



Maternal 1-nitropyrene exposure during pregnancy increases susceptibility of allergic asthma in adolescent offspring

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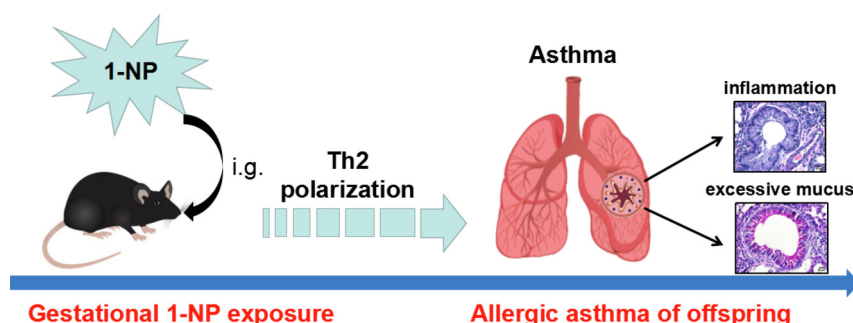
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HIGHLIGHTS

- Gestational 1-NP exposure aggravates airway inflammation in OVA-sensitized offspring.
- Gestational 1-NP exposure increases airway mucus secretion in OVA-sensitized offspring.
- Gestational 1-NP exposure accelerates pulmonary Th2 cytokine polarization in OVA-sensitized offspring.

GRAPHICAL ABSTRACT



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ABSTRACT

1-nitropyrene (1-NP) is widespread in the environment, as a typical nitrated polycyclic aromatic hydrocarbon. The purpose of this research was to explore the effects of gestational 1-NP exposure on susceptibility of allergic asthma in offspring. Maternal mice were exposed to 1-NP ($100 \mu\text{g kg}^{-1}$) by gavage throughout the whole pregnancy. Pups were sensitized by injecting with ovalbumin (OVA) on postnatal day (PND)23, 29, and 36, respectively. At 7 days following the last injection, sensitized mice were exposed to aerosol OVA. As expected, there were quite a few inflammatory cells in the lungs of OVA-sensitized pups, accompanied by bronchial wall thickening and hyperemia. Elevated goblet cells and overproduced mucus were observed in the airways of OVA-sensitized pups. Interestingly, gestational 1-NP exposure aggravated infiltration of inflammatory cells, mainly eosinophils, in OVA-sensitized offspring. Although it had little effect on airway smooth muscle layer thickening and basement membrane fibrosis, gestational 1-NP exposure aggravated goblet cell hyperplasia, *Muc5ac* mRNA upregulation, and mucus secretion in the airways of OVA-sensitized and challenged offspring. Mechanistically, gestational 1-NP exposure aggravated elevation of pulmonary IL-5 in OVA-sensitized pups. These findings suggest that gestational 1-NP exposure increases susceptibility of allergic asthma in offspring.

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1. Introduction

Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) are mainly generated by incomplete combustion of fossil fuel and biomass, vehicle emission of diesel and gasoline and secondary formation (Bandowe and Meusel, 2017; Li et al., 2019). 1-nitropyrene (1-NP) is a typical nitro-PAH emitted directly from combustion sources, especially from diesel and gasoline engine vehicles, not coal and biomass combustion (Tang et al., 2005). 1-NP is widespread existence in the environment such as diesel exhaust (Bamford et al., 2003; Holly et al., 2003), vehicle exhaust (Miller-Schulze et al., 2016), PM_{2.5} (Lin et al., 2015), soil (Yaffe et al., 2001), river (Ohe and Nukaya, 1996), sea-water (Murahashi et al., 2001), sediments (Ozaki et al., 2010), aquatic organisms (Uno et al., 2011) and food (Deng and Chan., 2017). Although general population are exposed to 1-NP through respiration (Tang et al., 2017) and digestion (Deng and Chan., 2017), the digestive tract is the primary route of exposure and cereals and vegetables are the greatest contributor (Deng and Chan., 2017; Yaffe et al., 2001). The mutagenicity and carcinogenicity of 1-NP have been demonstrated by many investigations in the past decades (Bacolod et al., 2017; Benbrahim-Tallaa et al., 2012; Chatel et al., 2014; Li et al., 2017; Zhao et al., 2004). In addition, 1-NP has been found to induce inflammation (Park and Park., 2009), emphysema (Li et al., 2017) and hepatocyte apoptosis (Podechard et al., 2011). Furthermore, our research team showed that exposure to 1-NP during pregnancy led to intrauterine growth restriction (IUGR) in mice (Li et al., 2018) and gender-dependent neurobehavioral disorders in mouse offspring (Li et al., 2019). However, little research has been conducted on the susceptibility of maternal 1-NP exposure to respiratory diseases in offspring.

