Lead exposure affects inflammatory mediators, total and differential white blood cells in sensitized guinea pigs during and after sensitization

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Abstract

Background: Serum inflammatory mediators and white blood cells (WBC) counts in the blood of sensitized and lead exposed guinea pigs were evaluated. Methods: Guinea pigs were randomly allocated into control (C), sensitized (S) and sensitized groups exposed to three lead concentrations (0.1, 0.2 and 0.4 M) during (DS) and after sensitization (PS), (n = 6 for each group). Animals were sensitized by intra-peritoneal injection and aerosol inhalation of ovalbumin (OA). Serum total protein, PLA2, IgE, histamine, total and differential WBC counts of blood were evaluated. Results: Serum PLA2, total protein, IgE, histamine, total and differential WBC counts of animals exposed to lead after sensitization were significantly increased, but the percentage of lymphocyte was decreased compared to control group (p < 0.05 to p < 0.001). Serum total protein and total WBC number in all animals exposed to lead as well as the eosinophils and histamine in animals exposed to highest lead concentration and IgE in sensitized groups exposed to lead after sensitization were significantly higher, but the lymphocyte in animals exposed to two higher lead concentrations was lower than non exposed sensitized group (p < 0.05 to p < 0.001). The changes in all parameters in lead exposed animals after sensitization were higher than those during sensitization which was statistically significant for total WBC counts in the animals exposed to low lead concentration (p < 0.05). Conclusion: Inhaled lead can increase serum total protein, PLA2, IgE and histamine levels, total and most differential WBC counts in sensitized animals which was more pronounced in animals exposed to lead after compared to those during sensitization.

Introduction

The widespread occurrence of lead pollution in the air is a major environmental concern especially for developing countries (Jacobs et al., 2009). However, the effects of lead exposure on respiratory system have been poorly studied (Joseph et al., 2005). Epidemiological studies have shown the high incidence of asthma in workers exposed to lead as well as children that live in places with high levels of lead pollution (Bener et al., 2001; Englysta et al., 2001; Gould, 2005). Lead induces cellular damage through accumulation in mitochondria and blocking the respiratory chain, intracellular depletion of GSH, increasing oxidative stress and apoptosis and enhancement of pro-inflammatory processes via activation of nuclear factor kappa B (NFkB) (Wang et al., 2009). There are reports regarding increase of immunoglobulin E (IgE) and some inflammatory cells in blood of laboratory models and children exposed to lead and increased inflammatory mediators from Th cells and macrophages exposed to lead in a cell culture model (Chen et al., 1997; Heo et al., 1996). Pb exposure increased plasma IL-4 and IgE Levels via skewing T-helper cell subsets toward Th2 (Heo et al., 1996). Our pervious study indicated that inhaled lead acetate exposure can increase total WBC percentage of eosinophil, neutrophil and basophil in broncho-alveolar lavage as well as IL-4 besides a reduction in percentage of lymphocyte, IFN-γ and IFN-γ/IL-4 ratio (Boskabady et al., 2012). In another study, we observed that lead can cause further increase in total and differential WBC counts of lung lavage as well as IL-4 with a reduction in percentage of lymphocyte, IFN-γ and IFN-γ/IL-4 ratio in sensitized guinea pigs (Farkhondeh et al., 2013). Increased respiratory symptoms and decreased pulmonary function tests in workers exposed to lead was also observed (Khazdair et al., 2012). However, controversies
exist about the effect of Pb exposure on immune system and leukocytes count (Hogan & Adams, 1979; Kasten-Jolly et al., 2010).

Asthma is a chronic disease characterized by widespread inflammation of the airway walls. Airway inflammation correlates closely with asthma severity and AHR (Louis et al., 2000). Increase in various inflammatory cells with preferential increase in the number of eosinophils are involved in the pathogenesis of airway inflammation in asthma (Kelly et al., 1998). Increased serum histamine level (Bickford et al., 2012), WBC and eosinophil counts were documented in both asthmatic patients and sensitized animals (Busse & Swenson, 1989; Chang et al., 1987; Hogan et al., 2003; Kashim et al., 1993). IgE levels correlate with asthma severity and bronchial hyperresponsiveness. However, the link between total IgE and asthma appears to be independent of allergen sensitization (Keyhanmanesh et al., 2009; Luksha & Jones, 1982). Increased inflammatory cells lead to overproduction of inflammatory mediators and total protein in serum of astmatic patients (Kelly et al., 1998).

