
DRUG BIOCHEMISTRY

EFFECT OF LEAD ON HUMAN ERYTHROCYTES: AN *IN VITRO* STUDY

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Abstract: The present study was an attempt to investigate toxic effects of lead on red blood corpuscle (RBC) under *in vitro* conditions. Venous blood samples from adult, healthy, well-nourished male donors falling in age group of 25–30 years were collected in EDTA vials for preparation of RBC suspension. These donors were the residents of an area of Bhuj, where soil was not contaminated with lead and also concentration of lead was below detectable limit in blood and drinking water. RBC suspension was treated with (0.0625 to 0.625 ppm) of Pb(NO₃) for 4 h at 37°C. The results revealed that addition of lead salt causes concentration-dependent increase in the rate of hemolysis, thus, proving Pb(NO₃) as cytotoxic agent even at very low concentration.

Keywords: cytotoxicity, hemolysis, red blood corpuscle (RBC), lead nitrate

Exposure to toxic metals remains a widespread occupational and environmental problem in the world. There have been a number of reports suggesting an incidence of childhood due to contaminated drinking water in many areas of West Bengal in India and Bangladesh where lead poisoning had become a national calamity (1). It has been found that once it is absorbed through either ingestion or inhalation, it enters the bloodstream where it is predominantly bound to erythrocyte proteins (2), with an average clearance half-life after a short time limited exposure of approximately 35 days from the whole blood (3). Paul et al. had studied detailed hematological and biochemical changes in patients with lead poisoning; they revealed that lead has direct effect on mature RBC, which occurs independently of the effects of lead on heme biosynthesis in erythroid precursors (4, 5).

Despite decades of intensive research, lead toxicity also remains one of the most studied subjects of all within the fields of environmental health and environmental medicine. It had become the most important problem in terms of prevalence and public health impact. So, the present study was undertaken to study the effects of lead at low level exposure on human RBC, under *in vitro* conditions.

MATERIALS AND METHODS**Chemicals**

Lead nitrate was procured from Hi-Media Laboratory Pvt. Ltd, Mumbai, India. All other chemicals used in present study were of analytical (AR) grade.

Sample selection

Blood samples were obtained with voluntary consent from 20 well-nourished, healthy adult male donors, age 25–30 years residing in Bhuj. Drinking water and soil samples from their residence have been analyzed for the presence of lead. Venous blood samples were taken by trained laboratory technician by disposable syringe in EDTA vials. After the collection of sample, lead concentration was analyzed prior of preparing RBC suspension.

Lead analysis*Soil sample*

Lead from soil was extracted by digesting soil sample with HNO₃. Twenty five grams of soil was taken in 100 mL beaker. It was mixed with 50 mL of concentrated nitric acid and kept on sand bath for 1.5 h at approximately 200°C. It was brought to

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room temperature, then 15 mL of distilled water was added and the mixture was filtered through Whatman filter paper no.1. If the odor of HNO₃ was still present, the sample was kept on sand bath for further digestion. The final volume was made up to 50 mL with distilled water. Concentration of lead was estimated using an atomic absorption spectrophotometer at 217.00 nm, Chemito – 201 (6).

Water sample

Samples were directly aspirated and estimated using an atomic absorption spectrophotometer at 217.00 nm, Chemito – 201 (6).

Blood sample

Five mL of blood was treated with 1 mL of 5% Triton-X and 1 mL of ammonium tetramethylene dithiocarbamate (APDC). After shaking, 4 mL of water saturated with methylisobutyl ketone (MIBK) was added. The solvent layer was separated and the absorbance was measured at 283.3 nm using atomic absorption spectrophotometer, Chemito – 201 (7).

RBC suspension

Blood samples were diluted with normal saline (0.9% NaCl) and centrifuged at 1000 × g for 10 min. The RBC pellets were washed twice and finally suspended in normal saline to have a cell density of 2 × 10⁴ cells/mL (8).

Lead nitrate solution

Stock solution of lead nitrate (1 mg Pb(NO₃)₂/mL i.e., 0.625 ppm of lead) was prepared.

To determine the effect of lead on RBC, the following sets of tubes were prepared.

- 1) Control tubes: These tubes contained 2.0 mL of RBC suspension and 2.0 mL of normal saline.
- 2) Lead nitrate treated tubes: Pb(NO₃)₂ (0.001 to 0.01 mg) were mixed with 2.0 mL of RBC suspension. The total volume was made up to 4.0 mL with normal saline.

All tubes were incubated at 37°C for 4 h with intermittent shaking. Thereafter, tubes were centrifuged at 1000 × g for 10 min and the absorbance of the supernatant was read spectrophotometrically at 540 nm. To achieve 100% hemolysis, 2 mL of distilled water was added to 2 mL of RBC suspension. Morphological alterations in RBC were observed under the microscope by staining RBC with Leishman's stain.