Allergic asthma is a chronic inflammatory disease characterized by airway hyperresponsiveness (AHR), excessive mucus secretion, airway remodeling, and airway stenosis (Lambrecht and Hammad., 2014). Th2 lymphocytes affect the pathogenesis of allergic asthma via producing type 2 (Th2) cytokines such as IL-4, IL-5, and IL-13. These cytokines induce the release of IgE and the infiltration of eosinophils into the lungs, leading to the development and progression of allergic asthma (Larché et al., 2003; Nakajima and Takatsu., 2007). Diesel exhaust particulate (DEP) is an important component of traffic pollutants in large European cities, various epidemiological evidences suggest DEP exposure increases the prevalence of allergic asthma (Muñoz et al., 2019; Robinson et al., 2018; Zuo et al., 2019). Animal studies have also demonstrated that DEP exposure significantly increases AHR and airway inflammation (Brandt et al., 2015; De Grove et al., 2017; Kim et al., 2016; Wang et al., 2019). Recent evidences have proved that air pollution exposure during pregnancy increases the risk of asthma in children (Jung et al., 2019; Lavigne et al., 2018; Lee et al., 2018; Sordillo et al., 2019). Perinatal air pollution exposure increases the incidence of asthma in preschool children with low birth weight (Sbihi et al., 2016). According to the CCAAPS birth cohort, high DEP exposure in early life (1 year and 4 years) is associated with an increased incidence of asthma at age 7 in the context of aeroallergen sensitivity (Brandt et al., 2015). Animal experiment has indicated that pregnant C57BL/6 mice exposure to DEP increased the susceptibility of allergic asthma during pubertal offspring (Manners et al., 2014). Various data indicate that DEP exposure is associated with an increase in allergic disease, but the specific effect of which component is unknown. As an important component of DEP, and exposure to 1-NP in early life, especially during fetal development, is rarely evaluated for postnatal offspring asthma.

In this study, maternal mice were exposed to 1-NP during pregnancy, allowing their offspring to receive sensitization and

stimulation of ovalbumin (OVA) during puberty, exploring whether gestational 1-NP exposure aggravates allergic asthma symptoms in adolescent offspring. This study aimed to investigate susceptibility of offspring to allergic asthma from maternal exposure to environmental pollutants, providing a new direction for exploring the etiology of allergic asthma.

2. Materials and methods

2.1. Chemical reagents

1-NP (N22959) and OVA (A-5503) were purchased from Sigma Chemical Co (St. Louis, MO, USA). Imject™ Alum Adjuvant (77161) was ordered from Thermo Scientific (China). Mouse IL-4 (E-EL-M0043c) and IL-13 (E-EL-M0727c) ELISA kits were obtained from Elabscience (Wuhan, China). Mouse IL-5 (CSB-E04637 m) and OVA specific IgE (CSB-E08914 m) ELISA kits were purchased from Cusabio (Wuhan, China). Diff-Quik Stain (G1541), Glycogen Periodic Acid Achiff (PAS/Hematoxylin) Stain Kit (G1281) and Modified Masson's Trichrome Stain Kit (G1345) were from Beijing Solarbio Science & Technology Co (China). TRIzol reagent was obtained from Molecular Research Center (Cincinnati, Ohio). RNase-free DNase was ordered from Promega Corporation (Madison, WI). All other chemicals were of reagent grade.

2.2. Animals and treatments

Specific pathogen free female and male C57BL/6 mice (8 weeks) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd (Beijing, China). Animals were maintained in a specific pathogen-free facility and provided with food and water ad libitum. The protocols and the use of animals were approved by the Anhui Medical University Animal Care and Use Committee. The procedure for obtaining pregnant mice was described in a previous study (Fu et al., 2019). Twelve pregnant mice were randomly divided into two groups. Mice in the 1-NP group were administered with 1-NP (100 µg kg⁻¹, dissolved in corn oil) by gavage daily from gestational day (GD)0 to GD18, while controls were performed with corn oil. Pregnant mice were kept in separate cages until offspring were weaned. No pups were exposed to 1-NP after birth. The first birth day of pups was recorded as postnatal day (PND)1. Within 3 days after birth, all pups were kept with their dams. Eight pups per litter were kept with their dams on PND4. At PND21, 48 pups in the 1-NP group were divided into two subgroups: 1-NP and 1-NP + OVA groups. At PND21, 48 pups in the Ctrl group were divided into two subgroups: Ctrl and OVA groups.

2.3. Ovalbumin sensitization and challenge

The pups in OVA and 1-NP + OVA groups were sensitized through injecting 10 ml kg⁻¹ OVA working fluid (20 µg OVA, dissolved in 0.1 ml sterilized saline and complexed with 50 µl Imject Alum Adjuvant) on PND23, 29, and 36. At 7 days following the last injection, sensitized mice were subsequently challenged with 1% OVA by nebulization for 30 min daily for three days. Mice in the Ctrl and 1-NP groups were performed with saline instead of OVA. All pups were sacrificed 24 h after the final challenge. Lungs of twelve mice from each group were collected for RT-PCR and ELISA, while other twelve mice were used for lung lavage. The detailed protocols are shown in Fig. 1.

