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).

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 helper T (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
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 ± 1 days, 4 min daily. The aerosol was administered in a closed chamber, dimensions 30 × 20 × 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 Mashh University of Medical Sciences.

2.2. Exposure of animals to lead

Animals were placed in a closed chamber (30 × 20 × 20 cm) connected to an ultra-nebulizer (Ultra-Neb 99 DeVilbiss) with an air flow of 10 L/min, which forms a tracheal chain (Boskabady et al., 2010, 2004). 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 tissues were washed with Krebs solution every 15 min.

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 M) 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 (EC50) 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 × 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 × 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 test 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
exposed to all lead concentrations and maximum response to OA in those exposed to its two higher concentrations (0.2 and 0.4 M) were significantly higher than group S ($p < 0.01$ to $p < 0.001$, Figs. 2 and 3).

Tracheal responsiveness to OA in animal exposed to high concentration and maximum response to OA in animals exposed to two higher concentrations (0.2 and 0.4 M) were also significantly higher than those exposed to low (0.1 M) lead concentration ($p < 0.01$ to $p < 0.001$ for 0.2 and 0.4 M lead concentrations, respectively, Fig. 3).

3.2. Total and differential WBC count in lung lavage

There were significant differences in total and all differential counts of WBC in lung lavage between groups S and C ($p < 0.001$ for all cases, Fig. 4a and b). Total and all differential WBC counts in lung lavage of all exposed animals to lead were also significantly higher than those of group C ($p < 0.001$ for all cases, Fig. 4). In addition, total and differential WBC counts in lung lavage of animals exposed to all three lead concentrations were significantly higher, than those of group S ($p < 0.001$ for all cases) except lymphocyte count in animals exposed to high lead concentration which was lower than group S ($p < 0.01$, Fig. 4).

Total WBC count in animal exposed to high lead concentration (0.4 M) was greater than those exposed to medium (0.2 M) concentration ($p < 0.05$, Fig. 4a). All differential WBC counts in lung lavage of animals exposed to high lead concentration (0.4 M) were significantly higher except lymphocyte which was lower than those exposed to medium (0.2 M) and low (0.1 M) lead concentrations ($p < 0.001$ for all cases, Fig. 4b). Lymphocyte, basophil and monocyte counts in animals exposed to medium concentration (0.2 M) were also different from those exposed to low (0.1 M) concentration ($p < 0.001$ for all cases, Fig. 4).

3.3. Serum level of IFN-$\gamma$ and IL-4 and IFN-$\gamma$/IL-4 ratio

Serum level of IFN-$\gamma$ and IL-4 in group S were significantly higher ($p < 0.01$ for IFN-$\gamma$ and $p < 0.001$ IL-4) but IFN-$\gamma$/IL-4 ratio was lower ($p < 0.05$) than those in group C (Fig. 5). Serum level of IFN-$\gamma$ and IL-4 in animals exposed to low and high lead concentrations (0.1 and 0.4 M) was significantly higher but IFN-$\gamma$/IL-4 ratio was lower than those in group C ($p < 0.05$ to $p < 0.001$, Fig. 5). Serum level of IFN-$\gamma$/IL-4 ratio and IFN-$\gamma$ in group exposed to high lead concentration (0.4 M) were significantly lower but IL-4 was higher than those in group S ($p < 0.05$ to $p < 0.001$, Fig. 5).

3.4. Lead concentration in lung tissues

Lead concentration in the lung of all animals exposed to lead (117.13 ± 2.76, 179.38 ± 5.12 and 313.16 ± 4.33 in animal exposed to 0.1 M, 0.2 M and 0.4 M lead concentrations respectively) was significantly higher than that in groups C (0.00 ± 0.00) and S (0.00 ± 0.00), ($p < 0.001$ for all cases). The lead concentration in the lung of animals exposed to high (0.4 M) and medium...
(0.2 M) lead concentration was significantly higher than those exposed to low (0.1 M) lead concentration \( (p < 0.001\) for both cases). The lead concentration in the lung of animals exposed to high lead concentration was also significantly higher than those exposed to medium lead concentration \( (p < 0.05)\).

4. Discussion

The results of the present study showed significant increase in tracheal responsiveness to methacholine and OA, total WBC number and percentage of eosinophil, neutrophil, lymphocyte and basophil as well as serum levels of IL-4 but significant decreased in IFN-\(\gamma/\)IL-4 ratio in sensitized guinea pigs. The similar findings were also observed in lead exposed animals. However, total and differential WBC counts in lung lavage and tracheal response to OA in animals exposed to lead were significantly higher than those in group S. Serum levels of IL-4 was significantly higher, but IFN-\(\gamma/\)IFN-\(\gamma\)-IL-4 ratio was lower in sensitized animals exposed to highest lead concentration (0.4 M) than group S.

The increased total WBC and eosinophils counts are characterized feature of sensitized animals and asthmatic patients \( (\text{Lukszaj and Jones, 1982})\). Increased total WBC and eosinophils counts in lung lavage of sensitized guinea pigs were seen in our previous studies using the same method of sensitization \( (\text{Keyhmanesh et al., 2009; Neamati et al., 2009})\). Therefore, further increase in total WBC number in animals exposed to all lead concentrations and eosinophil count in lung lavage of animals exposed to two higher lead concentrations compared to those in group S indicate that lead exposure could aggravate asthma severity after sensitization (after development of asthma).

There was increased specific tracheal responsiveness (tracheal response to OA) in animals exposed to inhaled lead compared to sensitized group. The increased airway responsiveness (AHR) to different stimuli is the main characteristic of asthma disease.
The relationship between elevation of blood lead level and respiratory system morphologic changes as well as increased inhaled lead exposure on the severity of airway responsiveness in animals. In experimental studies on animal models exposed to lead, inhaled lead exposure after sensitization mainly affected immunologic changes and specific tracheal responsiveness in asthma. The results of the present study suggest that inhaled lead exposure can cause lung inflammation (increased total and differential WBC count) and Th1/Th2 change (change in IFN-γ/IL-4 ratio) toward worsening of asthma condition. These changes may cause increased specific tracheal responsiveness. However, the reason of absence of increased non-specific tracheal responsiveness in lead exposed sensitized animal is uncertain to us and should be clarified in further studies.

The inhaled lead concentrations used in the present study were 60, 120 and 240 mg/m³ (0.38, 0.76 and 1.52 g nebulized with 6000 L air). Although inhaled lead concentrations used in the present study were much higher (less than ten times) than lead concentration in lead industrial environment of 0.089 mg/m³ to 0.092 mg/m³ (Ho et al., 1998; Ibiebele, 1994). However, the studied animals were exposed to lead for 14 days each day 60 min, but workers in industrial environment of lead pollution exposed to inhaled lead for several years each day about 8 h. In addition, 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).

The effect of non-sensitized animals exposed to the same inhaled lead concentrations showed increased in all measured parameters. However, the data in animals exposed to inhaled lead were significantly lower than sensitized animals for most cases and similar (non-significantly different) in few cases (submitted to Respirology). The findings of the present study and those in non-sensitized animals exposed to inhaled lead indicate additive of lead exposure to changes induced by sensitization of animals.

In conclusion, these results showed that inhaled lead acetate exposure in animals after sensitization can cause further increase in specific tracheal responsiveness, total and differential WBC count as well as cytokines and IFN-γ/IL-4 ratio changes. Therefore, the results may suggest increased severity of asthma due to environmental lead pollution after development of the disease.

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