Bull. Environ. Contam. Toxicol. (2003) 70:1189–1196
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DOI: 10.1007/s00128-003-0108-z

Bulletin of Environmental Contamination and Toxicology

Protective Role of Ascorbic Acid Against Lead Toxicity in Blood of Albino Mice as Revealed by Metal Uptake, Lipid Profiles, and Ultrastructural Features of Erythrocytes

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Received: 30 April 2002/Accepted: 2 March 2003

Lead is a relatively abundant metal in nature (Todd et al.1996). A biologically nonessential element, the metal finds extensive use in modern times (Landrigan et al. 1990). The build up and transportation of lead in water, atmosphere and sediment results in bioaccumulation of the metal in various pockets of food chain. The absorbed lead enters the blood stream where over 90 percent is bound to the red cells with a biological half life of 25-28 days (Azar et al.1975). Toxicological effects of lead have their basis in perturbation in cell function of various organ system. The major biochemical effects of lead is its interference with heme synthesis leading to hematological damage (Awad 1997). Lead is also known to intercept calcium metabolism (Goyer 1988). The toxicity due to lead causes neurological effects, renal effects, hypertension, immunological effects, carcinogenesis, sterility, neonatal mortality, morbidity etc. (Keogh 1992). The prenatal toxic effects of lead on erythrocytes have recently been studied by scanning electron microscopy (Dey et al. 1999). Lead induced toxicity during pregnancy also known to cause ocular damages in offspring (Dey et al. 1997).

Despite several published accounts on pathophysiology of lead toxicity and the cure of lead poisoning by sequestering agents (Royce and Rosenberg 1993), the approaches are limited in scope. Studies to explore newer protective and therapeutic agents with least side effects to combat lead induced toxicity has not been addressed adequately. In this context our attention was drawn to a very recent report (Simon and Hudes 1999) on a population based study in US where blood levels of lead was shown to be related to low level of ascorbic acid (Vitamin C) in the blood stream. Protective role of ascorbic acid towards plants from automobile exhaust that includes lead has also been noted in a current study (Mandal and Mukherjee 1998). Albeit, antioxidant property of ascorbic acid is known, it has not been exploited in detail for its potential as a protective or a therapeutic chemical aginst metal toxicity in general and lead toxicity in particular. We conjectured that if ascorbic acid, otherwise an essential dietary supplement, can provide the therapeutic action against the metal toxicity, much of the side effects of metal detoxification can clearly be avoided. A recent review highlighted the bioregulatory role of ascorbic acid to protect extracellular protein function through gene expression (Griffiths and Lunec 2001). Accordingly in this paper we report result of studies on lead uptake, homeostatic interaction with

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other essential metals such as calcium, zinc and iron ,ultrastructural features and lipid balance in blood of albino mice. The protective role of ascorbic acid in pre- and post-treated animals has been clearly demonstrated.

MATERIALS AND METHODS

Thirty adult albino mice of 6-8 weeks old and 20-25 grams were selected from the laboratory stock for experiments. The experiments were performed in a quiet room maintained under standard conditions (22 ± 2^0C, 45 ± 10% relative humidity and 12 hour light/ 12 hour dark cycles each day). The animals were fed commercially available balanced food pellets and drinking water *ad libitum.* The animals were divided into five groups according to randomised block design, each consisting of six animals. The animals were treated as follows:

Group 1: The animals in this group were used as control. They were maintained in the laboratory with normal diet for 28 days before sacrifice.
Group 2: Each animal was administered sub lethal doses of lead through oral feed as lead acetate, Pb (CH_3 COO)$_2$ for 14 days and sacrificed on the 15th day.
Group 3: Animals of this group were administered lead acetate for 14 days and then kept with normal diet for another 14 days and sacrificed on the 29th day.
Group 4: Animals pretreated with vitamin C for 14 days were administered lead acetate for another 14 days and sacrificed on the 29th day.
Group 5: Animals treated with lead acetate for 14 days were adminsitered vitamin C for another 14 days and sacrificed on 29th day

The dose of lead acetate and vitamin C were 5 mg kg $^{-1}$ body wt. d $^{-1}$ and 20 mg kg^{-1} body wt. d $^{-1}$ respectively.

Blood from mice of control group and all the experimental groups were collected from the heart and processed for ultrastructural study, elemental analysis and lipid estimation.

Ultrastructural features were investigated using scaning elecrton microscopic tenique (SEM). A thin conducting film of gold was applied to the sample using a fine coat ion sputter, JFC-1100 (Jeol), maintaning a low vacuucm. Observation were made with a JSM-35 CF (Jeol) microscope using the secondary electron emission mode. The accelerating voltage used was 15 KV.