2.4. Bronchoalveolar lavage fluid (BALF) leukocyte count

Lungs were flushed three times with 0.4 ml irrigation solution (1 mg BSA and 0.01 ml 0.5 mM EDTA were soluble in 0.99 ml cold

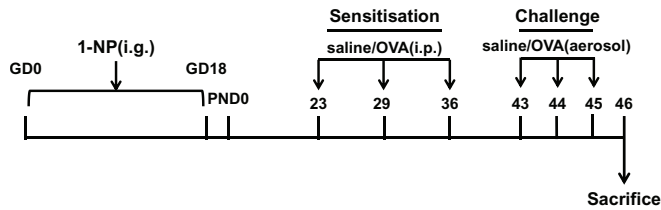


Fig. 1. Experimental protocols. Note: GD, gestation day; PND, postnatal day; i.g., intragastric; i.p., intraperitoneal.

PBS). BALF was obtained and then centrifuged at 1500 rpm at 4 °C for 15 min. The sediments were resuspended in 1 ml PBS and then total number of cells was counted in a hemocytometer. In addition, 300 µl suspension was evenly coated on three slides. Then Diff-Quik staining was used to distinguish eosinophil numbers in the BALF with an optical microscope.

2.5. OVA-special IgE and Th2 cytokine analysis

Serum samples were obtained from blood samples of twelve mice each group and centrifuged at 3500 rpm at 4 °C for 15 min. The levels of serum OVA-special IgE and pulmonary IL-4, IL-5 and IL-13 were analyzed with a commercial enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's instructions.

2.6. Histology and immunohistochemistry

The left lungs from six mice each group were fixed in 4% paraformaldehyde for one week, embedded in paraffin and cut into 5 µm sections. The dimethylbenzene was used to dewax for the lung slices and then rehydrated in a graded ethanol series. Hematoxylin and eosin staining were used to evaluate peritracheal and perivascular inflammatory infiltration in lung sections (Koltz et al., 2011). And a combined staining with Harris hematoxylin and Periodic Acid-Schiff (PAS) was used to analyze airways mucus production of lung slides (Campa et al., 2018). The smooth muscle thickness and collagen area of basement membrane were determined by Masson's trichrome-stained reagent staining. Three sections of bronchial wall with a diameter of 200–400 µm were randomly selected from each lung. The perimeter of basement membrane (Pbm) was measured. The smooth muscle and collagen were defined in a fixed color pattern and the area was measured. The results were expressed as smooth muscle area (WAm) and collagen area of airway basement membrane (WAc) per unit length (WAm/Pbm, WAc/Pbm, µm²/µm) (Li et al., 2013).

2.7. RNA isolation and real time RT-PCR

Total RNA of six lungs from each group was extracted using TRIzol reagent by manufacturer's instructions. Real-time RT-PCR was performed with a Light Cycler 480 SYBR Green I kit (Roche) using gene-specific primers as shown in Table 1. 18S was utilized to normalize the relative amount of target, as an endogenous

reference.

2.8. Statistical analysis

All statistical values were measured using SPSS 17.0. The data were presented as mean ± standard error. The differences among different groups were calculated with ANOVA and the Student-Newman-Keuls post hoc test. And the differences between two groups was determined by the unpaired Student *t*-test. Statistical significance is illustrated as **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.

3. Results

3.1. Effects of maternal 1-NP exposure on physical development of mouse offspring

No difference on maternal weight and dietary intake was observed between two groups, indicating that 1-NP exposure had no significant toxicity to the dams (Fig. 2A and B). As expected, a remarkable reduction of body weight at PND4 was observed in pups from 1-NP group (Fig. 2C). Until PND21, the weights of male and female pups from 1-NP-exposed group had not been able to catch up with those of control pups (Fig. 2D and E).

3.2. Maternal 1-NP exposure aggravates peribronchiolar and perivascular inflammation in OVA-sensitized offspring

No remarkable difference on absolute and relative weights of spleens and thymuses was observed among four groups (Table 2). However, absolute and relative weights of lungs were obviously increased in OVA-sensitized offspring (Fig. 3A and B). The results of inflammatory cell count and classification in BALF are shown in Fig. 3C and D. As expected, the numbers of total leukocytes and eosinophils in BALF were obviously elevated in OVA-sensitized offspring (Fig. 3C and D). Interestingly, the numbers of total leukocytes and eosinophils in BALF were elevated in offspring whose dams were exposed to 1-NP during pregnancy (Fig. 3C and D). Moreover, maternal 1-NP exposure during pregnancy aggravated

