

The Effect of Lead Exposure on Selected Blood Inflammatory Biomarkers in Guinea pigs

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Abstract: This study was aimed to examine the effect of lead acetate on inflammatory biomarkers in blood of guinea pigs in comparison with sensitized animals. Thirty guinea pigs were randomly allocated into control (group C), sensitized (group S), and three Pb-exposed (groups 0.1M Pb, 0.2M Pb and 0.4M Pb). Animal sensitized after aerosolized-ovalbumin (OA) challenge. Pb-exposed groups inhaled 0.1M, 0.2M and 0.4M lead acetate for 1 h, three times a week for two weeks. Total and differential WBC counts, PLA₂ activity and total protein levels were evaluated in blood of all animal groups. Serum PLA₂ activity, total protein and total WBC number in sensitized and animal exposed to lead were significantly higher as compared to control group ($p < 0.05$). When compared to control group, the percentages of eosinophil, neutrophil and basophiles were also increased in sensitized guinea pigs ($p < 0.01$). The percentage of eosinophil and basophile in animals exposed to high level of lead and that of basophile in animals exposed to middle lead concentration versus to control group significantly increased ($p < 0.05$). In addition, the significant difference between the lymphocyte percentages of Pb-exposed groups and control group were decreased in concentration dependent manner. The findings of the present study indicated that exposure to inhaled lead acetate may lead to asthma-like disease.

Keywords: Lead exposure, inflammatory biomarkers, guinea pig.

INTRODUCTION

One of the industrial environmental heavy metal pollution is lead [1]. However, the definite effect of lead on respiratory system is not well known [2]. There are evidences regarding the contribution of lead pollution in pathogenesis of pulmonary cancers, asthma, chronic obstructive pulmonary disease (COPD), but these effects are controversial [3, 4]. The increased incidence of asthma in workers exposed to lead and children living in high levels of lead polluted areas [5, 6] suggest that lead may play a role in causing asthma. There are reports regarding increase immunoglobulin E (IgE) and some inflammatory cytokines in serum of laboratory models and children exposed to lead and increased inflammatory mediator from the cells and macrophages exposed to lead in a cell culture model but some studies indicated no change or even decrease of serum immunoglobulins in laboratory models exposed to lead [7-11]. According to previous studies, phospholipase A₂ (PLA₂) catalyses the release of arachidonic acid from cell membrane phospholipids to generate lipid mediators of inflammation

and is crucial in diverse inflammatory processes. In addition, the contribution of the excess levels of total protein and total WBC especially eosinophil number in inflammatory disorders such as asthma have been documented [12-15]. Therefore, the present study has been designed to examine the effect of lead exposure on total and differential WBC blood count and as well as the PLA₂ activity and level of total protein in the serum of guinea pigs exposed to lead. This study is needed for evaluation the role of this metal in incidence and severity of asthma and other respiratory diseases.

MATERIALS AND METHODS

Animals Subjects

Thirty adult Dunkin-Hartley guinea pigs (400-700 g, both sexes) were used throughout this study. They were allowed to accommodate new situation for ten days. The animals were group-housed in individual cages (50 × 90 × 30 cm³) 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 5 groups as mentioned in Table 1 (n=6 for each group):

Sensitization of Animals

Sensitization of animals to OA was performed using the methods described previously [16, 17]. Briefly, guinea pigs

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were sensitized to ovalbumin (OA;Sigma Chemical Ltd, UK) by intraperitoneally (i.p.) injecting 10 mg OA and 100 mg AL(OH)₃ in saline on day one and a further i.p. injection of 2 mg OA and 100 mg AL(OH)₃ in saline 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, dimensions 30 x 20 x 20 cm³ using a nebulizer (CX3, Omron Healthcare Europe B.V., Netherlands). Control group was treated similarly but saline solution (%) was used instead of OA solution. The study was approved by the ethical committee of the Mashhad University of Medical Sciences.

Table 1. Animals and Experimental Design

Groups	Experimental Design
Control (C)	Non-sensitized and non-exposed guinea pigs to lead (Pb)
Sensitized (S)	sensitized guinea pigs to OA
0.1 M Pb	exposed guinea pigs to 0.1 M lead concentration
0.2 M Pb	exposed guinea pigs to 0.2 M lead concentration
0.4 M Pb	exposed guinea pigs to 0.4 M lead concentration

Exposure of Animals to Inhaled Lead

Three groups of studied animal were exposed to lead acetate (Sigma Chemical Co., St. Louis, MO, USA). The exposure protocol to lead was performed according to method describe by Fortoul and colleagues [18]. Briefly, animals were placed in a closed chamber (30 x 20 x 20 cm³) connected to an ultra-nebulizer (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. The study was approved by the ethical committee of the Mashhad University of Medical Sciences.

