Effect of lead in vitro on water metabolism and osmotic fragility of human erythrocytes

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ABSTRACT The addition of lead to normal human blood was previously found to cause a decrease in erythrocyte osmotic fragility in vitro. The mechanism of the decreased osmotic fragility caused by lead has not been completely clarified, but the following hypothesis has been proposed. Lead causes a leakage of water from erythrocytes, thus more water can enter the cell before haemolysis occurs. There has been no report, however, of the direct measurement of the intracellular water content of erythrocytes treated with lead. This study has tried to clarify the relation between intracellular water and the osmotic fragility of lead-treated erythrocytes in vitro. The results showed that 0.05 $\mu\text{M}/\text{ml}$ of lead decreased the osmotic fragility, the intracellular water content, and intracellular potassium and mean corpuscular volume, increased the plasma water content and trapped water content, and contracted the erythrocyte shape. These changes corresponded well with each other, and close coincidence of the osmotic fragility and the intracellular water content was also observed.

In previous papers^{1,2} we observed a decreased erythrocyte osmotic fragility in workers occupationally exposed to lead compared with controls and found a dose-response relationship between the blood lead concentrations and the osmotic fragility in lead workers using the coil planet centrifuge (CPC) method.³ We also observed, by an in vitro experiment,⁴ that the addition of lead to normal human blood caused a decrease in the osmotic fragility using the CPC method.

The exact mechanism of the decreased osmotic fragility is not completely understood, but the following hypothesis has been generally accepted. The addition of lead to blood causes a leakage of potassium accompanying water from the erythrocytes, which means that more water can enter the cell before haemolysis occurs.⁵ There has been no report, however, of the direct measurement of the intracellular water content of erythrocytes treated with lead. We have tried to clarify the relation between the intracellular water content and the osmotic fragility of erythrocytes treated with lead in vitro

and have studied the effects of lead on intracellular potassium and sodium, mean corpuscular volume (MCV), plasma water, trapped water, intracellular water contents, osmotic fragility, and erythrocyte appearance.

Methods

The experiment was performed in quadruplicate in this study. Water content was measured by Kageyama's method.⁶ One millilitre of heparinised normal human blood was incubated for two hours at 37°C in an atmosphere of 5% CO₂ and 95% air with 10 μl of lead acetate solution (lead concentrations in blood; 0, 10, 50, 100, 500, 1000 nM/ml) and 10 μl of physiological saline containing 2 μCi ³H-sucrose (Radiochemical Centre, Amsterdam) and 0.1 mg sucrose was added; this mixture was centrifuged for 30 minutes at 3000 rpm. The haematocrit value was read, and then 50 μl of the supernatant transferred with a micro syringe into a sample bottle covered with a rubber cap containing 1.95 ml of methanol and 4.1% of n-butanol as an internal standard with two glass beads to enhance the stirring efficiency. The sample bottle was shaken well and centrifuged for 10 minutes at 3000 rpm.

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The supernatant and buffy coat were completely removed from the haematocrit. The remaining packed erythrocytes were mixed well with a capillary pipette, and 50 μ l of the erythrocytes was injected quickly into the sample bottle with a microlitre syringe (Precision Sampling Co, USA). The sample in the bottle was shaken vigorously and centrifuged at 3000 rpm for 10 minutes. Next, 2 μ l of the supernatant was taken from each bottle containing plasma or packed erythrocytes and analysed for water content by gas chromatography (Shimadzu, thermal conductivity detector Model GC-4BPTE, Japan). The water content of the plasma and packed erythrocytes was estimated by the method described above and the calibration curve for water was determined using standard samples with known water contents.

Trapped water content—The radioactivity of 50 μ l of the methanol supernatant was measured with a liquid scintillation spectrometer (Packard, Model 3330, USA), and the trapped water content was estimated as: plasma radioactivity $\div$ packed erythrocyte radioactivity $\times$ plasma water content.

Intracellular water content was calculated by subtracting the trapped water content from the packed erythrocyte water content.

Osmotic fragility of erythrocytes was determined by CPC with a coil planet centrifuge, a coiled sample tube with a range of 150–30 mOsmol, a coil incubator, and a scanning photodensitometer (Sanki Engineering, Japan). Three haemolytic points of the haemoglobin distribution pattern in the coiled tube were determined (HSP, haemolysis of the starting point; HMP, haemolysis of the maximum point; HEP, haemolysis of the end point (fig 1).

Erythrocyte count—An automatic blood cell counter (Coulter Electronics, Coulter Counter Model ZF, USA) was used.

Mean corpuscular volume was calculated by dividing the haematocrit value by the erythrocyte count.

Intracellular potassium and sodium—A 20- μ l sample of blood was washed well with 0.1 M $MgCl_2$ and centrifuged. After decantation the blood was haemolysed with 4 ml of deionised water and centrifuged. Sodium and potassium in the supernatant were determined by flame photometry (Hitachi, Model 170-50A, Japan), and these values were divided by the haematocrit value.