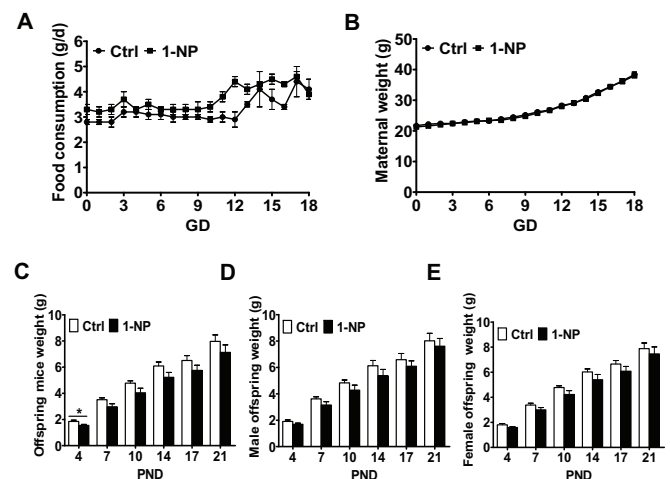


Fig. 2. Effects of maternal 1-NP exposure on body weight of dams and offspring. Twelve pregnant mice were randomly divided into two groups. Pregnant mice were treated with either corn oil or 1-NP (100 mg kg⁻¹ body weight) by gavage daily during the whole pregnancy (from GD0 to GD18). Daily food consumption and body weight of dams were measured. (A) Food consumption. (B) Maternal body weight. The results were indicated as means ± standard error (N = 6). (C–E) Body weight growth of offspring was monitored. The results were indicated as means ± standard error of six litters (N = 6).

Table 1
Sequences of the primers for real-time PCR.

Genes	Sequences	Product length (bp)
18S	Forward: 5'-GTAACCCGTTGAACCCATT-3' Reverse: 5'-CCATCCAATCGGTAGTAGCG-3'	151
Muc5ac	Forward: 5'-AATGACTCAATCTGCGTGCCTTCC-3' Reverse: 5'-CAGGTITAGCGTGGCTTCCTTACAG-3'	125

Table 2

Effects of maternal 1-NP exposure on weight of spleens and thymuses in OVA-induced offspring.

	Ctrl		1-NP		OVA		1-NP + OVA	
	Male	Female	Male	Female	Male	Female	Male	Female
spleen weight (g)	0.066 ± 0.003	0.075 ± 0.006	0.070 ± 0.003	0.068 ± 0.005	0.075 ± 0.003	0.083 ± 0.005	0.074 ± 0.002	0.078 ± 0.003
spleen index (%)	0.30 ± 0.01	0.42 ± 0.03	0.32 ± 0.02	0.38 ± 0.02	0.35 ± 0.02	0.46 ± 0.04	0.34 ± 0.01	0.45 ± 0.02
thymus weight (g)	0.056 ± 0.002	0.072 ± 0.004	0.060 ± 0.003	0.081 ± 0.003	0.065 ± 0.005	0.084 ± 0.006	0.062 ± 0.002	0.084 ± 0.003
thymus index (%)	0.26 ± 0.01	0.41 ± 0.02	0.28 ± 0.02	0.46 ± 0.02	0.30 ± 0.02	0.47 ± 0.03	0.29 ± 0.01	0.49 ± 0.02

All data were indicated as mean ± standard deviation (N=12).

