Effect of Aerobic Training and Diferuloyl Methane Supplement on the Hematotoxicity and Immunotoxicity

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Abstract: Environmental exposure to lead (pb) can result in hematotoxicity and immunotoxicity. However, individual and concomitant effects of regular aerobic training and diferuloyl methane (DM) antioxidant supplement on the hematological and immunological markers, particularly during chronic exposure to Pb, are not known. The purpose of the present study was to evaluate the effects of exercise training with and without DM supplement on hematological (red blood cell, hemoglobin, iron and ferritin) and immunological (IgA, IgG, IgM) markers during lead acetate-induced toxicity. For this purpose, 40 rats were randomly divided into five groups: (1) lead acetate, (2) DM, (3) aerobic training, (4) training + DM group, (5) sham group. The rats in the training groups experienced the treadmill running of 15 to 22 m/min for 25 to 64 minutes, 5 times a week for 8 weeks. Groups 1 to 4 received Pb (20 mg/kg), the sham group received ethyl oleate solvent and the DM and training + DM groups received DM solution (30 mg/kg). The results showed that lead administration resulted in significant increase in thiobarbituric acid and white blood cell (WBC) and significantly decreased total antioxidant capacity (TAC), immunoglobulin and hematological markers as compared to sham group. In contrast, aerobic training and/or DM supplement resulted in a reverse effect in the aforesaid markers. However, the combined strategy was more effective than DM supplement and aerobic training alone. These results suggested that chronic exposure to lead acetate induced hematoxocity and immunotoxicity. However, the combined strategy (aerobic training and diferuloyl methane) was more effective in ameliorating lead-induced hematotoxicity and immunotoxicity.

Key words: Antioxidant %Aerobic Training %Pollution %Hematological %Immunological

INTRODUCTION

Ambient air pollutants could have a significant effect on quality of life. The American Heart Association scientific statement reported that both short-and long-term exposure to the particulate component (particulate matter, PM) is associated with increased mortality and cardiovascular disease [1,2]. Lead acetate (pb) is a ubiquitous environmental toxin that is capable of causing numerous acute and chronic circulatory, neurological, hematological, gastrointestinal, reproductive and immunological pathologies [3,4,5]. A number of studies have documented the potential toxic effects of lead on the immune system [3]. Furthermore, lead is known to affect both humoral and cellular immune responses [6]. Also, it can suppress immune functions resulting in increased incidence and severity of infectious diseases [3]. On the other hand, exposure to arsenic, cadmium, copper, lead, mercury, tin or zinc has been shown to produce some effects on the hematological system [7]. Shah et al. (2010) [8] reported that the lead levels were significantly associated with biochemical indices in the blood which have the potential to be used as biomarkers of lead intoxication and Fe deficient anemia.

To prevent the effect of lead on the hematological and immune systems appears to be one of the major therapeutic goals. Researchers reported that lead exposed rats showed the generation of reactive oxygen species,
stimulation of lipid peroxidation and decreased antioxidant defense system, supporting the role of oxidative stress in lead toxicity [9]. Antioxidants provide protection for living organisms against damage caused by the uncontrolled production of ROS [10,11]. Consequently, the need to identify alternative natural and safe sources of food antioxidants arose and the search for natural antioxidants, especially of plant origin, has notably increased in recent years. Diferuloyl methane (DM) known as Curcumin \{1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione\} is the principal coloring agent present in the rhizomes of Curcuma longa (zingiberaceae) [12]. It also acts as a scavenger of oxygen free radicals [13]. Furthermore, DM induces protective effects in cardiovascular system [13]. On the other hand, there has been a great deal of attention paid to the role of lipid peroxidation and antioxidant effects of physical training in recent years. Dabidi Roshan \textit{et al.} (2011) reported that regular physical training increased the antioxidant system and reduced lipid peroxidation [14,15].

Despite the knowledge that lead can induce oxidative stress [3,9,12], there are few data available with respect to single and concomitant effects of regular aerobic training and DM antioxidant supplement on the hematological and immunological markers, particularly during chronic exposure to Pb. Hence, the aim of the current study was to examine the effects of aerobic treadmill running, herbal supplementation of diferuloyl methane or both on serum immunoglobulin levels (IgG, IgM, IgA) and hematological levels (hemoglobin, red blood cells, white blood cells, iron, ferritin) in rats that have been chronically exposed to lead acetate. In addition, the relationship between exposure to Pb and imbalance of the oxidant/antioxidant process, levels of thiobarbituric acid as marker of lipid peroxidation and levels of total antioxidant capacity (TAC) as marker of antioxidant were also assessed after aerobic training with and without herbal supplementation of DM.

