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# Tracheal responsiveness to methacholine and ovalbumin; and lung inflammation in guinea pigs exposed to inhaled lead after sensitization

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ABSTRACT

The association between lead exposure and asthma is controversial. The effect of inhaled lead acetate on lung inflammation, tracheal responsiveness and immune components in guinea pigs after sensitization was examined in this study. Five groups of guinea pigs were randomly allocated to control (group C), sensitized (group S), and three test groups exposed to inhaled lead concentrations 0.1, 0.2 and 0.4 M Pb after sensitization ($n=6$ for each group). The measured variables included tracheal responsiveness to methacholine and ovalbumin (OA); total and differential white blood cells (WBC) counts of lung lavage; serum cytokine levels (IFN-γ and IL-4); and lead concentration in lung tissue. Tracheal responsiveness to methacholine and OA; total and differential WBC counts; IL-4 and IFN-γ were significantly increased in sensitized animals compared to control group ($p<0.05$ to $p<0.001$). However, the ratio of IFN-γ/IL-4 were significantly decreased in group S ($p<0.05$). In addition, all measured parameters in animals exposed to highest lead concentration and most of them in animals exposed to medium lead concentration were significantly higher than group S, except for the IFN-γ and IFN-γ/IL-4 ratio, which were significantly decreased ($p<0.05$ to $p<0.001$). The lead concentration in lung tissues of all test animals was significantly higher than that of group C ($p<0.001$ for all groups). These results showed that lead acetate exposure can cause further increase in tracheal responsiveness to methacholine and OA, total and differential WBC count and IL-4, IFN-γ and IFN-γ/IL-4 ratio. Therefore, environmental exposure to lead may aggravate the severity of asthma.



## 1. Introduction

Lead (Pb) is a heavy metal that is well known to be highly toxic to humans and other animals (Jacobs et al., 2009; Needleman, 2004; Landrigan et al., 2002). Exposure to this toxic metal can produce alteration in physiological functions and is considered to be associated with many diseases, including respiratory disorders (Joseph et al., 2005; Call et al., 1992). The contribution of lead pollution in pathogenesis of pulmonary cancers, asthma and COPD is suggested, but there is not confident result in this regard (Gould, 2005; Englysta et al., 2001; Benera et al., 2001).

There is evidence that exposure to primary sources of lead poisoning increases asthma morbidity (Call et al., 1992; Kang et al., 1993; Eggleston et al., 1998). Previous epidemiological studies inferred a connection between lead exposure and the development of asthma, an IgE-mediated allergic disease (Lanphear et al., 1998). Increased IgE and some inflammatory cytokines in serum of laboratory models and also children exposed to lead and the release of inflammatory mediator from Th cells and macrophages exposed to lead in a cell culture model were reported although, some studies did not show similar results or even showed a decrease in serum immunoglobulins of laboratory animals exposed to lead (Miller et al., 1998; Chen et al., 1997; Heo et al., 1996; Zelikoff et al., 1993; Onarigilue et al., 1999; Gupta and Fahim, 2007). Experimental animals exposed to lead showed respiratory system morphological changes and increased tracheal responsiveness (Salovsky et al., 1994).

Asthma is a chronic respiratory disease characterized by inflammation, orchestrated by type 2 helperT (Th2) cells (Tagaya and Tamaoki, 2007). Persistent inflammation in asthma may lead to airway hyperresponsiveness to different stimuli (Tagaya and Tamaoki, 2007).

However, the association between lead exposure and asthma and the corresponding mechanism of this association is not clear. Determining the effect of lead poisoning on occurrence and development of symptoms of asthma may provide information to guide interventions aimed at preventing or reducing the

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severity or impact of lead exposure and asthma (Lanphear et al., 1998). Therefore, in the present study, the effects of inhaled lead exposure on tracheal responsiveness, total and differential inflammatory cell (white blood cells) count in lung lavage and serum cytokines levels in guinea pigs after sensitization were examined.

## 2. Materials and methods

### 2.1. Animal sensitization

Sensitization of animals to OA was performed using the method described previously (McCaig, 1987; Boskabady and Adel-Kardan, 1999; Boskabady et al., 2006). Briefly, guinea pigs were sensitized to OA (Sigma Chemical Ltd, UK) by i.p. injecting 10 mg OA and 100 mg $Al(OH)_3$ on day 1 and 8. Animals were exposed to an aerosol of 4 percent OA from day 14 for $18 \pm 1$ days, 4 min daily. The aerosol was administered in a closed chamber, dimensions $30 \times 20 \times 20$ cm using a nebulizer (CX3, Omron Healthcare Europe B.V., and the Netherlands). Control animals were treated similarly but saline was used instead of OA solution. The study was approved by the ethical committee of Mashha University of Medical Sciences.

### 2.2. Exposure of animals to lead

Animals were placed in a closed chamber ($30 \times 20 \times 20$ cm) connected to an ultra-nebulizer (Ultra-Neb 99 DeVilbis) with an air flow of 10 L/min, which produces particles of 1 μm. Animal were exposed to aerosol of three lead acetate concentrations of 0.1, 0.2 and 0.4 M (Sigma Chemical Co., St. Louis, MO, USA) for 1 h, three times a week for two weeks (Fortoul et al., 2005) from day 35 (after OA inhalation period). All measurements were made after the end of exposure of animals to lead (day 49). Animals were allowed to get in to the habit of new situation for ten days. They were group-housed in individual cages in climate-controlled animal quarters and were given water and food *ad libitum*, while a 12-h on/12-h off light cycle was maintained.