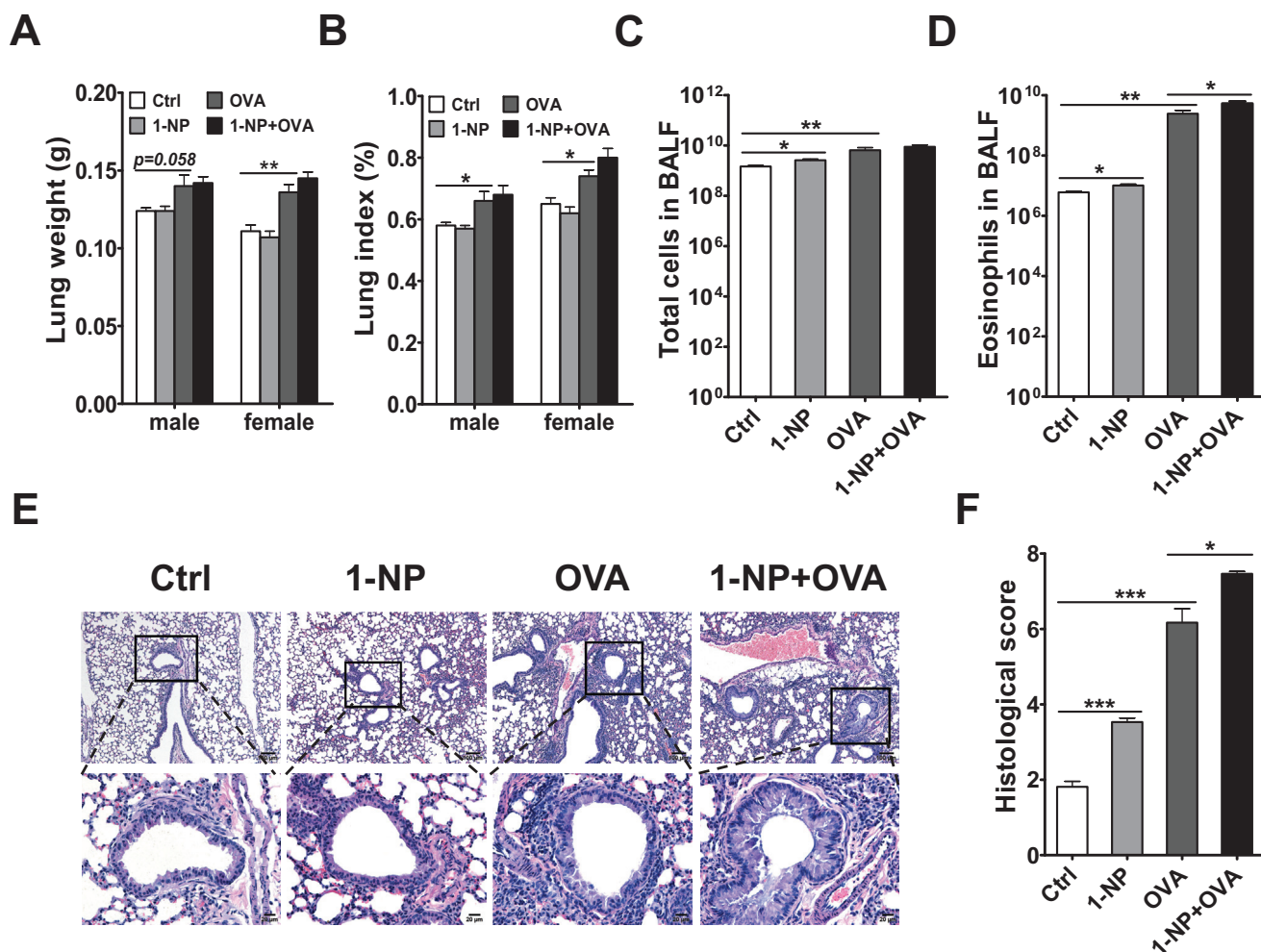


Fig. 3. Maternal 1-NP exposure aggravates airway inflammation in OVA-sensitized offspring. (A) Absolute weight of lungs. (B) Relative weight of lungs. The results were indicated as means ± standard error (N = 12). (C) The total number of cells in BALF was counted with a hemocytometer using an optical microscope. (D) Diff-Quik staining is used to distinguish eosinophil numbers in the BALF of OVA-sensitized and challenged offspring. The results were indicated as means ± standard error (N = 12). (E) H&E staining shows inflammatory cell infiltration in the airways of OVA-sensitized and challenged offspring. The upper of the picture is magnified 100, scale bars, 100 μ m. The lower is magnified 400, scale bars, 20 μ m. (F) Quantification of histological assessment of lung inflammation. The results were expressed as mean ± standard error (N = 6). * P < 0.05, ** P < 0.01 and *** P < 0.001 as indicated.

elevation of total leukocytes and eosinophils in BALF in OVA-sensitized pups (Fig. 3C and D). The effects of maternal 1-NP exposure during pregnancy on OVA-induced pulmonary inflammation were determined using histopathology. As expected, pulmonary inflammatory cells were abundant in OVA-sensitized pups, accompanied by the occurrence of thickened bronchial wall and hyperemic pulmonary vessels (Fig. 3E and F). Interestingly, there were a few inflammatory cells infiltrated around the airways in pups whose dams gestational were exposed to 1-NP (Fig. 3E). Moreover, gestational 1-NP exposure aggravated peribronchiolar and perivascular infiltration of inflammatory cells in OVA-

sensitized pups (Fig. 3E). Semi-quantitative analysis found that maternal 1-NP exposure aggravated pulmonary inflammatory scores in OVA-sensitized pups (Fig. 3F).

3.3. Maternal 1-NP exposure aggravates airway mucus secretion in OVA-sensitized offspring

PAS staining was used to determine airway mucus production in OVA-sensitized pups. As shown in Fig. 4A, there was scarcely mucus production and goblet cell hyperplasia in the lungs of control mice. By contrast, there was an obvious bright violet staining in the lungs

