Immunopathological Alterations Induced by Lead Exposure in Rat

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ABSTRACT

The impact of lead toxicity on human health is currently an area of intense interest due to ubiquity of exposure. Its presence is considered unique in the sense that it is difficult to remove from the environment once it enters in it. Hence, studies related to immunotoxic effects of chronic lead exposure were carried out in adult male inbred Wistar rats following 250µ2g of lead acetate per kg body weight, given daily for 60 days in drinking water. Organ weight/body weight and histology (light microscopic and electron microscopic studies) were used as major parameters. Mesenteric lymph nodes of exposed rats showed a decrease in somatic tissue index by 13%. Atrophy of lymph nodes as revealed by marked reduction in the number and size of follicles with inconspicuous germinal centre was observed. In comparison to cortex, paracortical and medullary regions showed higher degree of damage, resulting into loss of demarcation between the cortex and medulla. Under electron microscope, condensation of material in the vicinity of nuclear membrane and degeneration of mitochondrial cristae with increased electron dense material in mitochondrial matrix in most of the lymphocytes were observed. Chronic lead exposure induced significant increase in somatic tissue index of spleen i.e. 32%. Diffused atrophy of the white pulp with marked reduction in the number of lymphocytes within periarteriolar lymphocyte sheath was apparent. There was an increase in extramedullary haemopoiesis and hyperplasia of B lymphocytes in the marginal zone and follicles in red pulp. The presence of haemosiderin, a pigmented material of yellow–brown cytoplasmic granules was observed in macrophages of red pulp. The number of megakaryocytes significantly increased throughout the tissue section was seen under light microscope. Hypertrophy of rough endoplasmic reticulum, vesiculation of smooth endoplasmic reticulum, vacuolation in the matrix of mitochondria and loss of mitochondrial cristae were the remarkable ultrastructural changes observed in the different cells of the spleen. Finding of the present study indicates that chronic lead exposure adversely affects normal architecture of the lymphoid organs (lymph node and spleen) in rats.

Key Words: Lead, toxicity, lymph node, spleen.

INTRODUCTION

Among the toxic substances that man concentrates in his immediate environment, lead is one of the most ubiquitous metals. Therefore, its biological effects upon animal organism are of obvious interest (Chisolm 1971, Purves 1985, Flora 2002). Environmental contamination by lead has greatly increased as more and more lead is used in various industrial products such as storage batteries, paints and pigments, power cables etc. and an imminent danger of contamination and pollution from these industries exists. Lead compounds are known to exert toxic effects on the renal, haemopoietic, embryonic and central nervous system (Yamada et al 1984, Zaidi et al 1994, McCabe 1994, 1997, Woods1995, Marques2006). Though stressors and pollutants generally produce relatively rapid changes in immune response of rats (Laurence and McCabe 1995, DeJong et al 1999, Michielsen et al 1999), there is paucity of literature on cellular and subcellular studies in lymphoid organs of rats.

Lymph nodes serve as the site of reactivity following stimulation of antigen entering the node via the lymph or the blood. The central function of spleen is the formation of lymphocytes which pass into the blood. The central function of spleen is the formation of lymphocytes which pass
into the blood, clearance of particulate materials from the blood and concentration of blood-borne antigens. It is also the site of erythrocyte storage and of removal of effete erythrocytes and leukocytes.

In this study oral administration of lead was done, as this, in addition to inhalation is the most common mode of exposure in man. A dose of 250µg/kg body weight of lead acetate daily for 60 days was chosen, as this dose showed no apparent clinical signs of lead poisoning. In this study, cellular and subcellular changes in immune organs viz. lymph nodes and spleen of lead exposed rats were elucidated in detail together with alterations in somatic tissue indices.

MATERIALS AND METHODS
A total of 20 male wistar rats weighing 100-200gm were used for the experiment. The animals were kept under conventional conditions (2 animals /cage, 12 hours light-dark cycle, 60±10% humidity, 24±2°C) and they received compressed rodent food and water ad libitum.

First group of 10 animals were given 250µg/kg body weight of lead acetate dissolved in drinking water for 60 days. The other group of 10 animals was given equal quantity of sodium chloride in water and was used as controls. After completion of experiment, animals of both groups were sacrificed under deep anaesthesia and spleen and lymph nodes were taken immediately for light and electron microscopy.

For light microscopy, tissue samples were fixed in buffered 10% neutral formalin and Bouin’s solution separately for 8hrs. and after thorough wash, proceeded for paraffin sectioning and serial sections of 5-6µm thickness were obtained and stained with haematoxylin and eosin.