\textbf{MATERIALS AND METHODS}

40 adult male Wistar rats (initial body weight 240 ± 20 g) were obtained from the Laboratory of Animal Bearing and Multiplying at Pasture Institute of Iran. Each rat was housed in single standard cages of polycarbonate (20 × 15 × 15) made at Pasture Institute of Iran in a large air-conditioned room with controlled temperature of 22 ± 2°C, light-dark cycles of 12:12 hours and humidity of 50% ±5%. Rats were fed with a standard rat chow provided by Pars Institute for animals and poultry with a daily regimen of 10 g per 100 g of body weight for each rat. Water was available ad libitum.

The experimental protocol was approved by Department of Physiology, University of Mazandaran and was performed according to guiding procedures in the care and use of animals prepared by the Council of the American Physiological Society. Our study replicated a previously-reported lead-dosing regimen that caused oxidative stress so that the doses of diferuloyl methane (DM) and Pb were 30 and 20 mg/kg respectively [12,14,15]. Rats were familiarized with the laboratory environment and running on the treadmill, then were randomly assigned to 5 experimental groups of eight rats each. The groups were defined as follows:

\textbf{Group 1}: these animals were exposed to Pb at a concentration of 20 mg/kg in the form of a water solution (for intraperitoneal [ip] injection) three days weekly for eight weeks;

\textbf{Group 2}: diferuloyl methane (DM)-this group similarly received Pb as well as DM 30 mg/kg five days weekly for eight weeks (ip);

\textbf{Group 3}: aerobic training (Pb+ training)-these rats similarly received lead acetate; in addition, they performed progressive running exercise of 15 to 22 m/min for 25 to 64 minutes, five times a week for eight weeks;

\textbf{Group 4}: aerobic training and DM (training + DM + lead acetate)-the rats in this group performed a training protocol of running on the treadmill similar to that of group 3; in addition, they received Pb and DM supplementation;

\textbf{Group 5}: the sham-operate or control group (sham)-these rats received ethyl oleate in the same manner and for the same duration of time as other groups.

Pb (Sigma) was solubilized in Milli-Q water and Diferuloyl Methane (DM) was solubilized in 50% ethanol. In order to perform intraperitoneal injections, DM was solubilized in ethyl oleate and was injected at a dose of 30 mg/kg. DM was protected from light throughout the experiment [12,14,15]. All groups were anesthetized with ketamine and xylazine and decapitated after 10 to 12 hours of overnight fasting. Blood samples were collected 24 hours after the last dose of treatment. These blood
samples were initially centrifuged by a refrigerated centrifuge at 3000 rpm for 15 minutes within 30 minutes of collection and then stored at-80ºC for subsequent assay of immunoglobulin levels (IgG, IgM, IgA) and hematological levels (hemoglobin, red blood cells, white blood cells, platelet, iron). All samples were analyzed in duplicate. All samples were analyzed in the same assay to avoid inter-assay variations.

Serum IgA, IgG and IgM levels were estimated by a nephelometer as previously described by Ilsley et al. (2005) [16] and Mishra et al. (2006) [6]. A Sysmex SF-3000 automated hematology analyzer was used for whole-blood analysis of hematological parameters. The instrument automatically counts and gives a printout result of absolute numbers of leukocytes (WBC), erythrocytes (RBC), hemoglobin (g/dL), iron (µg/dL) and Ferritin (µg/d) [17,18].

