Effects of Di-(2-ethylhexyl) Phthalate on Ovalbumin-Immunized Rat Asthma Model: A Histopathological Study

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Abstract: Phthalate pollution becomes an environmental health problem in China recent years. In order to investigate the potential role of di-(2-ethylhexyl) phthalate (DEHP) in the pathogenesis of asthma and to establish the animal models of DEHP-induced asthma, 32 Wistar rats in four experimental groups were exposed to: (1) saline, (2) ovalbumin (OVA), (3) OVA+DEHP (0.7mg·kg⁻¹·d⁻¹) and (4) OVA+DEHP (70mg·kg⁻¹·d⁻¹). DEHP exposure treatment in this study was undertaken by gastric gavages and done before and during the process of OVA immunization. Lung histological analysis was conducted after. The results showed that, compared with the OVA exposure only, DEHP exposure could increase inflammatory cells infiltration and airway wall thickness significantly; these changes of airway structure in 70mg·kg⁻¹·d⁻¹ group were more serious than those in 0.7mg·kg⁻¹·d⁻¹ group. Therefore, we conclude that DEHP can induce airway remodeling in asthma model and provide an adjuvant role in the pathogenesis of DEHP-induced asthma.

Keywords: DEHP; asthma; rat model; adjuvant effect; phthalate

DEHP 对 OVA 致敏大鼠哮喘模型的影响：一项组织病理学研究

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摘要：近年来邻苯二甲酸酯污染已成为中国一个重要的环境问题。为探讨邻苯二甲酸二乙基酯（di-(2-ethylhexyl) phthalate, DEHP）在诱发哮喘方面的作用，及研制 DEHP 诱导大鼠哮喘模型，将 32 只 Wistar 大鼠随机分成 4 组（每组 8 只）；生理盐水对照组，卵清白蛋白（OVA）致敏组和 2 个 DEHP 染毒组，采用 OVA 致敏加激发的方法制作大鼠哮喘模型。2 个 DEHP 染毒组大鼠每天分别进行 0.7mg·kg⁻¹ 和 70mg·kg⁻¹ 邻苯二甲酸二乙基酯灌胃染毒。连续 30d. OVA 致敏组、DEHP 染毒组大鼠均在第 31~37 天给予 1% OVA 雾化，诱发哮喘。第 38 天取肺脏做组织切片，进行分析。结果表明，与 OVA 组相比，DEHP 染毒组气管壁增厚，细胞浸润增加，气道重塑，特别是在 70mg·kg⁻¹·d⁻¹ 组，变化更为明显。由此可以得出结论，邻苯二甲酸二乙基酯可诱导哮喘模型大鼠气道重塑，在诱发哮喘发病过程中可能起到佐剂作用。

关键词：邻苯二甲酸二乙基酯；哮喘；大鼠模型；佐剂效应；邻苯二甲酸酯

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1 Introduction

Phthalate esters (PAEs) are used for industrial chemicals which serve as important additives to impart flexibility to plastics. They are a key component in a wide range of products, including flexible polyvinyl chloride (PVC) plastics, vinyl tile, food packaging, insecticides, pharmaceuticals, and personal care products (ATSDR, 2001; NTP-CERHR, 2003; Schettler, 2006). Because they are not chemically bound to the plastics, phthalates can be released from the products that are used and discarded (Oie et al., 1997; Bornhag et al., 2005). Consequently they are widely distributed in the ecosystem and have been described as being among the most abundant man-made environmental pollutants which can cause widespread of toxicity (Wu et al., 2007; Li et al., 2007). In particular, di-(2-ethylhexyl) phthalate (DEHP) is the most commonly used plasticiser. Globally, more than 18 billion pounds of phthalates are used each year and well above 2 million tons of DEHP alone are produced annually worldwide (Lorz et al., 2002).

Epidemiological studies have suggested that DEHP has been associated with the development of wheezing, rhinitis, asthma or asthma symptoms (Jaakkola et al., 1999; Bornhag et al., 2004). However, animal study has shown that DEHP was not allergen, i.e. there was no specific anti-DEHP-IgE formed after DEHP treatment in the animal (Boulet et al., 2007). This has led to a conception that DEHP may cause asthma via a non-IgE-mediated mechanism. In this study, we purposed to establish the animal models of DEHP-induced asthma to examine the effect of DEHP on the respiratory systems. As regards, the lung histology was studied.

2 Materials and methods

Wistar rats (male, 5–6 weeks old and 70–80g) were purchased from the Hubei Experimental Animal Center (Wuhan, China) and maintained in pathogen-free room on environmental conditions that temperature was kept at 20–25°C and relative humidity at 50–70%.

32 Wistar rats were randomly divided into 4 groups. The saline group (group A) was administered with a hypodermal injection of 1mL saline at day 19 and 27. During the days of 31–37, animals placed in the glass chamber were challenged with an aerosol of saline for 30min per day using an ultrasonic nebulizer (Yuyue 402AL type, China); the OVA-immunized group (group B) was treated with a hypodermal injection (i.p.) of 100mg OVA (Sigma, Grade II) and 100mg aluminum hydroxide fine powder suspended in 1mL of saline at day 19 and 27. From day 31 to 37, animals were exposed to an aerosol of 1% OVA for 30min per day; the DEHP groups (group C and D), For preparation, DEHP (Sigma) was dissolved in TWEEN-80 (Sigma) in the ratio 1:1 and diluted by sterile water to the final concentration designed in advance, the rats were weighed, and oral dosing was initiated with 0.7mg and 70mg/kg·d, respectively and immunized and challenged with OVA following the protocol shown in Fig. 1.

![Fig. 1](image)

Fig.1 The exposure protocol

(GroupId: saline control; Group B: OVA-immunized only group; Group C: OVA+0.7mg/kg·d group; Group D: OVA+70mg/kg·d group)

3 Results

Fig. 2 showed lung histology results, it demonstrated airway remodeling in OVA-immunized group, including higher levels of mucus secretion, increased inflammatory cells infiltration and airway wall thickness, indicative of asthma. DEHP exposure aggravated the asthma syndromes in immunized rats, and the most serious change of the airway structure was shown in the OVA+70mg/kg·d group.
4 Discussion

Asthma becomes a global health problem, with the prevalence of it increasing steadily worldwide. Much of our understanding on the underlying mechanisms of chemical-induced comes from the studies of animal models (Dawkins et al., 2001). In this study, we establish the DEHP induce asthma model to observe the DEHP procession.

Airway remodeling refers to a complex typical airway structural changes that can be observed in chronic respiratory conditions like asthma (Elias, 2000). A series of events are involved in airway remodeling, including airway wall thickening, inflammatory cell infiltration, subepithelial fibrosis, mucus metaplasia, hyperproliferation and hyperplasia of smooth muscle and goblet cells (Yamauchi, 2006). It is believed that airway remodeling is a critical factor in the development of asthma syndromes such as AHR and airflow limitation, and close related to the aggravation of this respiratory disorder (Elias, 2000; Yamauchi, 2006; Leigh et al., 2002).

From the results of this study, we concluded that oral exposure to DEHP exhibits an adjuvant effect in the pathogenesis of DEHP-induced asthma and results in obvious airway remodeling of the tested rats.

References

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Fig.2. Histological examination of lung tissue airway remodeling (A: the saline group; B: the OVA-immunized group; C: OVA+0.7mg/kg•kg•d•d group; D: OVA+70mg/kg•kg•d group. Lung tissue was fixed, stained with H&E, and sectioned in 10μm slices)