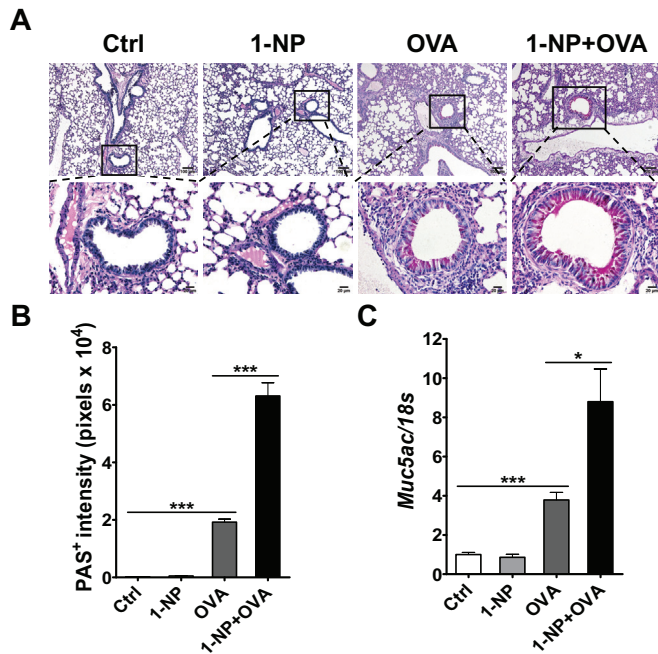


Fig. 4. Maternal 1-NP exposure aggravates secretion of airways mucus in OVA-sensitized offspring. (A) PAS staining shows airway goblet cells and mucin secretion in asthmatic offspring. The upper of the picture is magnified 100, scale bars, 100 μ m, while the lower is magnified 400, scale bars, 20 μ m. (B) Quantification of airway mucin secretion in OVA-sensitized offspring. (C) Pulmonary *Muc5ac* mRNA expression was analyzed using real-time RT-PCR. All results were expressed as mean $\pm$ standard error (N = 6). * P < 0.05, ** P < 0.01 and *** P < 0.001 as indicated.

of OVA-sensitized pups (Fig. 4A), indicating mucus overproduction in bronchial airways. Interestingly, the intensity of mucus staining was obviously elevated in OVA-sensitized pups whose dams were treated with 1-NP (Fig. 4B). The expression of pulmonary *Muc5ac*, a mucoprotein gene, was analyzed. As expected, pulmonary *Muc5ac* was markedly upregulated in OVA-sensitized pups. Of interest, maternal 1-NP exposure aggravated upregulation of pulmonary *Muc5ac* in OVA-sensitized offspring (Fig. 4C).

3.4. Effects of maternal 1-NP exposure on smooth muscle proliferation and collagen deposition in airway basement membrane of OVA-sensitized offspring

Masson's trichrome staining was used to determine smooth muscle proliferation and collagen deposition in airway basement membrane in OVA-sensitized pups (Fig. 5A). Unexpectedly, airway smooth muscle layer thickening and basement membrane fibrosis didn't occur in OVA-sensitized pups (Fig. 5B and C). Moreover, maternal 1-NP exposure didn't aggravate airway smooth muscle layer thickening and basement membrane fibrosis in OVA-sensitized pups (Fig. 5B and C).

3.5. Effects of maternal 1-NP exposure on serum OVA-specific IgE and pulmonary type 2 cytokines in OVA-sensitized offspring

The effects of maternal 1-NP exposure on OVA-evoked serum OVA-specific IgE was analyzed by ELISA. As expected, serum OVA-specific IgE was markedly elevated in OVA-sensitized pups (Fig. 6A). Interestingly, maternal 1-NP exposure had no effect on OVA-evoked elevation of serum OVA-specific IgE in pups (Fig. 6A). The impacts of gestational 1-NP exposure on pulmonary type 2 cytokines were measured in OVA-sensitized offspring. Despite no distinct difference on pulmonary IL-4 among different groups

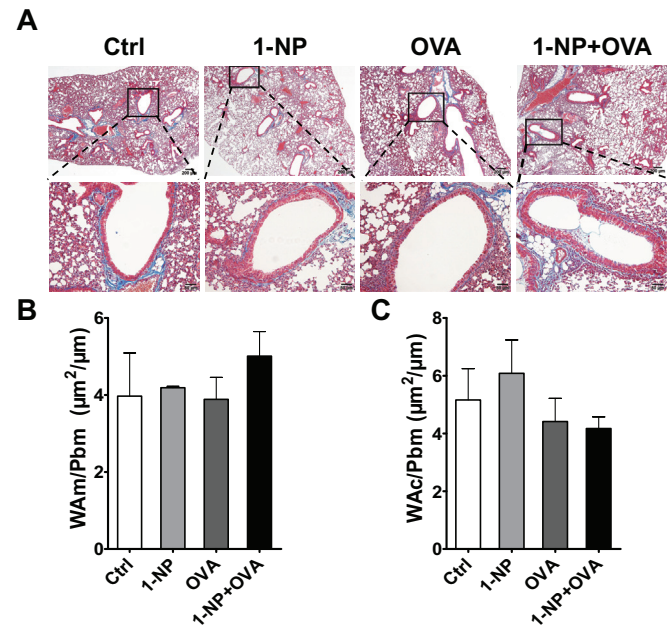


Fig. 5. Effects of maternal 1-NP exposure on smooth muscle and collagen deposition around airway basement membrane in OVA-sensitized offspring. (A) Masson's trichrome staining is used to determine airway smooth muscle thickness and subepithelial fibrosis around airways of asthmatic offspring. The upper of the picture is magnified 40, scale bars, 200 μ m, while the lower is magnified 200, scale bars, 50 μ m. (B) Airway smooth muscle thickness was quantized for WAm/Pbm. Pbm: bronchial basement membrane perimeter; WAm: smooth muscle area. (C) Collagen area around airway basement membrane was quantized for WAc/Pbm. WAc: airway basement membrane collagen area. All results were expressed as mean $\pm$ standard error (N = 6).

(Fig. 6B), pulmonary IL-5 and IL-13 levels were obviously elevated in OVA-sensitized pups (Fig. 6C and D). Maternal 1-NP exposure aggravated elevation of pulmonary IL-5 in OVA-sensitized pups (Fig. 6C).