Serum total antioxidant capacity (TAC) was measured using a commercially available kit (Randox Laboratories, Crumlin, UK) as previously described by Dabidi Roshan et al. (2011) and Asali et al. (2011) [14,15]. In this method, the most potent radical, hydroxyl radical, is produced. First, a ferrous ion solution is mixed with hydrogen peroxide. The sequentially produced radicals such as the brown colored dianisidinyl radical cations produced by the hydroxyl radical are potent radicals. The antioxidant effect of the sample against the potent free radical reactions is then measured. The assay has excellent precision which has been shown to be lower than 3%. The results are expressed in mmol/mL. Thiobarbituric acid levels were measured with a thiobarbituric acid reaction by the method of Ohkawa et al. (1979) [19]. The quantification of thiobarbituric acid reactive substances was determined at 532 nm by comparing the absorption to a standard curve of malondialdehyde equivalents generated by acid catalyzed hydrolysis of 1, 1, 3, 3 tetramethoxypropane. The values of thiobarbituric acid were expressed as nmol/ml. In accordance with the protocols of Daniel et al. (2004), Dabidi Roshan et al. (2011) and Asali et al. (2011), the pb concentration was analyzed using a spectrophotometer method only in the Pb group [12,14,15].

Statistical analysis was performed using a commercial software package (SPSS version 16.0 for Windows). Results are expressed as mean ±SD. Data for biomarkers related to immunological and hematological systems were normally distributed after log-transformation. A two-way analysis of variance (ANOVA) was used to detect statistical differences of diferuloyl methane supplement and exercise training in rats chronically exposed to lead acetate. A post-hoc test (Tukey test) was performed to determine differences in the various markers among groups. The differences were considered significant at P<0.05.

RESULTS

Mean values of the immunological markers (IgA, IgG and IgM) in the various groups are shown in Table 1. Intra-peritoneal administration of lead acetate (20 mg/kg) in lead acetate (Pb) group decreased the concentration of IgA, IgG and IgM levels by 52.4%, 8.6% and 39.6% respectively as compared to the sham group (all significantly increased, p<0.05). In contrast, the trainingþþlead and/or D þþtrainingþþlead treatment for 8 weeks significantly increased IgG (28.6% and 17.8% respectively) in comparison with the sham group (both p<0.5). However, the combination of DM+trainingþþlead treatment had a far better effect on immunological markers rather than using each of them separately (Fig. 1). There was no significant difference in any of the aforesaid markers between the trainingþþlead group and DM+trainingþþlead group.

Changes in the hematological parameters in the rats exposed to Pb are shown in Table 2. The administration of Pb for 8 weeks significantly increased white blood cell (WBC) (50.7%) and significantly decreased levels of red blood cell (RBC) (32.2%), hemoglobin (Hb) (34.2%), Fe (46%) and ferritin (84%) as compared to sham group.

Although changes in the hematological markers were in whole compensated only following the combined strategy (DM+trainingþþlead treatment), there was no significant difference in any of the hematological markers between the trainingþþlead group and DM+trainingþþlead group (Fig. 2).

Table 1: Levels of serum immunoglobulins (IgA, IgG and IgM) in rats exposed to lead acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>IgA(mg/L)</th>
<th>IgG(mg/L)</th>
<th>IgM (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>0.32 ± 0.19</td>
<td>4.41 ± 2.9</td>
<td>1.51 ± 0.9</td>
</tr>
<tr>
<td>Pb</td>
<td>0.21 ± 0.16</td>
<td>4.06 ± 2.55</td>
<td>1.01 ± 0.89</td>
</tr>
<tr>
<td>Trainingþþlead</td>
<td>0.42 ± 0.14</td>
<td>6.18 ± 0.12</td>
<td>2.16 ± 0.82</td>
</tr>
<tr>
<td>DM+þleadd</td>
<td>0.34 ± 0.22</td>
<td>5.57 ± 1.59</td>
<td>2.00 ± 1.14</td>
</tr>
<tr>
<td>Dmþ+trainingþþlead</td>
<td>0.47 ± 0.15</td>
<td>6.30 ± 0.52</td>
<td>2.46 ± 0.74</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD for 8 rats; Abbreviation: lead acetate (pb), Diferuloyl methane (DM), immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA).
Table 2: Levels of hematological markers in rats exposed lead acetate