Percent hemolysis was calculated by the formula (9):

$$\text{Percent hemolysis} = \frac{\text{Absorbance of individual tube}}{\text{Absorbance with 100\% hemolysis}} \times 100$$

Data analysis

Statistical analysis of the data was done using Student's *t*-test. Values of *p* < 0.05 were considered statistically significant. Data were expressed as the mean ± SEM.

Table 1. Hemolytic effect of lead on RBC suspension.

| No. | Samples | Pb Concentration |
|-----|----------------|---------------------|
| 1. | Blood | BDL* |
| 2. | Drinking water | BDL* |
| 3. | Soil | 13.88 ppm (average) |

n = 20 ; *Below detection limit (BDL)

Table 2. Hemolytic effect of lead on RBC suspension.

| No. | Concentration of lead (ppm) in final solution | Hemolysis % |
|-----|---|---------------|
| 1. | 0.0 (Control) | 00.08 |
| 2. | 0.015 | 35.46 ± 3.65* |
| 3. | 0.031 | 48.94 ± 2.60* |
| 4. | 0.062 | 64.57 ± 2.22* |
| 5. | 0.092 | 71.25 ± 3.87* |
| 6. | 0.125 | 78.75 ± 1.83* |
| 7. | 0.156 | 85.50 ± 1.10* |

*Values are significant at both *p* < 0.001 and *p* < 0.05

RESULTS

As it was imperative to measure the level of lead in volunteers before taking their blood samples, therefore, drinking water and soil samples from their residence were analyzed. Further, lead concentration in blood samples were also examined before preparing RBC suspension. All the measurements of lead are shown in Table 1.

In control tubes, RBC appeared as spheres or biconcave disks. The cells remained settled at the bottom of the tubes with almost clear and ambient solution above indicating lesser hemolysis. With the addition of 0.0625 ppm $\text{Pb}(\text{NO}_3)_2$, a tinge red color appeared in the medium; most of the cells remained settled at the bottom of the tube leads. Incubation of RBC suspension with different concentration of lead nitrate caused pronounced disruption of RBC. However, 0.625 ppm – the highest concentration of $\text{Pb}(\text{NO}_3)_2$, caused the maximum hemolysis showing the maximum appearance of red color in the incubation medium. Thus, the results revealed dose dependent significant increase in the rate of hemolysis.

DISCUSSION

Present investigation shows the significant cytotoxic effect of lead on RBC at low level exposure which may be due to its high potential in changing osmotic fragility, interaction of lead with membrane protein, an increase of oxidative stress or may interact with some essential trace metals in the blood which can be correlated with following studies. It is well documented that more than 95% of lead, an environmental heavy metal, entering into blood accumulates in erythrocytes suggesting erythrocytes as an important target of lead toxicity (10). In addition to killing cells *via* exotoxicity and apoptosis, lead also causes toxic effects by oxidative stress either directly or by indirectly produced lipid peroxidation (11). Lead alters lipid metabolism, enhances lipid peroxidation and decreases cell membrane fluidity of developing rats (11).

M. Ahamed et al. have studied the interaction of lead with some essential trace metals in the blood of anemic child from Lucknow and revealed that elevated blood lead levels ($= 10 \mu\text{g}/\text{dL}$) in children were significantly associated with the risk of anemia and that blood lead levels also influence the status of essential trace metals (12).

Apostoli et al. have studied the effects of lead on red RBC membrane proteins in two groups of workers with different lead exposure levels and con-

cluded that the effects of lead on RBC membrane proteins seem to be evident at blood-lead levels higher ($> 50 \mu\text{g}/100 \text{ mL}$) than those previously reported in the literature. These results confirm the effects of lead on erythrocytes membrane proteins, although the exact mechanism of proteolysis of erythrocytes membrane protein is not completely understood (13).

Lead particles adhered to the external and internal surfaces of the human erythrocyte membrane; lead ions distributed the lamellar organization of isolated unsealed erythrocyte membranes (IUM) and dimyristoylphosphatidylcholine (DMPC) large unilamellar vesicles (LUV) studied at sublethal dose i.e., 100 mg/kg body weight (14). There are several studies that confirm the effects of lead on red blood cell membrane proteins at different lead exposure levels. Lead may be responsible causing osmotic change in blood hence osmotic fragility is the reason for hemolysis (15). The results of present study revealed that lead is heavy metal having harmful effects on human beings. The comparison of clear supernatant of untreated RBC suspension and red-dish supernatant of lead treated tubes gave the evidence for the toxic effects of lead on red blood cells which leads to cell damage at very low dose. Therefore, from the present study it is concluded that lead destroys the red blood cells and can cause anemia even at very low level exposure and its detailed mechanism of action needs to be studied thoroughly.

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