A defining event in asthma is the liberation of bioactive lipid metabolites of AA (arachidonic acid), the eicosanoids. The eicosanoids play an essential role in the inflammatory response, ultimately mediating vasodilation, vascular permeability, bronchoconstriction, chemotaxis and the transcription of pro-inflammatory enzymes. AA, the precursor of the eicosanoids, is produced by hydrolysis of membrane phospholipids by the PLA2 (phospholipase A2) (Neamati et al., 2010). Activated inflammatory cells, such as neutrophils and alveolar macrophages, and tracheal epithelial cells release PLA2 into interstitial or intravascular compartments (Neamati et al., 2010; Yssel et al., 1998). PLA2 has been shown to be released from activated mast cells which are mainly involved in the allergic inflammation of bronchial asthma (Hamelmann, 2007). Increased PLA2 activity has been demonstrated in serum and bronchoalveolar lavage fluid from asthmatics (Hamelmann, 2007).

Therefore, the present study was designed to examine the effect of lead exposure on total and differential WBC blood count as well as the levels of PLA2 and total protein in serum of sensitized guinea pigs exposed to lead during or after sensitization compared to non-exposed sensitized animals.

Materials and methods

Animals and Groups

Forty eight Dunkin-Hartley guinea pigs (400–700 g, both sexes) were used throughout the study. They were allowed to accommodate in the new situation for ten days. The animals were group-housed in individual cages (50 × 90 cm²) in climate-controlled animal quarters and were given water and food ad libitum, while a 12-h light/12-h dark cycle was maintained. Animals were randomly divided to 8 groups as follows (n = 6 for each group); (1) Control (non sensitized non exposed to lead, group C) (2) Sensitized (sensitized non exposed animals to lead, group S) (3) Sensitized animals exposed to 0.1 M lead concentration during sensitization (group DS + 0.1 M Pb) (4) Sensitized animals exposed to 0.2 M lead concentration during sensitization (group DS + 0.2 M Pb) (5) Sensitized animals exposed to 0.4 M lead concentration during sensitization (group DS + 0.4 M Pb) (6) Sensitized animals exposed to 0.1 M lead concentration after sensitization (group PS + 0.1 M Pb) (7) Sensitized animals exposed to 0.2 M lead concentration after sensitization (group PS + 0.2 M Pb) (8) Sensitized animals exposed to 0.4 M lead concentration after sensitization (group PS + 0.4 M Pb)

Sensitization of animals

Sensitization of animals to OA was performed using the method previously described (Boskabady & Adel-Kardan, 1999; Boskabady et al., 2006; Vosooghi et al., 2013). Briefly, guinea pigs were sensitized to OA (Sigma Chemical Ltd, UK) by intra-peritoneal (i.p.) injection of 10 mg OA and 100 mg AL(OH)₃ dissolved in saline on day one and a further i.p. injection of 2 mg OA and 100 mg AL(OH)₃ on day 8. From day 14, sensitized animals were exposed to an aerosol of 4% OA for 18±1 days, 4 min daily. The aerosol was administered in a closed chamber with dimensions of 30 × 20 × 20 cm³, using a nebulizer (CX3, Omron Healthcare Europe B.V., the Netherlands). Control group was treated similarly but saline was used instead of OA solution. The study was approved by the ethical committee of the Mashhad University of Medical Sciences.

Exposure of animals to inhaled lead

The protocol of animal exposure to lead (Sigma Chemical Co., St. Louis, MO) was performed according to the method describe by Fortoul et al. (2005). Briefly, animals were placed in a closed chamber (30 × 20 × 20 cm³) connected to an ultranebulizer (Ultra-Neb 99 DeVilbis) with a air flow of 10 l/min, which produces particles of <1 µm. Different animal groups were exposed to aerosol of three lead acetate concentration of 0.1, 0.2 and 0.4 M for 1 h, thrice a week for two weeks.

White blood cells count

Blood samples were collected into test tubes containing anticoagulant EDTA by cardiac puncture immediately after anesthesia and exposing the animals’ chest. Blood sample was stained with Turk solution (1:10 dilution) and total white blood cell (WBC) was counted in duplicate in a hemocytometer (in a Burker chamber). The Turk solution consisted of 1 ml of glacial acetic acid, 1 ml of gentiac vialet solution 1% and 100 ml distilled water. Differential cell counts were done on thin slide, prepared with smearing blood sample, using Wright-Giemsa’s stain. According to staining and morphological criteria, differential cell analysis was carried out under a light microscope by counting 100 cells and the percentage of each cell type was calculated.