Erythrocyte shape was observed by scanning electron microscopy. The erythrocytes were fixed with 0.1M phosphate buffer solution containing 1% glutaldehyde and with 0.5% osmic acid solution. The sample was then dehydrated with alcohol, dried at critical temperature, placed on the sample stage, ion-coated, and observed with a scanning electron microscope (JEOL, JSM-50A, Japan).

Fig 1 Haemolysis of normal human blood samples in coiled tube determined by CPC after treatment with lead *in vitro* at 37°C for two hours. Three haemolytic points of haemoglobin distribution pattern were recorded by scanning photodensitometer (haemolysis of starting point, HSP; haemolysis of maximum point, HMP; haemolysis of end point, HEP). Greatly decreased osmotic fragility in 500 nM/ml are clear.

Results

Figure 1 shows the haemolysis of normal human blood samples in the coiled tube determined by CPC after treatment with lead at 37°C for two hours: fig 2 shows the change of three points of haemolysis. The osmotic fragility at all three points of haemolysis decreased with 50 nM/ml of lead; it was greatly decreased with 500 nM/ml of lead.

The intracellular water content decreased considerably with 50 nM/ml of lead, then gradually decreased with increased blood lead concentration (fig 3). Plasma water content, however, gradually increased with increasing blood lead concentration (fig 4). The trapped water content increased with 50 nM/ml of lead and increased greatly with 500 nM/ml of lead (fig 5).

The changes in intracellular sodium and potassium with lead treatment are shown in fig 6. Intracellular potassium decreased with 50 nM/ml of lead and decreased greatly with 500 nM/ml of lead.

Fig 2 Change in osmotic fragility of erythrocytes. Normal human blood was incubated for two hours at 37°C with lead.

Fig 3 Change in intracellular water content. Normal human blood was incubated for two hours at 37°C with lead.

Fig 4 Change in plasma water content. Normal human blood was incubated for two hours at 37°C with lead.

Intracellular sodium did not change even with 1000 nM/ml of lead.

Figure 7 shows that MCV was decreased greatly with 50 nM/ml of lead, then decreased gradually with increasing blood lead concentration.

Figure 8 shows the morphological changes in erythrocytes observed with scanning electron micrographs. Erythrocytes were slightly flattened with lead compared with the controls but retained a discocyte appearance. Erythrocytes were flattened

Fig 5 Change in trapped water content. Normal human blood was incubated for two hours at 37°C with lead.

Fig 6 Change in intracellular sodium and potassium. Normal human blood was incubated for two hours at 37°C with lead.

more with 1000 nM/ml of lead, and most of the erythrocytes were contracted and looked like flat discs without concaveness. The presence of echinocytes was observed.

Discussion

Changes in osmotic and mechanical fragilities of erythrocytes have been observed in vitro and in vivo, and these changes showed alterations in the morphology and permeability of the erythrocyte membrane.^{7,8} The mechanism for the decrease in osmotic fragility has not been completely clarified, however. Griggs⁵ proposed that lead disturbs the active transport of potassium leading to its leakage and an accompanying water loss from the erythrocytes and then a decrease in MCV. These changes indicate that more water can enter the erythrocyte before the critical spherical form and lysis occur.

Measurement of the intracellular water content

and the water metabolism of lead-treated erythrocytes have not previously been reported. In this study we found that the addition of lead to normal human blood causes a disturbance of the water metabolism, and we also found close relationships between the change in osmotic fragility and those in intracellular water content, intracellular potassium, MCV, trapped water content, and erythrocyte shape. Therefore, our results seem to agree well with Grigg's hypothesis.⁵

The contracted shape of erythrocytes observed by

Fig 7 Change in mean corpuscular volume of erythrocytes. Normal human blood was incubated for two hours at 37°C with lead.

scanning electron microscope indicates a decrease in erythrocyte flexibility, which leads to increases in intercellular space and trapped water content of packed erythrocytes. We assume that a pronounced increase in trapped water content with 500 and 1000 nM/ml of lead is related to the formation of echinocytes with crenation of cell membrane surface and generation of spicules caused by a pronounced decrease in intracellular potassium accompanying the water loss from the erythrocytes.

The MCV value increased a little with 1000 nM/ml of lead, and was not correlated with the results of intracellular water and potassium, trapped water content, and osmotic fragility. The MCV value consists of cell volume and intracellular space. Therefore, we assume that the increased MCV with 1000 nM/ml of lead is due to the considerably increased intercellular space and trapped water content.

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Fig 8 Scanning electron micrographs of erythrocytes. Normal human blood was incubated for two hours at 37°C with lead. (a) control, (b) 50 nM/ml of lead, (c) 100 nM/ml of lead, (d) 500 nM/ml of lead, (e) 1000 nM/ml of lead.

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