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (mm³)</th>
<th>WBC (mm³)</th>
<th>Ferritin (µg/d)</th>
<th>Hb (g/dL)</th>
<th>Fe (µg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>6.28 ± 0.3</td>
<td>2.86 ± 0.7</td>
<td>47.43 ± 15</td>
<td>12 ± 0.66</td>
<td>47.43 ± 14</td>
</tr>
<tr>
<td>pb</td>
<td>4.75 ± 0.6</td>
<td>5.80 ± 1.7</td>
<td>25.7 ± 9.3</td>
<td>8.94 ± 1.12</td>
<td>25.2 ± 9</td>
</tr>
<tr>
<td>Training+ lead</td>
<td>6.01 ± 0.8</td>
<td>3.97 ± 1.6</td>
<td>55.14 ± 8</td>
<td>9.16 ± 0.54</td>
<td>55 ± 8</td>
</tr>
<tr>
<td>DM + pb lead</td>
<td>6.80 ± 0.7</td>
<td>4.53 ± 1.1</td>
<td>47.3 ± 8.8</td>
<td>8.86 ± 0.77</td>
<td>47 ± 8</td>
</tr>
<tr>
<td>DM + training+ lead</td>
<td>6.64 ± 0.5</td>
<td>4.77 ± 1.6</td>
<td>57 ± 4.1</td>
<td>8.67 ± 0.80</td>
<td>57 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± SD for 8 rats; Abbreviation: lead acetate (pb), Diferuloyl Methane (DM), red blood cells (RBC), white blood cells (WBC), Hb (hemoglobin), iron (Fe).

Fig. 1: Levels of serum immunoglobulins (IgA, IgG and IgM) in rats exposed to lead acetate. Data are presented as the mean ± SD for 8 rats; Abbreviation: immunoglobulin G (IgG), immunoglobulin M (IgM), immunoglobulin A (IgA), lead acetate (pb), Diferuloyl Methane (DM); ¥ significantly different than sham group; ‡ significantly different than lead group; _ significantly different than combined (diferuloyl methane + training+ lead) group; $ significantly different than Diferuloyl methane + lead group; Statistical significance p<0.05.

Fig. 2: Levels of hematological markers in rats exposed to lead acetate. Data are presented as the mean ± SD for 8 rats; Abbreviation: red blood cells (RBC), white blood cells (WBC), Hb (hemoglobin), iron (Fe), lead acetate (pb), Diferuloyl methane (DM); ¥ significantly different than sham group; ‡ significantly different than lead group; Statistical significance p<0.05.
Fig. 3: Levels of markers related to the oxidative stress in rats exposed to lead acetate. Data are presented as the mean ± SD for 8 rats; Abbreviation: total antioxidant capacity (TAC), lead acetate (pb), Diferuloyl methane (DM); ¥ significantly different than sham group; ‡ significantly different than lead group; _ significantly different than combined (diferuloyl methane þ+ trainingþ+ lead) group; Statistical significance p<0.05.

Fig. 3 shows significant changes in the markers related to oxidant (thiobarbituric acid) and antioxidant (total antioxidant capacity (TAC) following the administration of Pb for 8 weeks. Administration of Pb for 8 weeks decreased TAC levels by 27%, but increased thiobarbituric acid levels by 71% when compared to the sham group (both P<0.01). The trainingþ+lead, DM+lead and/or DM +training +lead treatments significantly decreased thiobarbituric acid levels and significantly increased TAC levels (p<0.001, P < 0.01 respectively). However, DM +trainingþ+lead treatment was more effective than DM+lead and training+lead alone (p<0.001, P<0.001 respectively) (Fig. 3).

DISCUSSION AND CONCLUSION

The novel finding in the present study was that the administration of Pb for 8 weeks decreased levels of red blood cell (RBC), hemoglobin (Hb), Fe and ferritin as compared to sham group which suggests the evidence of lead acetate-induced anemia in rats. In contrast, aerobic training and/or diferuloyl methane supplement resulted in a reverse effect in the aforesaid markers. However, the combined strategy was more effective than diferuloyl methane supplement and aerobic training alone. Other finding in the present study was that levels of the immunological markers and white blood cell (WBC) were elevated in the adult male rats by imbalance in biomarkers related to oxidative stress (thiobarbituric acid and TAC) during exposure to Pb as compared to sham group. This may reflect an important role of air pollution in inducing immunotoxicity and hematotoxicity. Researchers reported that lead is an environmentally persistent toxin that causes immunological, hematological, circulatory, gastrointestinal, reproductive and neurological pathologies [14]. Recent studies suggest that one of the mechanisms by which lead can exert some of its toxic effects is the disruption of the delicate pro-oxidant/antioxidant balance that exists within mammalian cells. In this current study, we observed that exposure to lead significantly decreased TAC (27%) and significantly increased thiobarbituric acid (71%) levels.