Elemental analysis of blood samples were performed using PERKIN -ELMER 3110 atomic absorption spectrometer. The total lipid was determined by Bligh and Dyer method extracting with a mixture of chloroform and methanol (2:1 v/v).

The results were expressed as mean ± standard deviation (SD) of six animals in each of the analysis. The different sets of data were examined for statistical significance using students 't' test. All reagents used in the experiment were of analytical grade.

RESULTS AND DISCUSSSION

The amount of lead, iron, calcium and zinc in blood samples of control and all treated animals of different groups are displayed in Table 1.

Lead acetate treated mice of Group 2 revealed 2.61 $\mu g\ ml^{-1}$ lead absorption in blood. Lead is thought to enter RBC through anion exchange and remain bound to thiol(–SH) groups (Lal et al. 1996). Lead transport through the erythrocyte membrane is also mediated by Ca-ATPase pump. Fourteen days lead acetate treated mice kept with normal diet for further fourteen days (Group 3) and when examined for lead, a significant hike in its concentration was noticed. This observation is accounted by presuming greater mobilisation of lead from liver to blood during the additional time they survived. Elevated blood lead concentration are known to have detrimental effects on neurophysiological functions (Muldoon et al. 1996). Pertinent here is to mention that the presence of lead in the blood stream (inside the red blood cells and mostly linked to hemoglobin) provokes anaemea. The disease cannot be considered a symptom but rather a delayed sign of lead poisoning. Ascorbic acid pretreated mice when injested with lead (Group 4) recorded significant decrease in concentration of lead in blood. The absorption of lead was found to be about 45% less compared to animals of group 2 receiving only lead doses. Vitamin C post treated animals also registered about 36% clearance of the metal from blood compared to those of Group 2. It is therefore evident that both pre-and post treatment with vitamin C work against lead accumulation in blood. It is however, not clear at this stage whethervitamin C directly binds with lead or not. In order to determine the homeostatic relationship of lead uptake with iron, calcium and zinc, the concentration of these elements were also monitored as a function of lead absorption. The iron level in blood exhibited rather characteristic trends. Lead treated mice (Group 2) showed relatively lower level of iron compared to control animals. That lead impairs the enzymatic activity of delta - aminolevulinic acid dehydratase which is involved in heme synthesis is known (Satija and Vij 1995). This accounts for the observed trend in iron concentration in blood. Pre- or post vitamin C treatment has substantially restored the iron levels in blood (Table 1). The calcium level in blood of lead treated mice appeared to be significantly lower. Owing to chemical resemblence of Ca^{2+} to Pb^{2+}, the later competitively inhibits the uptake of calcium in mitochondrial Ca^{2+} transport proteins (Goyer 1988). Lead may also compete with calcium for binding to the triphosphate chain of ATP (Goyer 1988). However, when lead treated mice were administered normal diet (Group3) blood calcium level dropped further. Pertinent here is to note that blood lead was found to increase under similar condition. Considering chemical analogy of two bipositive metal ions, this homeostatic relationship do not appear to be unexpected. Both pre- and post treatment with vitamin C rectified the calcium level to near normal values simultaneously causing decline in lead levels as noted earlier. All these clearly suggest direct relation between lead and calcium competing with each other for a biochemical matrix. Infact lead's toxicity is largely due to its capacity to mimic calcium and substitute it in many of the fundamental cellular process. However the chemical basis for lead mimicing calcium is not obvious. Zinc an essential element is known to offer structural

Table 1. Metal concentration in whole blood.

Group	Metal concentration (Mean ± SD)			
	Lead ($\mu g\ ml^{-1}$)	Iron ($mg\ 100\ ml^{-1}$)	Calcium ($mg\ 100\ ml^{-1}$)	Zinc ($mg\ 100\ ml^{-1}$)
Group 1	Not detected	135.47 ± 3.34	366.42 ± 28.36	8.01 ± 0.22
Group 2	2.61 ± 0.20	106.63 ± 2.78[a]	287.48 ± 16.27[a]	8.78 ± 0.20[a]
Group 3	3.22 ± 0.19[b]	110.24 ± 3.36[d]	262.22 ± 7.62[d]	9.43 ± 0.26[b]
Group 4	1.47 ± 0.07[c]	129.52 ± 5.13[c]	342.34 ± 14.76[c]	8.29 ± 0.04[c]
Group 5	1.68 ± 0.22[f]	119.29 ± 4.49[g]	316.23 ± 9.61[f]	8.55 ± 0.05[f]

[a] $p < 0.001$ compared to Group 1

[b] $p < 0.001$, [c] $p < 0.001$, [d] $p < 0.001$, [e] $p < 0.01$ compared to Group 2

[f] $p < 0.001$, [g] $p < 0.01$ compared to Group 3

Table 2. Lipid Profile in blood and diameter of red blood cell (RBC)

Group	Amount of lipid (Mean ± SD) (g 100 ml $^{-1}$)	Diameter of RBC (Mean ± SD) (micron)
Group 1	1.30 ± 0.12	3.61± 0.08
Group 2	2.64 ± 0.20 [a]	4.05± 0.05 [a]
Group 3	2.28 ± 0.15 [b]	3.91± 0.02 [e]
Group 4	1.83 ± 0.06 [c]	3.75± 0.04 [c]
Group 5	1.77 ± 0.03 [d]	3.82± 0.06 [f]

[a] $p < 0.001$ compared to Group 1.

