findings demonstrated that SB-431542 or CZP effectively reversed airway damage, providing insight into how dermal DINP exposure exacerbates allergic asthma.

CRediT authorship contribution statement

Qi Peng: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Yang Wu:** Conceptualization, Formal analysis, Writing – original draft, Writing – review & editing. **Yan Li:** Investigation. **Chan Lu:** Conceptualization, Writing – review & editing. **Runming Yao:** Conceptualization, Funding acquisition, Writing – review & editing. **Siyuan Hu:** Investigation. **Ning Ma:** Investigation. **Shaohui Chen:** Formal analysis, Validation. **Xu Yang:** Conceptualization, Validation, Writing – review & editing. **Ping Ma:** Conceptualization, Formal analysis, Funding acquisition, Project administration, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare no competing financial interest.

Data availability

Data will be made available on request.

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