Measurement of serum PLA2 and total protein

A total of 5 ml peripheral blood was obtained immediately after sacrificing the animals 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 analysis. Finally, blood PLA2 activity and total protein were measured using the enzyme-linked immunosorbent assay (ELISA) sandwich method according to the manufacturer’s instructions (PLA2 Assay Kit, E10217, Co., Invitrogen, England; Total protein assay kit, PT1 500 028, Co., Pars Azmoon, Iran). Serum PLA2 and total protein were measured in control and sensitized animals and the ones exposed to low and high lead concentrations.

Measurement of serum IgE and histamine

IgE level was measured using the enzyme-linked immunosorbentassay (ELISA) sandwich method according to the manufacturer’s instructions (IgE Assay Kit, PT-IgE-96, Co., Pishtaze., Iran). Serum histamine level was also measured using the enzyme-linked immunosorbentassay (ELISA) sandwich method according to the manufacturer’s instructions (Histamine Assay Kit, BAE-1100, Co., LDN, England).

Statistical analyses

The data were expressed as mean ± SEM. Statistical analysis was performed by Instat statistical software. Kolmogorov–Smirnov tests showed that the data were normally distributed. The data of sensitized group were compared with control and guinea pigs using unpaired ‘t’ test. The data between different groups of animals exposed to lead acetate were compared using one way analysis of variance (ANOVA) with Tukey–Kramer post hoc test. Statistical significance was accepted at p < 0.05 level.

Results

Comparison of WBC count, serum total protein, PLA2, IgE and histamine levels between control and other groups

Total WBC number in blood, serum total protein, PLA2, IgE and histamine levels of non exposed sensitized and all sensitized and exposed groups to lead were significantly higher compared to those of control group (p < 0.05 to p < 0.001, Figures 1–3). Compared to the control group, the percentages of eosinophil, neutrophil and basophil were also increased in non exposed sensitized guinea pigs and all sensitized animals exposed to lead (p < 0.05 to p < 0.001, Figure 4). Monocyte percentage was also significantly increased in animals exposed to two higher lead concentrations after sensitization (p < 0.05 for both cases, Figure 4). However, the percentage of lymphocyte in all sensitized groups was decreased compared to control group (p < 0.001 for all cases, Figure 4).

Comparison of WBC count, serum total protein, PLA2, IgE and histamine between sensitized animals exposed to lead and non exposed sensitized group

Total WBC number and serum total protein in all animals exposed to lead were significantly higher compared to non-exposed sensitized group (p < 0.05 to p < 0.001, Figures 1, 3 and 4). In addition, the percentage of eosinophil and level of IgE and histamine in animals exposed to the highest lead concentration after sensitization was higher but percentage of lymphocyte in those exposed to two highest lead concentrations (both after and during sensitization) was lower than non exposed sensitized group (p < 0.05 to p < 0.01, Figures 2 and 4). However, there was not significant difference between serum PLA2 level of animals exposed to three lead concentrations and non exposed sensitized animals (Figure 2).

Comparison of WBC count, serum total protein, PLA2, IgE and histamine levels between animals exposed to lead during and after sensitization

Serum total protein, PLA2, IgE and histamine levels, total and differential WBC counts in animals exposed to lead after sensitization were higher compared to those exposed during sensitization but this differences were only statistically significant for total WBC number exposed to low lead concentration (p < 0.05, Tables 1 and 2).

Figure 1. Serum total protein, (a) and PLA2 (b) of control (C), sensitized (S), lead exposed during sensitization (DS + Pb) and post sensitized (PS + Pb) guinea pigs exposed to 0.1 and 0.4 M lead concentration, (for each group, n = 6). Values are presented as mean ± SEM. Statistical significance for the difference between the data of control versus other groups: ***; p < 0.001. Statistical significance for the difference between the data of sensitized versus lead exposed sensitized groups: +; p < 0.05, ++; p < 0.01, +++; p < 0.001.
Comparison of WBC count, serum total protein and PLA2 levels between animals exposed to different lead concentration

Serum total protein, histamine, total WBC number and the percentage of eosinophil in animals exposed to high lead concentration (0.4 M) both during and after sensitization were significantly higher compared to animals exposed to low (0.1 M) concentration of lead. Values are presented as mean ± SEM. Statistical significance for the difference between the data of control versus other groups: ***; p < 0.001. Statistical significance for the difference between the data of sensitized versus lead exposed groups: ++; p < 0.01, +++; p < 0.001.