Thirty adult Dunkin-Hartley guinea pigs (400–700 g, both sexes) were randomly divided into five groups as follows ($n=6$ for each group);

1. Control group (not sensitized and exposed to the nebulized distilled water alone similar to lead exposure, group C)
2. Sensitized and exposed to the nebulized distilled water alone similar to lead exposure after sensitization (group S)
3. Exposed to 0.1 M lead concentration post sensitization (group PS+0.1 M Pb)
4. Exposed to 0.2 M lead concentration post sensitization (group PS+0.2 M Pb)
5. Exposed to 0.4 M lead concentration post sensitization (group PS+0.4 M Pb)

The lead concentrations used in the present study were chosen according several previous animal studies (Fortoul et al., 1999, 2005; Miller et al., 1998; Onarigilue et al., 1999; Zelikoff et al., 1993).

### 2.3. Tissue preparation

Trachea was removed after sacrificing guinea pigs by a blow on the neck and was cut into eight rings (each containing two to three cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain (Boskabady et al., 2010, 2004).

Tissue was then suspended in a 20 mL organ bath (Schuler organ bath type 809, March-Hugstetten, Germany) containing Krebs–Henseliet solution of the following composition (mM): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, $KH_2PO_4$ 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11. The Krebs solution was maintained at 37 °C and gassed with 95 percent $O_2$ and 5 percent $CO_2$. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

Responses were measured using vernier control type 850 N sensor with sensitivity range: 0–20 g and resolution: 0.2 mm/turn (Hugo-Sachs Elektronik, Germany) and amplified with amplifier (ML/118 quadribridge amp, March-Hugstetten, Germany) and recorded on powerlab (ML-750, 4 channel recorder, March-Hugstetten, Germany).

### 2.4. Assessment of tracheal response to methacholine

A cumulative log concentration–response curve of methacholine hydrochloride (Sigma Chemical Ltd, UK) was obtained in each tracheal chain by adding consecutive concentrations ($10^{-7}$ to $10^{-1}$ mM) to organ bath every 3 min. The contraction due to each concentration was recorded at the end of 3 min. The percentage of contraction of the tracheal smooth muscle due to each concentration of methacholine in proportion to the maximum contraction obtained by its final concentration was plotted against log concentration of methacholine to obtain the curve. The effective concentration of methacholine, causing 50 percent of maximum response ($EC_{50}$) was measured from methacholine response curve in each experiment using 50 percent of maximum response in the *Y* axis and measuring the dose of methacholine causing this response in the *X* axis. Contractility response to 10 μM methacholine as the magnitude of contraction was also measured.

### 2.5. Measurement of tracheal response and contractility response to OA

Tracheal response to 0.1 percent solution of OA was measured as follows: 0.5 mL of 4 percent OA solution (dissolved in saline) was added to the 20 mL organ bath. Tracheal smooth muscle contraction was recorded after 15 min and expressed as the proportion (in percentage) of contraction obtained by 10 μM methacholine. Contractility response to OA was the maximum contractility response of tracheal smooth muscle to 0.1 percent solution of OA. The measurement of tracheal response to methacholine and OA was performed in random order.

### 2.6. Lung lavage and its white blood cells count

A cannula was located into the remaining trachea coincident with preparing the tracheal chain and lungs were lavaged four times with 5 mL of saline (total: 20 mL). A volume of 1 mL of lung lavage fluid (LLF) were stained with a Turk solution and counted in duplicate in a hemocytometer (in a Burker chamber). The Turk solution consisted of 1 mL of glacial acetic acid, 1 mL of 1 percent gentian violet solution in 100 mL distilled water. The remaining LLF was centrifuged at $2500 \times g$ at 4 °C for 10 min. The supernatant was removed. The smear was prepared from the cells and stained with Wright-Giemsa. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 400 cells in each sample and the percentage of each cell type was calculated.

### 2.7. Measurement of blood IL-4 and IFN-γ

After sacrificing the animals, 5 mL of peripheral blood were obtained immediately and placed at room temperature for 1 h. The samples were then centrifuged at $3500 \times g$ at 4 °C for 10 min. The supernatant was collected and immediately stored at −70 °C until analyzed. Finally, blood IL-4 and IFN-γ were measured using Elisa sandwich (Ab Sandwich) method and the ratio of IFN-γ/IL4 as an index of Th1/Th2 was calculated.

### 2.8. Measurement of lead concentration in lung tissue

Lung samples were analyzed using a graphite furnace atomic absorption spectrometer (Perkin-Elmer Mod. 2380). The light source came from a hollow cathode lamp. Accuracy was assured by three random determinations of seven different standard solutions, prepared with the same chemical reactive used during the metal analysis. For Pb, the wavelength was 318.4 nm, the detection limit was 0.37 ppm, and the slit was 0.7 nm. Each sample was analyzed in triplicate (Fortoul et al., 2005).

### 2.9. Statistical analysis

The data were quoted as mean ± SEM. Comparison of the data between different groups was made using one way analysis of variance (ANOVA) with Tukey-Kramer post-test. Significance was accepted at $p < 0.05$.

## 3. Results

### 3.1. Tracheal responsiveness to methacholine and ovalbumin

There were leftward shifts in methacholine concentration response curves in group S and all test groups compared to those in group C. (Fig. 1).

Tracheal responsiveness and contractility to both methacholine and OA were significantly higher in sensitized group compared to group C ($p < 0.05$ to $p < 0.001$, Figs. 2 and 3). Tracheal responsiveness and contractility to both agents in guinea pigs exposed to all lead concentrations were also significantly higher than those in group *C* ($p < 0.05$ to $p < 0.001$, Figs. 2 and 3). In addition, tracheal responsiveness to OA in all test animals