4. Discussion

In this study, we were to explore whether gestational 1-NP exposure sensitizes offspring to OVA-evoked allergic asthma in mice. We found that maternal 1-NP exposure increased the susceptibility of mouse offspring to OVA-induced allergic asthma. The specific findings include: (1) maternal 1-NP exposure aggravated infiltration of airway inflammatory cells in OVA-sensitized offspring; (2) maternal 1-NP exposure aggravated excessive secretion of airway mucus in OVA-sensitized offspring; (3) maternal 1-NP exposures aggravated elevation of Th2 cytokine IL-5 in OVA-sensitized offspring.

1-NP, as a nitro-PAHs, is extensively located in environment (Bandowe and Meusel, 2017). Many studies have been conducted on its mutagenicity (Chatel et al., 2014) and carcinogenicity (Imaida et al., 1995). Recently, our results found that maternal 1-NP exposure induced fetal IUGR and even disturbed neurobehavioral development of female progeny (Li et al., 2018). The fetal stage serves as a key time window for lung development (Lacasana et al., 2005). Neonatal growth and immune system development can be influenced by maternal polluted air exposure (Vieira, 2015; Wong et al., 2015). A recent epidemiological study showed that maternal exposure to air pollutants, such as NO₂, SO₂, PM_{2.5}, PM₁₀, was positively correlated with childhood asthma (Zhang et al., 2017). Similarly, several animal experiments found that gestational NO₂ and DEP exposure aggravated the susceptibility of progeny to allergic asthma (Manners et al., 2014; Yue et al., 2017).

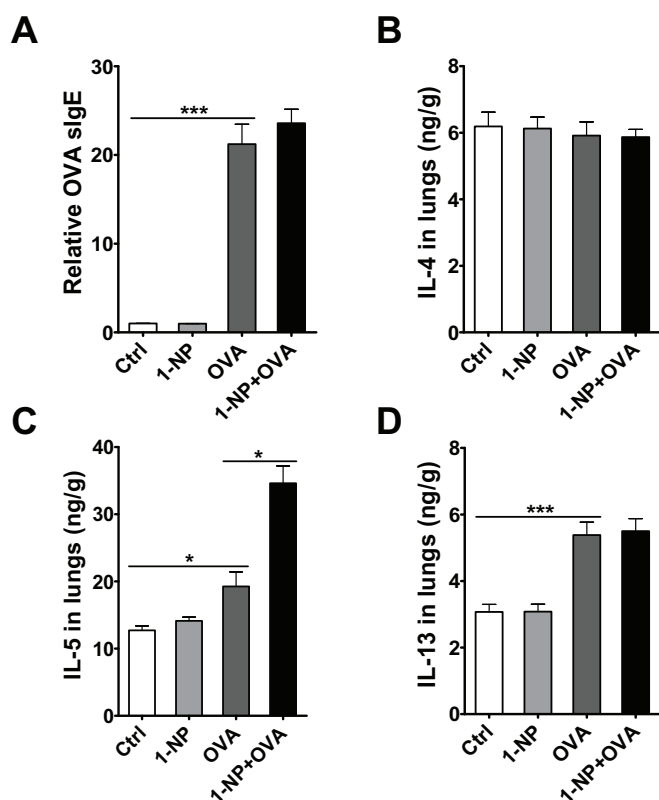


Fig. 6. Effects of maternal 1-NP exposure on serum OVA specific IgE and pulmonary Th2 cytokines in asthmatic offspring. (A) Serum OVA specific IgE level was detected by ELISA. The results were indicated as means $\pm$ standard error (N = 12). (B–E) Pulmonary IL-4, IL-5 and IL-13 levels were measured by ELISA. The results were expressed as mean $\pm$ standard error (N = 6). * P < 0.05, ** P < 0.01 and *** P < 0.001 as indicated.

However, whether maternal exposure to 1-NP, a representative component of DEP, aggravates the susceptibility of offspring to allergic asthma remains to be determined. To address this implication, this study established an allergic asthma model of mouse using OVA sensitization and challenge, and then evaluated the effects of maternal exposure to 1-NP on asthmatic pups. In our study, we defined the susceptibility of allergic asthma as more serious allergic asthma symptoms such as airway inflammation, airway remodeling, excessive mucus secretion, and AHR under allergen stimulation.