White Blood Cells Count

Blood samples were taken by cardiac puncture immediately after anesthesia and exposing the animals' chest and were collected into test tube containing anticoagulant EDTA. Blood sample was stained with Turk solution (1:10 dilution) and total white blood cell (WBC) counted in duplicate in a hemocytometer (in a Burker chamber). The Turk solution consisted of 1 ml of glacial acetic acid, 1 ml of gentian violet solution 1 % and 100ml 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 Activity 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 serum 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).

Statistical Analysis

The data are expressed as mean± SEM. Statistical analysis was performed by InStat statistical software. The data of sensitized group were compared with control guinea pigs using unpaired "t" test. The data of exposed animals to lead were also compared with control and sensitized group 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. Significance was accepted at p<0.05 level. Kolmogorov Smirnov test showed that these data were normally distributed.

RESULTS

Serum total protein level and total WBC number in sensitized and Pb-exposed groups were significantly higher compared to control group (p<0.05), Fig. (1, 2a). When compared to control group, the percentages of eosinophil, neutrophil and basophil and serum PLA₂ activity were also increased in sensitized guinea pigs. In addition, the percentages of eosinophil in animals exposed to two higher

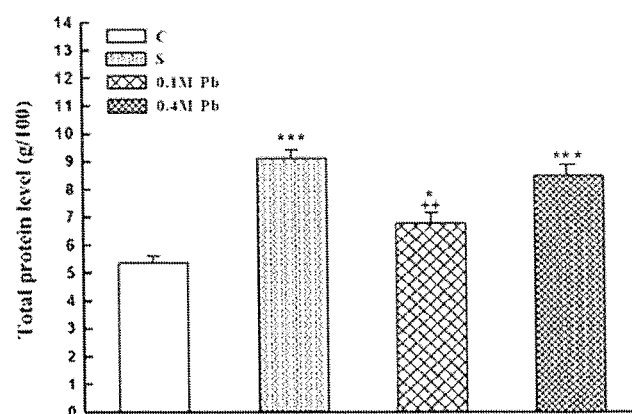


Fig. (1). Level of total protein (g/100) in serum of control (C), sensitized (S), and Pb-exposed (Pb) guinea pigs to 0.1 and 0.4 M lead concentration, (for each group, n=6). Values are presented as mean±SEM. Statistical significance for the difference between the data of control vs other groups: *, P<0.05, ***, P<0.001. Statistical significance for the difference between the data of sensitized vs treated groups: ++; p<0.01.

