Ultrastructural Study of Neutrophils in Fetal Rat Spleen Following Lead Intoxication

Hamid Reza Mahmoudzadeh Sagheb, PhD.[☆ ⚷], *Zahra Heidari, Ph.D.*[☆], *Mohammad Barbarestani, Ph.D.*[★]
Mohammad Hosein Noori Mugahi, Ph.D.[★],

☆ Histology Department, Zahedan Medical Sciences University

★ Anatomy Department, Tehran Medical Sciences University

★ Histology Department, Tehran Medical Sciences University

⚷ **P.O.Box: 98165-488, Histology Department, Zahedan Medical Sciences University, Zahedan, Iran**

Abstract

Received 13/Aug/2003, Accepted 31/Dec/2003

Introduction: Lead is one of the heavy metals that have adverse effects on blood cells and hemopoiesis. In this study the ultrastructure of neutrophils in fetal rat spleen were investigated following lead intoxication.

Material and Methods: Thirty female and 6 male Sprague-Dawley rats were chosen by simple random sampling. After mating the pregnant rats were classified into test and control groups. From the first day of pregnancy the test group was provided ad lib with water containing 0.13% lead acetate and the control group had access to distilled water. After birth 10 newborn in each group were chosen by systematic random sampling. The spleens of the newborn rats were fixed in a solution of 2% glutaraldehyde, and after processing, sections were studied by a transmission electron microscope.

Results: The ultrastructural changes included: irregular nuclei with deep invagination, plasma membrane pockets, presence of vacuoles with a heterogeneous material and an increasing incidence of rough endoplasmic reticulum with dilated cisternae. No differences between the groups were observed in the mitochondrial morphology and pattern of cytoplasmic granules (primary granules with electron dense appearance and specific or secondary granules with less electron density and heterogeneous appearance).

Conclusion: Lead transmitted via the placenta can affect the ultrastructure, and most probably the function, of fetal neutrophils. More attention must be given to the dangers of lead pollution of the environment and the need to eliminate exposure to lead in work places.

Key words: Ultrastructure, Neutrophil, Lead, Spleen, Embryo, Rat

Introduction

Lead from industrial processes and from petrol-powered vehicles is considered a major environmental pollutant (1). Although lead has been eliminated from petrol in many countries industry is still a major source of pollution (1,2). Occupational lead exposure may occur during the manufacture of batteries, painting, printing, pottery glazing, and lead smelting processes (2-4). Exposure may also occur during the construction of tank linings, piping and other equipment that carries corrosive gases and liquids, superconductors, and fiber optics. (2-4). Magnetic resonance imaging and nuclear medicine procedures may also be sources of lead exposure (4). All sources of lead are considered in the permissible exposure limits for metallic lead, lead oxide, lead salts and soaps that has been set by WHO and other health organizations (3). Lead is absorbed through the digestive and respiratory tracts, and skin. After absorption into the blood, 99% of lead is bound to erythrocytes and the remaining one percent is carried by the plasma to other tissues (4,5). Lead has many target organs such as the hematopoietic system, immune system, kidneys, and nervous system (4).

Studies of chronic lead intoxication on immature rat leukocytes have shown that blood eosinophils, lymphocytes, monocytes and neutrophils increase significantly following lead exposure (5). Neutrophils are white blood cells that are essential in defending animals from invasive bacteria such as Staphlococcus, Streptococcus and gram negative bacilli that infect them (6). It has been shown that the ability of neutrophils to respond to an immunological stimulus and to kill bacteria is inhibited in workers with high blood lead levels(6). Furthermore there is an immunosuppressive effect of relatively low-level lead absorption which suggests that immune dysfunction may be a sensitive indicator of lead exposure (6). Another study suggested that actin redistribution does not regularly occur within neutrophils in lead-containing medium (7). Since previous investigations indicated that lead inhibits chemotaxis of neutrophils, these results suggest that one possible cause of this inhibition is related to an effect of lead on actin reorganization within the cells (7,8). It has been shown that polarization of neutrophils supplemented with lead is abortive (7,8).