Our previous study indicated that maternal 1-NP exposure in the late pregnant period induced fetal IUGR (Li et al., 2018). In the present study, pregnant C57BL/6 mice were exposed to 1-NP in the whole pregnancy, with no effect on diet and maternal body weight, but the body weight of pups on PND4 was significantly reduced in 1-NP-exposed mice. Moreover, the body weights of male and female pups from 1-NP-exposed group had not been able to catch up with those of control pups. After weaning, mice were subjected to OVA sensitization and challenge to establish an asthma model. The results suggest that maternal 1-NP exposure had no significant influence on the development of immune organs in offspring. In addition, maternal 1-NP exposure did not aggravate the elevation of lung weight and lung coefficient in OVA-sensitized offspring. Interestingly, maternal 1-NP exposure aggravated airway eosinophil infiltration in OVA-sensitized pups. Increasing evidence demonstrates that eosinophils are the central effector cells that, when localized in the airways of allergic asthma, result in bronchial epithelial damage and airflow obstruction (Kim et al., 2010). It has been demonstrated that pulmonary eosinophilia is driven by IL-5,

which recruits eosinophils to pulmonary mucosa and interstitium via producing chemokines (Lambrecht and Hammad, 2014). In the present study, we found that gestational 1-NP exposure aggravated elevation of pulmonary IL-5 in OVA-sensitized offspring. These results provide mechanistic explanation for which gestational 1-NP exposure aggravates airway eosinophil infiltration in OVA-sensitized offspring.

Accumulating data demonstrate that airway goblet cell hyperplasia depends on Th2-biased inflammation (Rothenberg and Hogan, 2006). Th2 cells can directly promote Th2 cytokine-mediated airway goblet cell hyperplasia and mucus formation in asthmatic mice (Taube et al., 2002). Mucin is a heavily glycosylated glycoprotein, as the major component of mucus (Hollingsworth and Swanson, 2004). Increased goblet cells always occurred with changes in mucin gene expression, which results in alteration of mucus composition and airways organization. These changes are linked with elevated airway mucus gel viscoelasticity, which impedes mucus transport and results subsequent airway obstruction and mucus plugging (Bonser and Erle, 2017). It is known that airway mucus viscoelasticity depends on MUC5AC and MUC5B (Thornton, 2004). Indeed, the present study showed that pulmonary *Muc5ac* mRNA was upregulated in OVA-sensitized offspring. Moreover, gestational 1-NP exposure aggravated upregulation of pulmonary *Muc5ac* mRNA in OVA-sensitized offspring. These results suggest that gestational 1-NP exposure increases mucus production through upregulating pulmonary *Muc5ac* mRNA in OVA-sensitized offspring.

An early study has demonstrated that IL-4 increases airway reactivity through promoting OVA-evoked eosinophils to enter the airways and B cells to produce IgE (Brusselle et al., 1994). However, another study has confirmed that the key role of IL-4 is its effect on Th2 development rather than IgE synthesis (Coyle et al., 1995). This study showed that pulmonary IL-4 level was not enhanced in OVA-sensitized pups, just like shown in other experiments (Ano et al., 2013; Liu et al., 2016; Ren et al., 2014). Indeed, IgE is an important biomarker for asthma. According to a recent report, the levels of serum IgE were elevated in asthmatic animals and patients (Froidure et al., 2015). Accumulating data indicate that increased level of IgE could cause different immune effector cells to release different mediators that promote AHR (Rabe et al., 2011). In the present study, we detected serum IgE in OVA-sensitized asthmatic offspring. We found that serum OVA-specific IgE was elevated in pups. Unexpectedly, gestational 1-NP exposure did not aggravate the level of serum IgE in OVA-sensitized offspring. These results suggest that gestational 1-NP exposure exacerbates OVA-evoked allergic asthma independent of increase in IgE.

The present study has several limitations. Firstly, the present study played emphasis on the effects of gestational 1-NP exposure by gavage on susceptibility of adolescent offspring on allergic asthma. Indeed, recent urban atmospheric concentrations of 1-NP are in the range of several hundred ng/m³ (Tang et al., 2017). Thus, additional experiment is necessary to investigate whether gestational 1-NP exposure through inhalation increases susceptibility of allergic asthma. Secondly, the exact mechanism through which gestational 1-NP exposure elevates susceptibility of adolescent offspring on allergic asthma remains obscure. Several animal studies showed that female adult mice exposure to indeno[1,2,3-cd]pyrene (IP), a representative of polycyclic aromatic hydrocarbons (PAHs), aggravated cockroach allergen extract (CRE) or OVA-induced asthma through elevating inflammatory response in an aryl hydrocarbon receptor (AhR)-dependent manner (Sun et al., 2019; Wong et al., 2018). Moreover, Benzo(a)pyrene (BaP), another prominent of PAHs facilitated dermatophagoides group 1 allergen (Der f 1)-induced asthma through the AhR-ROS axis (Wang et al., 2019a). Another study showed PAHs upregulated Th2-

related inflammatory cytokines through epigenetic modification (Klingbeil et al., 2014). Thus, further study is required to explore whether gestational 1-NP exposure increases susceptibility of allergic asthma in adolescent offspring through epigenetic reprogramming.

In summary, the current research found that gestational 1-NP exposure aggravated infiltration of airway inflammatory cells in OVA-sensitized offspring. In addition, gestational 1-NP exposure aggravated excessive secretion of airway mucus in OVA-sensitized offspring. Moreover, gestational 1-NP exposure promoted Th2 polarization in OVA-sensitized offspring. Our results provide evidence that maternal 1-NP exposure may increase susceptibility of allergic asthma in adolescent offspring, providing a new etiological direction for the onset of allergic asthma.

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