Researchers reported that variability of the blood system parameters depends on the level of toxic load [20]. One week following removal of lead from the drinking water, significant reductions in serum levels of IgA, IgM and IgG were detected. Levels of blood lead as low as 10-15 Ag/ml have been associated with reductions in cognitive and behavioral capabilities [20]. In the present study, we have found increased serum immunoglobulin levels particularly in IgG level in a group of lead-exposed rats, all of whom had blood lead levels more than the accepted safe limit of 10 mg/dL. Low exposure to lead has been reported to exert immune-stimulating effects, in contrast to higher exposure which usually leads to immunosuppression. Oxidative stress is an important physiologic modifier of immune and inflammatory mechanisms [3,6]. Lead potentially induces oxidative stress and evidence is accumulating to support the role of oxidative stress in the pathophysiology of lead toxicity. Lead is capable of inducing oxidative damage to brain, heart, kidneys and reproductive organs. Some evidences suggest that cellular damage mediated by oxidants may be involved in some of the pathogenesis associated with lead intoxication. The mechanisms for lead-induced immunotoxicity and hematotoxicity include the effects of lead on cell membranes, DNA and antioxidant defense systems of cells [21]. One of the hallmarks of lead-induced
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Researchers showed that the intense aerobic activity decreases the amount of immunoglobulins and sets the body exposed to injury, especially in the upper respiratory tract infection while the physical activity with average intensity causes the increasing of IgA amount and diminishes the danger of suffering from infection [29]. Changes in the immune functions due to acute exercise and training have been attributed to the increased secretion of cortisol, catecholamine and the neuropeptides. During exercise, when the max O2 consumption exceeds 60%, an increase in the epinephrine and cortisol concentrations occurs. Under any kinds of stress, vasopressin stimulates the release of corticotrophin-releasing factor, which in turn leads to the release of ACTH. Exercise increases the number of lymphocytes in the circulation by acting as a lymphocytic â2-adrenergic agonist. Cortisol on the other hand blocks the entry of lymphocytes which would otherwise lead to strong neutrophilia in the circulation, thereby facilitating the passage of lymphocytes from the lymphoid compartments [30]. In contrast, regular exercise has been reported to have several favorable effects on physiological, psychological and immunological functions and increase in the resistance to infections [30]. Regular and moderate exercise has been reported to improve the ability of the immune system to protect the host from infection. Resting levels of natural killer cells are enhanced as a result of training. Leucocyte number is clinically normal and remains unchanged with training [28]. So, the response of the immune system to exercise is varied, with different behaviors for each cell type and dependent on the intensity and duration of the exercise test and on the training of the subject [28].

Besides these factors, nutritional factors clearly play a key role in hematological and immunological deficiencies, but there has been little investigation of nutritional actors and their relationship to Pb toxicity. Diferuloyl methane (DM) known as curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione) extracted from the plant Curcuma longa is compounded of immunomodulatory properties. It has been ascribed a multitude of therapeutic activities and has been associated with suppression of inflammation, angiogenesis, tumorigenesis and diabetes and with therapeutic effects in diseases of the cardiovascular, pulmonary and neurological systems and skin and liver. Moreover, nutritional deficiencies alter immunocompetence and increase the risk of infection. Both heavy exercise and nutrition exert separate influences on immune function, appearing to be greater when acting synergistically [28]. Exercise training increases the body requirement for most nutrients. However, some athletes adopt an unbalanced dietary regimen predisposing them to immunosuppression. Several elements are known to exert modulatory effects on immune function including zinc, iron, selenium, calcium, copper and magnesium [28].

In general, most of these effects can be attributed to the antioxidant, anti-inflammatory and anti-cancer activities of DM. It is an effective scavenger of reactive oxygen species and reactive nitrogen species [31]. Ilsley et al. (2005) [16] reported that dietary DM at 40 mg/kg, fed for 5 weeks, enhanced plasma IgG concentrations in rats. Also, Churchill et al. (2000) [32] found that mice fed 1 g of DM/kg of feed had increased mucosal CD4+ T cells and B cells in the intestine, indicating DM can modulate lymphocyte mediated immune functions.

In conclusion, the present study demonstrated an important role of air pollution and Pb chronic exposure in inducing hematotoxicity and immunotoxicity. Moreover, the combined strategy (training + diferuloyl methane) was more effective than diferuloyl methane and training per se.

REFERENCES