Based on previous ultrastructural studies on blood neutrophils (9), cells of the liver (10), kidney (11), and splenic macrophages (12) the involved organelles in lead toxicity are probably lysosomes, mitochondria, microsomes, and the endoplasmic reticulum (9-12). Studies on the effects of lead on rat peripheral blood neutrophils have shown ultrastructural changes such as swelling of the cisternae of the endoplasmic reticulum (9). Nuclear pockets and special crystalline inclusions have also been reported in the matrix of some neutrophils after increased exposure to lead. Changes in mitochondrial morphology and cytoplasmic granular pattern have not been observed (9). In another study, decreased neutrophil index in the bone marrow and morphological changes such as irregular nuclei with deep invaginations, plasma membrane pockets, and vacuoles rich in heterogeneous materials have been reported (2).

To understand the reason for lead induced functional impairments, more ultrastruchural studies need to be done. Lead induced inflammation in lymphoid tissues (12), may be associated with changes in ultrastructure and function of neutrophils which are among the main cells contributing to inflammatory processes Previous studies suggested that lead can pass through the blood- placental barrier in both human and rats (13,14). In the present study ultrastructural changes of the neutrophils in the full-term fetal rat spleen following maternal lead intoxication have been investigated by means of an electron microscope.

Material and Methods

Thirty female and 6 male Sprague-Dawley rats weighing 160-235 gm and 230-270 gm respectively were randomly selected from the animal house of the research institute of Tehran University of Medical Sciences. They were maintained under a constant cycle of 12 h light and 12 h darkness and temperature of 18-23° C with food and water ad libitum. After an adaptation period of one week, male rats were

introduced to the females cages for mating. The female rats were examined for vaginal plugs each morning and pregnant rats (n=14) were divided into experimental and control groups.

From the beginning of pregnancy, rats in the experimental group had 0.13% lead acetate (15) in their drinking water whilst the control group had distilled water. Soon after delivery, newborn rats were anesthetized by ether and their spleens were removed. Ten spleens were selected from each group through a systematic random sampling method and from each spleen a one mm^3 sized specimen was prepared. The specimens were fixed in 2% glutaraldehyde solution (in 0.1 M phosphate buffer, pH 7.2) for 2-3 hours at 4C, rinsed several times in phosphate buffer, then post-fixed in 1% osmium tetroxide for 2 hours at room temperature. After washing in phosphate buffer, dehydration in ethanol and impregnation in Epoxy resin, the specimens were embedded with Epon 812 in plastic capsules. The blocks were trimmed and ultra-thin sections were prepared. The sections were stained with lead citrate and uranyl acetate and then examined using a transmission electron microscope (Zeiss 902). In each specimen of control and experimental groups three neutrophils were studied.

Results

The cytoplasm of the control fetal rat neutrophils appeared pale with a few primary and secondary granules.

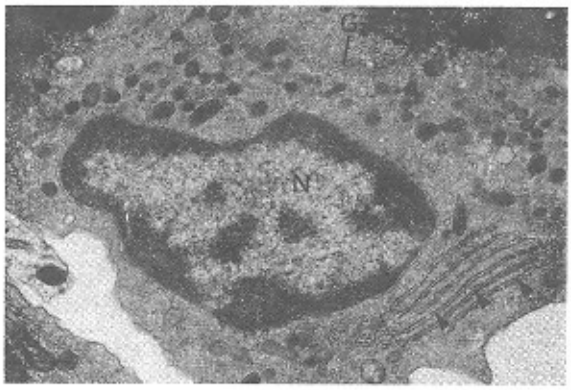

Fig 1: A micrograph of a neutrophil in the splenic red pulp of the control group. Heterochromatin nucleus(N), a segment of which is evident, cisterns of rough endoplasmic reticulum(arrow head) Golgi apparatus(G), and primary & secondary cytoplasmic granules(arrows) are clearly apparent (*10000)

Cisternae of the endoplasmic reticulum, the Golgi apparatus and the mitochondria appeared normal (Fig 1). The nuclei had a circular lobulated heterochromatin appearance that is normal in rats.