[b] $p < 0.01$, [c] $p < 0.001$, [e] $p < 0.001$ compared to Group 2.

[d] $p < 0.001$, [f] $p < 0.01$ compared to Group 3.

rigidity to metalloprotein in biological systems in addition to other biochemical roles . The balance of zinc also has been found to be disturbed by lead. Unlike calcium, there is a clear increment in zinc concentration following lead toxicity. Pre- and post vitamin C treatment resurrected the zinc levels (Table 1) .The protective efficacy of zinc against hemo and hematotoxicity induced by lead is known (Satija and Vij 1995) .

Lipids content in blood of various experimental groups of mice is displayed in Table 2. A twofold increase in blood of lead treated animal indicates that lead

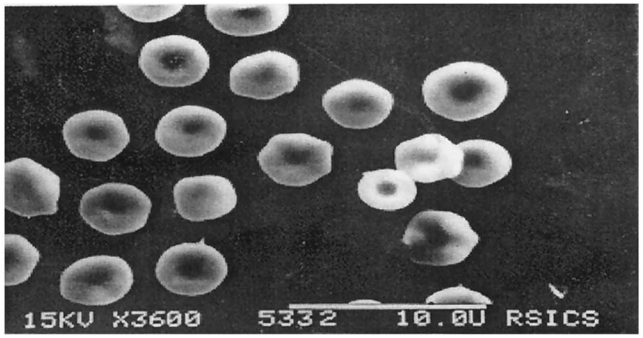

Figure 1. Electron micrograph of normal blood cells showing well defined biconcave RBC.

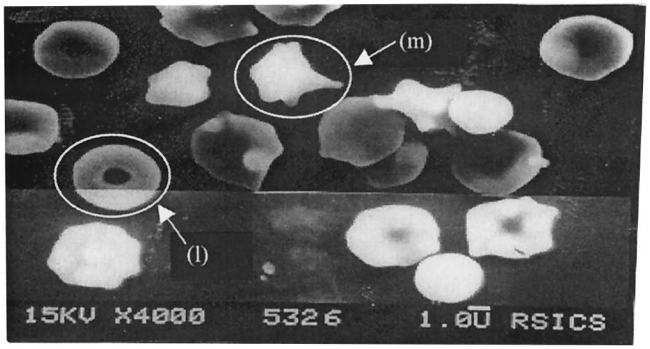

Figure 2. Electron micrograph showing development of ring shaped cell (l), tear drop shaped cell (m) RBC.

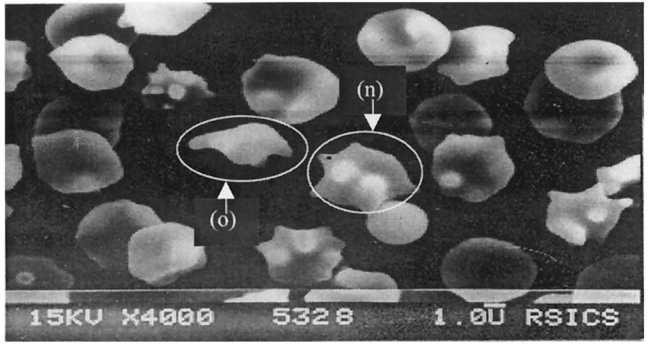

Figure 3. Electron micrograph showing development of echinocytes (n), mexican hat shaped cell (o) RBC.