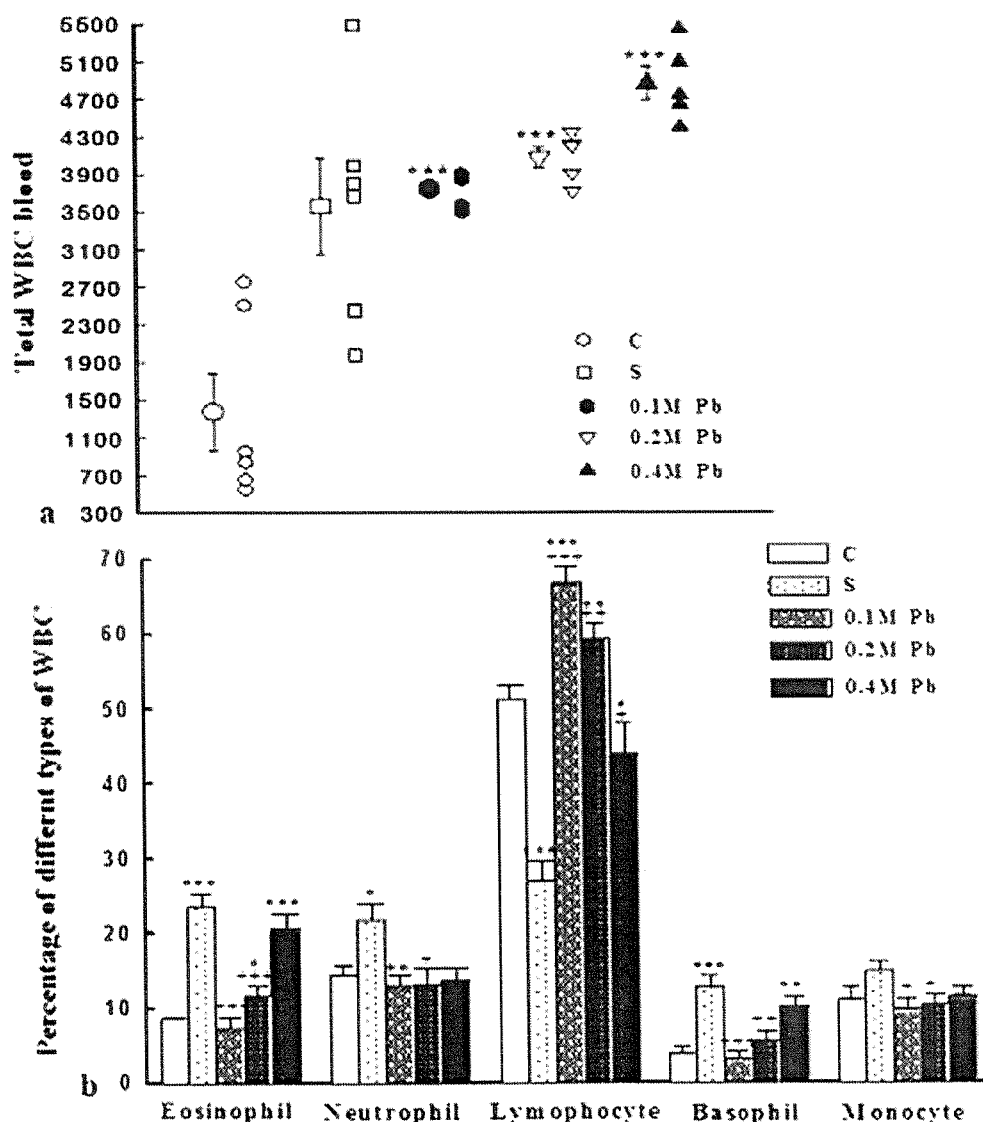


Fig. (2). Total and differential count of WBC in blood of control (C), sensitized (S), and Pb-exposed (Pb) guinea pigs to 0.1, 0.2 and 0.4 M lead concentration, (for each group, n=6). Values are presented as mean±SEM. Statistical significance for the difference between the data of control vs other groups: *, $P<0.05$, **, $P<0.01$, ***, $P<0.001$. Statistical significance for the difference between the data of sensitized vs treated groups: +, $p<0.05$, ++, $p<0.01$, +++, $p<0.001$.

concentrations of lead and that of basophil and serum PLA₂ activity in animals exposed to high lead concentration were also increased ($p<0.01$), Fig. (2b, 3). However, the percentage of lymphocyte in sensitized group and animals exposed to two high lead concentration was decreased compared to control group $p<0.001$ for sensitized and $p<0.05$ for Pb-exposed group, Fig. (2b).

There was not difference in total WBC number of animals exposed to three lead concentrations compared to sensitized group Fig. (2a). In addition, statistical difference was not observed in serum total protein and the percentage of eosinophil, neutrophil, basophil and monocyte and serum PLA₂ activity between animals exposed to high lead concentration (0.4 M) and group S Fig. (1, 2b, 3). However,

the percentage of lymphocyte in animals exposed to all lead concentrations was significantly higher than sensitized group ($p<0.05$), Fig. (2b).

Serum total protein level, PLA₂ activity and total WBC number in animals exposed to high lead concentration (0.4 M) were significantly higher compared to animals exposed to low concentration (0.1 M), ($p<0.05$, Tables 2 and 3). The percentage of eosinophil, basophil as well as reduction in the percentage of lymphocyte were also higher in animals exposed to high lead concentration (0.4 M) compared to animals exposed to low concentration (0.1 M) ($p<0.05$, Table 3). In addition, total WBC number, percentage of eosinophil and basophil in animals exposed to high lead

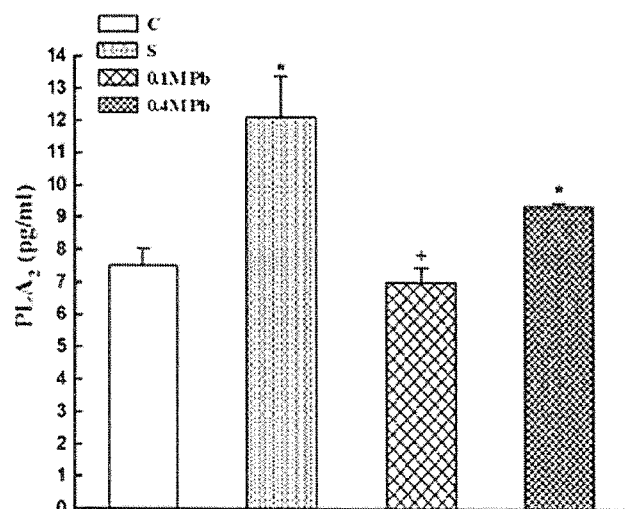


Fig. (3). PLA₂ activity (pg/mL) in serum of control (C), sensitized (S), and Pb-exposed (Pb) guinea pigs to 0.1 and 0.4 M lead concentration, (for each group, n=6). Values are presented as mean±SEM. Statistical significance for the difference between the data of control vs other groups: *, P<0.05. Statistical significance for the difference between the data of sensitized vs treated: +; P<0.05.

were significantly different with animals exposed to middle lead concentration (0.2M), (p<0.05, Table 3).