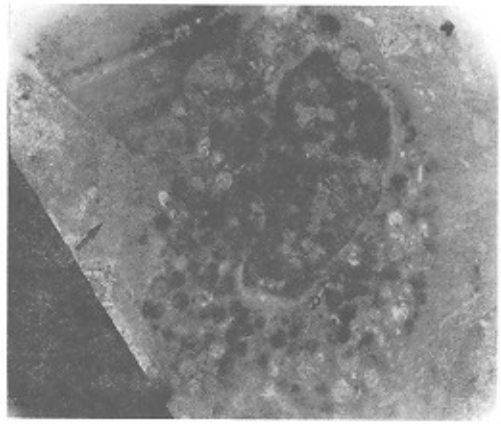

Fig 2: A micrograph of a neutrophil in the splenic red pulp of the experimental group. Heterochromatin nucleus (N), a segment of which appears eccentrically. There is heavy accumulation of granules in the cytoplasm. Electron dense primary granules or lysosomes (P), and specific or secondary granules (S) with less electron density are evident. In the gaps between this cell and other cells a meshwork of delicate connective tissue can be seen (*10000)

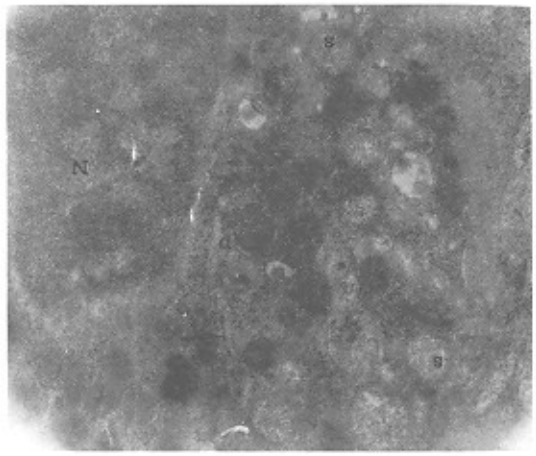

Fig 3: A micrograph shows a neutrophil in splenic red pulp in experimental group. A segment of nucleus (N), cytoplasm rich in granules, relatively homogenous electron dense primary granules (P), and secondary granules with less electron density (S). Cisterns of rough endoplasmic reticulum (arrow) can also be detected among the granules (*25000)

There were no differences between the experimental and control groups in the morphology of mitochondria and Golgi apparatus or the pattern of cytoplasmic granules (Figs 2 & 3). Some ultrastructural alterations of the splenic neutrophils of the experimental group were observed. Nuclear membranes were often irregular with deep invaginations (in 60% of cases) (Fig 4), the plasma membrane had developed pockets and vacuoles were rich in heterogeneous materials (in 40%

of cases) (Fig 5, 6), and the incidence of dilated cisternae was increased in the rough endoplasmic reticulum (in 30% of cases) (Fig 6).

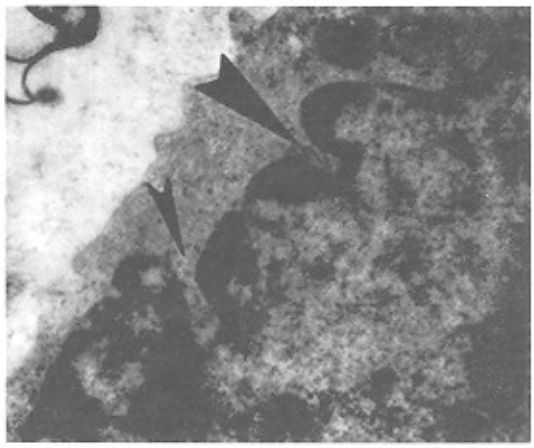

Fig 4: A micrograph shows nucleus of a neutrophil in spleen of experimental group. A segment of the nucleus(N) with irregular border and deep invaginations (arrow heads) is seen. Another small nuclear segment can be seen in the left lower part (star). (*25000)

