Original Article

Maternal Disononyl Phthalate Exposure Activates Allergic Airway Inflammation via Stimulating the Phosphoinositide 3-kinase/Akt Pathway in Rat Pups*

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Abstract

Objective To evaluate the effect of diisononyl phthalate (DINP) exposure during gestation and lactation on allergic response in pups and to explore the role of phosphoinositide 3-kinase/Akt pathway on it.

Methods Female Wistar rats were treated with DINP at different dosages (0, 5, 50, and 500 mg/kg of body weight per day). The pups were sensitized and challenged by ovalbumin (OVA). The airway response was assessed; the airway histological studies were performed by hematoxylin and eosin (HE) staining; and the relative cytokines in phosphoinositide 3-kinase (PI3K)/Akt pathway were measured by enzyme-linked immunosorbent assay (ELISA) and western blot analysis.

Results There was no significant difference in DINP's effect on airway hyperresponsiveness (AHR) between male pups and female pups. In the 50 mg/(kg·d) DINP-treated group, airway response to OVA significantly increased and pups showed dramatically enhanced pulmonary resistance (RI) compared with those from controls (P<0.05). Enhanced Akt phosphorylation and NF-κB translocation, and Th2 cytokines expression were observed in pups of 50 mg/(kg·d) DINP-treated group. However, in the 5 and 500 mg/(kg·d) DINP-treated pups, no significant effects were observed.

Conclusion There was an adjuvant effect of DINP on allergic airway inflammation in pups. Maternal DINP exposure could promote OVA-induced allergic airway response in pups in part by upregulation of PI3K/Akt pathway.

Key words: Allergic airway inflammation; Asthma; DINP; Maternal exposure; PI3K/Akt

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INTRODUCTION

Allergic asthma is a chronic airway disorder characterized by chronic eosinophilic airway inflammation, reversible airway obstruction, and non-specific airway hyperresponsiveness (AHR). A great deal of evidence indicated that these inflammatory responses are mediated by T-helper type 2 (Th2) and T-helper type 1 (Th1) cells together with mast cells, bronchial epithelial cells, and eosinophils, as well as a number of inflammatory cytokines and chemokines[1-2]. Epidemiological studies have shown that the prevalence of allergic disease, such as asthma, increased among children and adolescents during the past 30 years, in parallel with the period of increasing production of chemicals in industrialization[1]. This suggests that the prevalence

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of allergic airway disease might be associated with the exposure to environmental contaminants[3-7].

Diisononyl phthalate (DINP) is one plasticizer used in soft poly vinyl chloride (PVC) materials, which is a substitute for di-2-ethylhexyl phthalate (DEHP), a reproductive toxicological chemical. Humans are exposed to DINP via ingestion, inhalation, and dermal contact. Ingestion is considered to be the major route, accounting for over 90% of total DINP intake[8]. Moreover, as the restriction of DEHP use in Europe, DINP has replaced DEHP to be the most commonly used plasticizer. It has been reported that the use of DINP and diisodecyl phthalate (DIDP) is now threefold higher than the use of DEHP in Europe[9]. A human biomonitoring study showed a doubled DINP exposure from 1988 to 2003, and with the recent change in use pattern from DEHP to DINP in Europe, the risk of human DINP exposure may be rapidly increasing[10].

Allergic asthma and allergy have been reported in industrial workers and children exposed to phthalates and PVC materials, suggesting a possible association between chronic phthalate exposure and immune-mediated disease[11-16]. Phthalate plasticizers and metabolites have been shown to enhance or suppress the effect of immunogens, which means these chemicals have adjuvant effects on anaphylactic reaction[17-18]. Several *in vitro* and animal studies have examined the consequences of DINP exposure on mast cell degranulation, eosinophilic inflammation, and regulatory T cells, the results suggest that the developing immune system might be a particular sensitive target of DINP[19-20].

However, there are limited data determining co-effect of prenatal and postnatal DINP exposure on the developing immune system. So we designed this *in vivo* study to evaluate the effect of DINP on the development of allergic asthma in rats and the underlying mechanism. Our hypothesis is that maternal DINP exposure during the critical period of fetal immune system and respiratory system development could affect allergic inflammation response in pups, and modulate the severity of the effect through the phosphoinositide 3-kinase/akt pathway.

METHODS

Chemicals

Hen egg ovalbumin (OA grade V, >98%, CAS No. 9006-59-1, Cat. No. A5503) was from Sigma (St. Louis, Mo., USA). Diisononyl phthalate, CAS No 68515-48-0, AR, purity 99% was from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Aluminium hydroxide (CAS No 21645-51-2, AR, >97%) was from Shanghai Meixing Chemical Co., Ltd (Shanghai, China).

Animals

Specific-Pathogen Free (SPF) female Wistar rats at age of 50-60 d, obtained from Animal Center of Fudan University, Shanghai, China, were housed in plastic cages in an air-controlled room at 21±3 °C with relative humidity of 55%±15%. The animals were maintained on a 12 h light/12 h dark cycle and had free access to tap water and standard laboratory animal feed. Nulliparous rats were mated overnight and checked in the next morning for sperm in the vaginal smear. The morning when sperm was detected in the vaginal smear was defined as gestational day (GD) 0.5, and postnatal day (PND) 0.5 was the day after birth.

DINP Treatment

Pregnant rats were randomly assigned to treatment groups (5-6 animals) and administrated once a day by oral gavage with vehicle (corn oil from supermarket), 5, 50, or 500 mg DINP/kg bw from GD 7 to PND 21. According to Organization for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals, a part of litters were culled on PND 4 to balance provision of nutrition[21-22].

Allergic Airway Inflammation Model[23-24]

On PND 22, 23, and 37, the pups (n=6-8 per group) in groups of corn oil and DINP treatment were sensitized by subcutaneous injection of 1 mg OVA and 22.5 mg $Al(OH)_3$ suspended in 0.5 mL saline. The rats were challenged with 1% OVA aerosol for 30 min on PND 44 and 45. In addition, 6 pups in the control group were sensitized and challenged with saline, which were used as negative control model. Every group had the same quantity of male and female pups.

Measurements of Respiratory Function

The method for the invasive respiratory function measurements used in this study has been described previously[25]. Briefly, the pups were anesthetized 24 h after the last aerosol challenge, and were placed in a supine position and warmed with an incandescent lamp. At the upper part of the trachea, a T-shape incision was made and a T-shape cannula, which was directly attached to a heater-controlled pneumotachograph (Fleisch model 000, Hans