Discussion

The results of the present study showed significant increase in serum total protein, PLA2, IgE and histamine levels, total WBC number and percentage of eosinophil in animals exposed to high lead concentration compared to animals exposed to low concentration. However, the results of the present study do not show the type of lymphocytes which should be investigated in further studies.

The increased serum total protein, PLA2, IgE and histamine levels, total WBC number and eosinophil count are well known changes in asthma disease (Chang et al., 1987; Hogan et al., 2003; Kelly et al., 1998; Luksza & Jones, 1982). Our previous studies also showed increased total WBC and eosinophil but decreased lymphocyte count in lung lavages of sensitized guinea pigs (Keyhanmanesh et al., 2009; Neamati et al., 2010). The results of the present study also showed increased serum total protein, PLA2, IgE and histamine levels, total WBC and eosinophil count but decreased...
lymphocyte count in sensitized animals which confirm sensitization (induction of an animal asthma model) of guinea pigs. In addition, further increase of serum total protein and total WBC in sensitized animals exposed to all lead concentrations as well as the levels of IgE and histamine and eosinophil count in animals exposed to high lead concentration compared to non exposed sensitized group showed that lead exposure may cause enhanced inflammation in sensitized animals. These results suggest that exposure of asthmatic patients to environmental lead pollution could increase lung inflammation and asthma severity. The results of the present study are supported by previous studies indicating that exposure of asthmatic patients to environmental lead pollution in industrial areas may increase severity of asthma (Ho et al., 1998; Stata & Araki, 1997). This conclusion is also supported by the results of a previous study showing higher prevalence of respiratory symptoms, sputum production, shortness of breath and diagnosed asthma in industrial workers exposed to lead (Bener et al., 2001). The increased incidence of asthma in workers exposed to lead and children who live in places with high lead pollution is documented which supports the results of the present study (Ho et al., 1998; Stata & Araki, 1997). The results of our recent study also showed increased respiratory symptoms and decreased pulmonary function tests in workers exposed to lead in workplace (Khazdair et al., 2012).

There are reports regarding increase in the release of inflammatory mediators from the cells and macrophages exposed to lead in a cell culture model (Chen et al., 1997; Heo et al., 1996). These observations also support increased serum PLA2 seen in the present study.

Higher values of total protein, total WBC and eosinophil counts in sensitized animals exposed to high lead concentration both during and after sensitization as well as the percentage of basophil in sensitized animals exposed to high lead concentration after sensitization showed a concentration dependent effect of inhaled lead exposure which are further evidences of the effect of environmental lead pollution on respiratory status.

The results of this study also showed that all measured parameters were higher in sensitized animals after sensitization compared to those exposed to lead during sensitization. These findings may indicate that the effect of environmental lead pollution in increasing asthma severity is more prominent in patients with manifested asthma than those during development of the disease.

Regarding the mechanism of the findings of the present study, it is well known that the early allergic responses are mediated primarily by IgE-dependent processes (Holgate et al., 1993). This antibody binds to high-affinity receptors (FcγRI) located on the surfaces of mast cells and basophils and is subsequently cross-linked by allergen, causing cell activation with the release of inflammatory mediators (Metzger, 1992) such as histamine, and chemotactic factors.