Table 2. PLA₂ Activity (pg/ml) and Total Protein (g/100) in Serum of Control (C), Sensitized (S) and Pb-Exposed (Pb) Guinea pigs, (for Each Group, n=6)

Parameters	C	S	0.1M Pb	0.4M Pb
PLA ₂ (pg/ml)	7.49±0.54	12.10±1.30	7.03±0.43	9.37±0.11 *
Total Protein (g/100)	5.35±0.25	9.10±0.32	6.77±0.34	8.47±0.38 *

Values are presented as mean±SEM.

Statistical significance for the difference between the data of two Pb concentrations: *, P<0.05

Table 3. Total and Differential WBC Count in Blood of Control (C), Sensitized (S), and Pb-exposed (Pb) Guinea pigs (for Each Group, n=6)

Parameters	C	S	0.1M	0.2M	0.4M
Eosinophil (%)	8.55±0.42	23.66±1.50	7.33±1.54	11.66±1.22	20.66±1.94 ***, ++
Neutrophil (%)	14.51±1.33	21.83±2.71	13.00±1.46	13.16±2.16	13.83±1.44
Lymphocyte (%)	51.20±1.97	26.91±2.55	66.91±2.18	59.30±2.11	43.95±4.10 *
Basophil (%)	3.88±1.01	12.88±1.53	3.16±0.99	5.51±1.33	10.16±1.24 **, +
Monocyte (%)	11.00±1.77	14.81±1.40	9.61±1.77	10.30±1.38	11.44±1.35
Total WBC	1370±402	3563±509	3739±85	4082±111	4780±183 ***, +

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 0.1M vs 0.2 and 0.4M Pb groups: *, P<0.05, **, P<0.01, ***, P<0.001.

Statistical significance for the difference between the data of 0.2M vs 0.4M Pb groups: +, P<0.05, ++, P<0.01.

DISCUSSION

The results of the present study showed significant increase in serum total protein and PLA₂ levels, total WBC number and percentage of eosinophil, and basophil but significant decrease in percentage of lymphocyte in lead exposed guinea pigs as compared to control animals. The similar findings were also observed in sensitized animals.

The increased serum total protein level and PLA₂ activity [19], total WBC number and eosinophil count are well known changes in asthma disease [12, 13]. Previous studies have been shown to increase in total WBC count and eosinophil percentage with decrease of lymphocyte percentage in bronchoalveolar lavage fluid of sensitized guinea pigs [20-22]. The results of the present study also showed increased serum total protein level, PLA₂ activity, total WBC count; eosinophil count and decreased lymphocyte count in sensitized which confirm sensitization (induction of an animal asthma model) of guinea pigs. Increased serum total protein level, PLA₂ activity, total WBC and eosinophil count in Pb exposed animals indicates that inhaled lead exposure is able to induce asthma-like change in this group [12-14]. This conclusion is supported by the results of a previous study showing higher prevalence of respiratory symptoms for phlegm, shortness of breath and diagnosed asthma in industrial workers exposed to lead [4,

23]. The increased incidence of asthma in workers exposed to lead and children that live in high lead polluted places is documented which supports the results of the present study [5, 6].

There are reports regarding increase in the release of inflammatory mediator from the cells and macrophages exposed to lead in a cell culture model [7-11]. These observations also support increased serum PLA₂ activity seen in the present study [15]. Airway hyper-responsiveness (AHR) is associated with lipid inflammatory mediator, arachidonic acid (AA) metabolites, including thromboxane A₂ (TXA₂) and leukotriene B₄ (LTB₄) after exposure to allergen. It is concluded that PLA₂ plays an important role in bronchoconstriction by releasing thromboxane A₂ (TXA₂) and leukotriene B₄ (LTB₄) from the membrane phospholipids of various activated inflammatory cells, such as neutrophils and alveolar macrophages, and tracheal epithelial cells [24].

The greater values of total protein, PLA₂ activity, total WBC and eosinophil count in animals exposed to high concentrations of lead showed a concentration response effect of inhaled lead exposure. These results are further evidence of concentration dependent effect of environmental lead pollution on respiratory status.

In conclusion, the results of the present study showed that lead acetate can cause increased serum total protein level, PLA₂ activity, total WBC percentage of eosinophil and basophil but reduction in percentage of lymphocyte. Therefore, the findings of the present study indicate that exposure to inhaled lead may lead to asthma like disease.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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