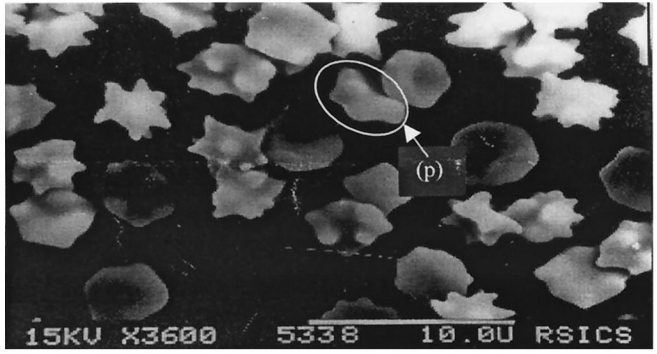

Figure 4. Electron micrograph of blood cells showing development of stomatocytes (p)

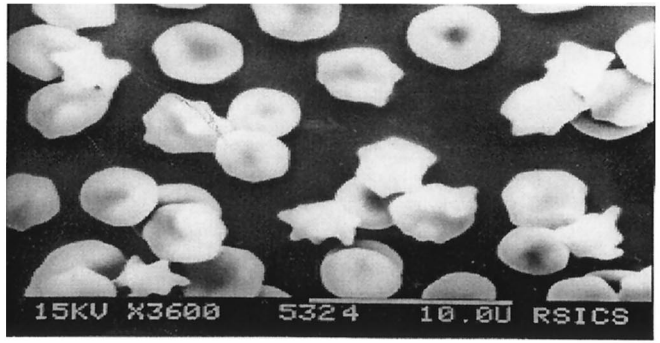

Figure 5. Blood cells when lead treatment is followed by normal diet.

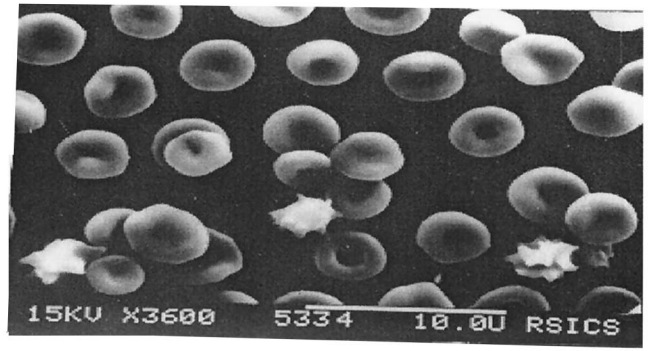

Figure 6. Blood cells of mice receiving vitamin C treatment followed by lead.

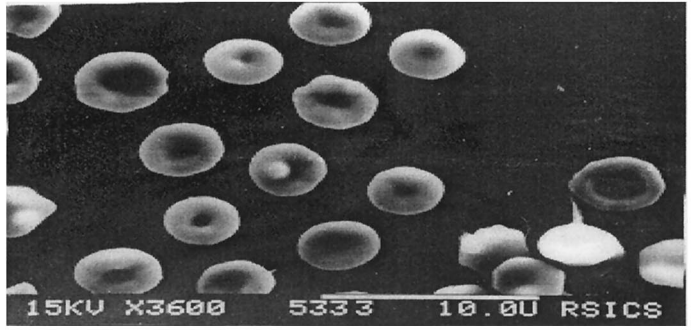

Figure 7. Blood cells of mice receiving lead doses followed by vitamin C treatment.

might have enhanced accumulation of fatty mass. Lead–phosphate (from phospholipid) interaction may be partly responsible for this. Pre- and post- vitamin C treatment (Group 4 and 5) restored the lipid balance substantially.

Scanning electron microscopy of blood revealed that red blood cell of mice receiving oral dose of lead were severely affected. On scrutiny, abnormalities such as developement of echinocytes, stomatocytes, mexican hat shaped cell, tear drop shaped cell and ring shaped cell were evident (Fig .2, 3 and 4) .In addition, significant increase in mean diameter of RBC were observed (Table 2). Lead being a surface active poison ,occurence of increased red cell fragality and acute hemolysis is not unlikely.Normally red cell suspended in isotonic medium maintain their circular biconcave shape. Cooccurence of hemolysis and expansion of RBC indicates that the medium in which red cell are suspended has become hypotonic.Thus it may be conjectured that lead is possibly involed in osmotic regulation between plasma and corpuscles. No significant alteration was noticed in the ultrastructural features of blood when lead treated animals were kept alive on normal diet for additional days although the number of abnormal cells increased (Fig .5).Treatment with vitamin C,either prior to lead administration or later seemed to offer protection to the cell from expansion or abnormalities in their structural features. In addition, distinct regenerative changes in cell shape was noticed though hemolysis still persisted to a lesser extent (Fig 6 and 7). On the whole, vitamin C showed marked efficacy towards protection of blood cell damage caused by lead.

Thus it is evident that vitamin C not only confer protection against lead toxicity but it can also perform therapeutic role against such toxicty . It is most likely that vitamin C could provide a tangible solution to heavy metal toxicty in general . Further biochemical work are needed to understand the precise mechanism of the action of vitamin C aganist lead toxicity.

Acknowledgement. Regional Sophisticated Instrumentation Centre (North Eastern Hill University) is thanked for spectroscopic and some analytical work.

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