Table 1. PLA2 (pg/ml), total protein (g/100 ml), histamine (ng/ml), and IgE (ng/ml) in serum of control (C), sensitized (S) lead exposed during (DS + Pb) and post sensitization (PS + Pb) groups, (for each group, n = 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total protein (g/100 ml)</th>
<th>PLA2 (pg/ml)</th>
<th>Histamine (ng/ml)</th>
<th>IgE (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>5.35 ± 0.25</td>
<td>7.49 ± 0.54</td>
<td>5.74 ± 1.12</td>
<td>29.95 ± 2.77</td>
</tr>
<tr>
<td>S</td>
<td>9.11 ± 0.33</td>
<td>12.11 ± 1.30</td>
<td>35.99 ± 1.42</td>
<td>109.22 ± 3.22</td>
</tr>
<tr>
<td>DS + 0.1 M Pb</td>
<td>11.07 ± 0.66</td>
<td>11.43 ± 1.10</td>
<td>40.64 ± 1.98</td>
<td>118.35 ± 3.40</td>
</tr>
<tr>
<td>DS + 0.4 M Pb</td>
<td>12.67 ± 0.24*</td>
<td>11.33 ± 1.77</td>
<td>49.97 ± 2.11*</td>
<td>125.72 ± 2.09</td>
</tr>
<tr>
<td>PS + 0.1 M Pb</td>
<td>11.45 ± 0.52</td>
<td>13.58 ± 1.77</td>
<td>38.22 ± 1.54</td>
<td>116 ± 1.33</td>
</tr>
<tr>
<td>PS + 0.4 M Pb</td>
<td>13.07 ± 0.22*</td>
<td>12.94 ± 1.22</td>
<td>50.11 ± 1.22*</td>
<td>136 ± 1.67*</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. Statistical significance for the difference between the data of two lead concentrations: *; p < 0.05.
Histamine regulates granulocyte accumulation in tissues in distinct ways which can lead to eosinophil migration to airways. Allergen-induced accumulation of eosinophils in the skin, nose and airways is potently inhibited by H1-antihistamines (Buckland et al., 2003). Eosinophilic inflammation of the airways is the key feature of asthma (Humbert et al., 1999). Increased eosinophil is a well known change both in asthmatic patients and animal model of asthma (Boskabady et al., 2011). Significant correlation between the activation of eosinophils and the severity of asthma was reported (Humbert 1996). Eosinophils are a rich source of cytotoxic proteins, lipid mediators, oxygen free radicals and cytokines (Ricci et al., 1997). The secretory PLA2s (sPLA2), secreted by activated leukocytes (Yedgar et al., 2000), act as receptor ligands to induce cytokine production (TNFα, IL-6, IL-8), mast cell survival, CD44 expression by eosinophils, and airway smooth cell proliferation (Triggiani et al., 2003). Therefore, PLA2s are involved in inducing inflammatory processes (Yedgar et al., 2000). Therefore the results of the present study suggest that lead exposure can lead to increased serum IgE level as well as augmented serum total protein. Increased IgE can lead to increased histamine and chemotactic factors. This can induce eosinophilic inflammation of the airways and other inflammatory processes. Increased PLA2 seen in the present study can also enhance lung inflammatory processes.

In conclusion, the results of the present study showed that inhaled lead acetate can cause further increase in serum levels of total protein, PLA2, IgE, histamine, total WBC and the percentage of eosinophil and basophil but reduction in the percentage of lymphocyte in blood of sensitized animals. The findings of the present study suggest that exposure to inhaled lead can increase severity of asthma both during development or after manifestation of the disease which is more pronounced in patients with manifested asthma.

Acknowledgements
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Table 2. Total and differential WBC count in blood of control (C), sensitized (S), lead exposed during (DS + Pb) and post sensitization (PS + Pb) groups, (for each group, n = 6).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>C</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 M</td>
<td></td>
<td>0.2 M</td>
</tr>
<tr>
<td>Total WBC</td>
<td>DS + Pb</td>
<td>1370 ± 402</td>
<td>3565 ± 509</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophil</td>
<td>DS + Pb</td>
<td>8.55 ± 0.42</td>
<td>23.66 ± 1.50</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>DS + Pb</td>
<td>14.50 ± 1.33</td>
<td>21.83 ± 2.77</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>DS + Pb</td>
<td>62.07 ± 1.97</td>
<td>26.91 ± 2.50</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophil</td>
<td>DS + Pb</td>
<td>3.88 ± 1.01</td>
<td>12.80 ± 1.53</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monocyte</td>
<td>DS + Pb</td>
<td>11.00 ± 1.77</td>
<td>14.80 ± 1.44</td>
</tr>
<tr>
<td></td>
<td>PS + Pb</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as mean ± SEM. The data of WBC is their count in one ml of blood and those of each type is the percentage of total WBC. Statistical significance for the difference between the data of 0.1 M versus 0.2 M and 0.4 M Pb groups: *; p < 0.05, **; p < 0.01, ***; p < 0.001. Statistical significance for the difference between the data of DS + Pb versus PS + Pb groups: ×; p < 0.05.

Declaration of interest
The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

References

Notice of Correction
The Early Online version of this article published online ahead of print on 12 December 2013 contained an error on page 1. The author “Morteza Boskabady” should be removed. The corrected version is shown in this issue.