For electron microscopy, animals of both groups were perfused transcardially and tissue samples were taken out and fixed in 3% glutaraldehyde in phosphate buffer solution for 2 hrs and then rinsed in 0.2M phosphate buffer three times. After cutting the tissues in required thickness (about 1mm), post fixed in buffered 1% OsO₄ for 2hrs and washed with 0.1M phosphate buffer solution and dehydrated with graded ethanol and embedded in araldite (epon). Thin sections were cut with LKB Ultratome III automatic ultramicrotome and counter stained with lead citrate and uranyl acetate and examined under electron microscope.

RESULTS
Body and Organ weight

Table 1 summarizes the body weight and selected organ weight following a treatment with lead acetate. Lead exposure at the levels employed had no significant on the body weights of rats. Actual lymph node weights were reduced in exposed group, as were relative lymph node weights. Also there was an increase in actual (P<0.10) and relative (P <0.01) spleen weights in the exposed group.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Lead Exposed</th>
</tr>
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<tbody>
<tr>
<td>Body weight (gms)</td>
<td>113.33±20.63</td>
<td>113.48±20.26</td>
</tr>
<tr>
<td>Lymph node wt. (gms)</td>
<td>0.013±0.009</td>
<td>0.002±0.007</td>
</tr>
<tr>
<td>Lymph node/body wt.(x10⁻³)</td>
<td>0.115±0.001</td>
<td>0.015±0.10</td>
</tr>
<tr>
<td>Spleen wt.(gms)</td>
<td>0.423±0.127</td>
<td>0.48±0.194</td>
</tr>
<tr>
<td>Spleen wt./body wt.(x10⁻³)</td>
<td>3.73±0.65</td>
<td>4.23±1.01</td>
</tr>
</tbody>
</table>

Histopathological Findings

Light microscopic examinations revealed marked alterations in their normal architecture. Moderate to severe atrophy of lymphoid nodules with depleted cell population of paracortex together with the loss of demarcation between cortex and medulla could be seen in lead exposed group. There was a marked reduction in the number and size of follicles resulting in inconspicuous germinal centers. Necrotic lymphoid cells that had pyknotic or fragmented nuclei and degenerated cytoplasm were observed. The chromatin was condensed in the vicinity of nuclear membrane. Degeneration of mitochondrial cristae and occasional presence of electron dense material in the mitochondrial matrix in most of the lymphocytes were the other changes observed under electron microscope.
DISCUSSION

Male wistar rats dosed with 250µg/kg body weight of lead acetate in their drinking water daily for 60 days showed no inhibition of growth as revealed by body weight. In addition, the animals showed no other overt signs of toxicity when experimental lymphoid organs were examined. A decrease in actual lymph node weight was observed in rats of exposed group. This confirms the findings of Gupta et al 1973, Seinen and Williams 1976, Dziedzic et al 1986, Venketeswaran 1994 and van Birgelen 1997 who have also observed the reduction in the lymph node weight of experimental animals exposed to various toxic compounds. Lead exposure produced a significant increase in splenic weight in this study as also reported by Liao et al 1995 and Fuzitani et al 2000. This may be due to increased extramedullary haematopoeis, occurring in spleens of these animals.

Histopathological changes showed severe atrophy of the lymph nodes as revealed by marked reduction in the number and size of follicles together with the loss of demarcation between cortex and medulla. B lymphocytes areas also showed low level activity as indicated by fewer follicles and inconspicuous germinal centers in lymph nodes and spleen of experimental rats.

The ultrastructural studies revealed mitochondrial vacuolation with loss of cristae, hypertrophy of rough endoplasmic reticulum with increased size of lysosomes were observed in our findings. Also pronounced changes in the nucleus - fragmentation of chromatin material, disappearance of nucleoli and dissolution of nuclear membrane clearly indicated cellular death. These findings also comply with the earlier studies by Hoffman et al 1972 and Deveci, 2006. Electron- dense materials in macrophages and lysosomes were a significant finding in our study, which was also observed by Hamasaki et al 2000 and Turkay et al 2015. Hamada et al 1998 observed histiocytosis in the parietal lymphatic sheath and hyperplasia of B lymphocytes in the red pulp. They observed marked poikilocytosis and Heinz body formation in red blood cells in both the sinus and cord of spleen under electron microscope. The alterations in lymphocyte population and morphology were apparently correlated to tissue damage and haemorrhage due to lead accumulation (Ohsawa et al 1983 and Deveci, 2006).

From these results it can be concluded that lead affects the lymphoid system as revealed by cytopathologic alterations and degenerative changes in lymph node and spleen. Lead intoxication even in low doses can lead to severe drastic toxicological effects and immunomodulations in the living beings.

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How to cite this article: Anjali. Immunopathological Alterations Induced by Lead Exposure in Rat. International Journal of Science & Healthcare Research. 2018; 3(3): 7-10.

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