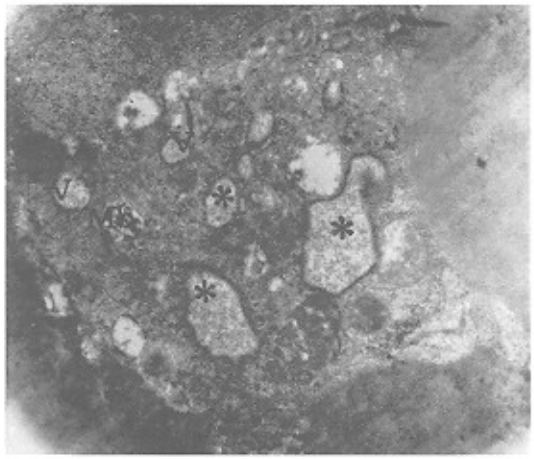

Fig 5: A photomicrograph shows cytoplasm of the neutrophil in experimental group. Several multivesicular bodies (Mb), containing heterogeneous materials and some plasma membrane pockets (star) and vacuoles with heterogeneous materials (V). (*30000)

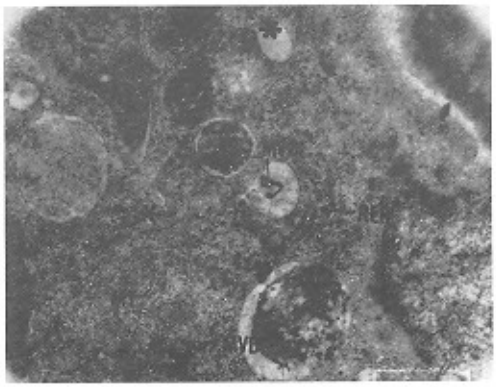

Fig 6: A photomicrograph shows cytoplasm of the neutrophil in experimental group. Rough endoplasmic reticulum (RER) with dilated cisterna, mitochondria (MT), and several multivesicular bodies (MVB), containing heterogeneous materials and some membrane pockets without specific content (star) (*30000)

Discussion

In the present study, ultrastructural changes in fetal splenic neutrophils following maternal lead intoxication included irregular nuclei with deep invaginations, plasma membrane pockets, presence of vacuoles with heterogeneous materials, and dilated cisternae of the rough endoplasmic reticulum. Based on previous studies, the presence of heterogeneous vacuoles and multi-vesicular bodies can be a sign of increased phagocytic activity of cells and lead-induced inflammatory effects (12).

In previous studies on the concentration of lead in kidney and liver cells, high lead concentrations were found in the microsomes, mitochondria, and lysosomes (10,11). High affinity of lead for sulfhydryl groups and the great amount of sulfhydryl enzymes in mitochondria are the reasons for lead aggregation mainly in these organelles. Lead steadily separates from the microsomal fraction and specifically concentrates in the mitochondria (10). Although these studies have reported lead-induced morphologic changes in mitochondria such as organelle swelling or edema (10,11), in the present study no obvious change was observed regarding morphology of the mitochondria. This observation, however, was completely in accordance with another report that quoted no alterations in mitochondrial and cytoplasmic granules in peripheral blood neutrophils of adult rats (9). The reaction of neutrophils present in the splenic pulp cords and peripheral blood were similar (16). Since the origin of splenic neutrophils is peripheral blood (14), such results were expected. Although in a previous study nuclear pockets and crystalline inclusions in the cytoplasmic matrix of some neutrophils were reported (12), such changes were not observed here. A possible explanation could be the fact that the previous study used adult rats, whereas the present study was carried out on fetal rats.

Development of pockets and deep invagination in the nuclear membrane, plasma membrane pockets, and heterogeneous vacuoles that were seen in the present study, is in accordance with Sudakova,s study (17). It seems that ultrastructural changes are due to the metabolic effects of lead, altered enzyme activity in

response to lead toxicity, interaction of lead in reactions with calcium as their second messenger, and the accumulation of lead in these cellular organelles (18).

Recent studies have shown that lead causes a decrease in chemotactic activity and random migration of the neutrophils (6). The results reported here suggest that one possible cause of this inhibition is related to the effect of lead on actin reorganization. It has been shown that polarisation of neutrophils supplemented with lead is abortive (7). Neutrophils are the body's first line of defense against microbial challenge and in response to microbes in tissue they interact with the endothelium lining the blood vessel wall, migrate across the endothelium and move towards the offending stimuli to eliminate the microbes by ingesting them (6,16). In addition, neutrophils serve an important role in maintaining homeostasis and thus impaired neutrophil recruitment can lead to a compromised immune state. Based on this and previous studies (6,7,8) it is concluded that lead can affect the immune system and may lead to immune dysfunction with consequences such as recurrent infections and poor wound healing. For these reasons more attention should be given to environmental pollution by lead and the necessity to eliminate exposure to lead in work places.

Acknowledgments

The authors wish to thank Ms. Amini, chief executive of electron microscopy of Daru-pakhsh Pharmaceutical Research Institute Co. for her skillful cooperation. Special thanks go to Dr Mohammad Abdollahi, from the Toxicology Dept. Faculty of Pharmacology in Tehran University of Medical Sciences, Dr. Rezayat and Dr. Dehpour, from Pharmacology Dept. -Tehran University of Medical Sciences. We are grateful to Dr Ali Ghanbari and Miss F Heidari for their forbearance in reading the manuscript and providing their comments.

References

1. Ghorbel F, Boujelbene M, Makni-Ayadi F, Guermazi F, Kammoum A, Murat JC: Effect of chronic lead exposure on kidney function in male and female rats: Determination of a lead exposure biomarker. Arch Physiol Biochem 2001; 109: 457-463

2. Patel AB, Williams SV, Frumkin H, Kondawar VK, Glick H, Ganju AK: Blood lead in children and its determinants in Nagpur India. Int J Occup Environ Health 2001; 7: 119-126

3. Harbison RD, Lead. In: Harbison RD: Hamilton & Hardy s Industrial Toxicology, 5th ed, Mosby, Philadelphia, 1998; 70-76

4. Sanborn MD, Abelsohn A, Campbell M, Weir E: Identifying and managing adverse environmental health effects: 3. Lead exposure. J Canad Med Assosi 2002; 166: 1287-1292

5. Tchernitchin AN, Villagra R, Tchernitchin NN: Effect of chronic exposure to lead on immature rat leucocytes.Med Scien Res 1997; 25: 355-357

6. Queiroz MLS, Almeida M, Gallao ML, Hoehr NF: Defective neutrophil function in workers occupationally exposed to lead. Pharm Toxicol 1993; 72: 73-77

7. Governa M, Visona I, Valentino M, Marchiseppe I: Lead interference in actin redistribution induced by chemotactic peptide in human neutrophils. ATLA 1989; 17: 16-21

8. Basaran N, Undeger U: Effects of lead on immune parameters in occupationally exposed workers. Am J Med 2000; 38: 349-354

9. Silwa-Tomczok W, Tomczok J, Matysiak N: Effects of acute lead intoxication on the ultrastructure of neutrophils in the peripheral blood of the rat. Exp Pathol 1991; 43: 149-154

10. Mahaffy K: Concurrent exposure of Lead, Cadmium and Arsenic: Effects on toxicity and tissue metal concentration in rat. J Lab Clin Med 1981; 98: 463-481

11. Krigman M, Lysosomal alternation in Lead neuropathy, Fed Proc 1981; 410-427

12. Lee J, Battles A: Lead Toxicity via arachidonate signal transduction to growth responses in the splenic macrophage. Environmental Research 1994; 67: 209-219

13. Gulson BL, Mahaffey KR, Mizon KJ, Korsch MJ, Cameron MA, and Vimpani G: Contribution of tissue lead to blood lead in adult female subjects based on stable lead isotope methods. J Lab Clin Med 1995; 125: 703-712

14. MacClain R, Becker B: Teratogenicity, fetal toxicity and placental transfer of lead nitrate in rats. Toxicol Appl Pharmacol 1975; 31: 72-82

15. Bashir M, Jarrar Z, Mahmoud N: Histochemical characterization of renal phosphatases in rats treated with Lead. Internat J Toxicol 1999; 18: 231-238

16. Gartner LP, Hiatt JL: Color textbook of Histology, Second

edition,W.B.Saunders Company 2001; 291-295
17. Sudakova AI, Shevchenko ZT, Nosova LI, Peripheral blood and bone marrow cell status of white rats with long-term lead exposure, Tsitol Genet 1983; 17: 3-7
18. Calderon Salinas V, Hernandez-Luna C, Maldonado M, Saenz D: Mechanisms of the toxic effect of lead. I- Free Lead in erythrocyte. J Expo Anal Environ Epidemiol 1993; 3 Suppl 1: 153-164