Mechanism of increased osmotic resistance of red cells in workers exposed to lead

I KARAI,¹ K FUKUMOTO,² AND S HORIGUCHI¹

From the Department of Preventive Medicine and Public Health,¹ Osaka City University Medical School, Abeno-ku, Osaka, 545, and Osaka Prefectural Institute of Occupational Health,² Higashi-ku, Osaka, 540, Japan

ABSTRACT In order to clarify the mechanism of the increased osmotic resistance of red blood cells in lead workers 19 men employed in a lead refining factory and 18 control male workers employed in railway construction were examined for red cell count, haematocrit, MCV, blood and urine lead concentrations, urine coproporphyrin and δ -aminolevulinic acid, osmotic resistance of red cells, lecithin-cholesterol acyltransferase (LCAT) activity in serum, and cholesterol content and cholesterol-to-phospholipid ratio of the red cell membrane. The results were: (1) The osmotic resistance of the red cells ($p < 0.05$), cholesterol content of the red cell membrane, blood and urine lead, urine coproporphyrin, and urine δ -ALA concentrations ($p < 0.01$) were higher in the lead workers than in the controls. (2) In the lead workers close relationships were observed between the osmotic resistance and the blood lead concentration ($r = -0.515$, $p < 0.05$), osmotic resistance and LCAT activity ($r = 0.596$, $p < 0.01$), and osmotic resistance and cholesterol of the red cell membrane ($r = -0.492$, $p < 0.05$).

An increase in the osmotic resistance of red blood cells has been observed in lead workers using a coil planet centrifuge system.¹⁻⁴ There is a dose-response relationship between the osmotic resistance of red cells and the blood lead concentration. The mechanism of the increased osmotic resistance in lead poisoning has not been completely clarified, however.

Recently, Cooper *et al*⁵⁻⁷ reported an increase in osmotic resistance of red cells in patients with obstructive jaundice and also a close relationship between increased osmotic resistance and an increased cholesterol-to-phospholipid ratio in the red cell membrane. Cooper concluded that membrane cholesterol was the major factor affecting osmotic resistance.

The present report describes changes in red cell osmotic resistance and cholesterol in lead workers and clarifies the mechanism of the increased osmotic resistance.

Materials and methods

Nineteen men aged 32-73 (mean 48) exposed to lead

in a scrap lead refining factory were studied, while 18 men aged 30-57 (mean 41.1) employed in railway construction served as controls. Workers with disordered liver function were excluded.

Blood sample were taken from a cubital vein into heparinised test tubes for the following examinations.

Osmotic fragility of red cells—The coil planet centrifuge system (Sanki Engineering Ltd, Japan) was used. Three haemolysis points (of the starting point, HSP; of the maximum point, HMP; and of the end point, HEP) of the haemoglobin distribution pattern in the coil tube were determined by scanning photodensitometer² (fig 1).

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

Haematocrit—The capillary tube method was used. The mean corpuscular volume was calculated by dividing the haematocrit value by the red cell count.

Lecithin-cholesterol acyltransferase (LCAT) activity in serum—The enzymatic method⁸ without EDTA was used.

Preparation of the red cell membrane—After removal of the serum and buffy coat, red cells were washed three times with 0.15 M NaCl and lysed with deionised water. The ghosts were washed with 2.5 mM Tris-HCl buffer (pH 7.5) several times to

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Fig 1 Osmotic fragility curve of normal human blood determined with a coil planet centrifuge. Ten microlitres of blood was put into a coiled tube and forced by centrifugation through a saline solution having a linear osmotic gradient with a range of 150-30 mOsmol. Thus red cells were exposed to gradually changing osmotic pressure down to a point where haemolysis occurred. The haemoglobin distribution pattern in the coiled tube was recorded by scanning photodensitometer. Three points of haemolysis (of the starting point, HSP; of the maximum point, HMP; and of the end point, HEP) were observed.

remove haemoglobin completely from the membrane.

Cholesterol of the red cell membrane—Five microlitres of 10% dodecyl sodium sulphate (SDS) were added to 20 μ l of dispersed membrane fragments and total cholesterol was measured by an enzymatic method.⁹

Protein of the red cell membrane—After dissolving the membrane fragments with 10% SDS, the protein content was measured by the method of Lowry *et al.*¹⁰ The amount of total cholesterol of red cell membrane was calculated by dividing the total cholesterol value by the protein concentration of the membrane.

Phospholipid of the red cell membrane—Total phospholipid was determined for inorganic phosphorus by the method of Shin.¹¹ The ratio of cholesterol-to-phospholipid of red cell membrane was calculated by dividing the total cholesterol value by the phospholipid value of red cell membrane.

Blood and urine lead—The dithizone-polarography¹² was used.

Urine δ -aminolevulinic acid (δ -ALA)—The method of Tomokuni and Ogata¹³ was used.

Urine coproporphyrin—The method of Rimington modified by Sano and Granick¹⁴ was used.

Statistical significance was tested by Student's *t*-test.

Results

The osmotic resistance of the red cells of the two

Table 1 Osmotic resistance (mOsm) of red blood cells by CPC method in lead workers and controls

	Lead workers (n = 19)	Controls (n = 18)
Haemolysis of starting point	56.7 $\pm$ 3.89*	59.2 $\pm$ 2.41
Haemolysis of maximum point	73.6 $\pm$ 4.12*	77.0 $\pm$ 3.25
Haemolysis of end point	94.5 $\pm$ 5.29	95.4 $\pm$ 4.68

*Significant difference at $p < 0.05$.

groups is shown in table 1; tables 2 and 3 show the results of the other tests. The values for cholesterol and cholesterol-to-phospholipid ratio of the red cell membrane, blood and urine lead concentrations, and urine coproporphyrin and δ -ALA concentrations were higher in the lead workers than in the controls, while the values for osmotic fragility, red cell count, haematocrit, and LCAT activity were lower. The mean corpuscular volumes were almost the same for the two groups. We found significant differences in osmotic resistance (HMP and HEP) ($p < 0.05$), membrane cholesterol ($p < 0.01$), blood and urine lead ($p < 0.01$), urine copropor-

Table 2 Clinical laboratory data for lead workers and controls

	Lead workers (n = 19)	Controls (n = 18)
Red blood cell ($10^6/\text{mm}^3$)	467 $\pm$ 40	493 $\pm$ 47
Haematocrit (%)	43.6 $\pm$ 3.1	46.0 $\pm$ 3.3
Mean corpuscular volume (μ^3)	93.8 $\pm$ 9.1	93.6 $\pm$ 5.3
Lecithin-cholesterol acyltransferase in serum (U)	105 $\pm$ 46	127 $\pm$ 57
Cholesterol in red cell (mg/protein mg)	244 $\pm$ 17**	226 $\pm$ 15
Cholesterol/phospholipid ratio in red cell	0.83 $\pm$ 0.033	0.79 $\pm$ 0.022

**Significant difference at $p < 0.01$.

Table 3 Lead exposure levels for lead workers and controls

	Lead workers (n = 19)		Controls (n = 18)	
	Geometric mean	Dispersion factor	Geometric mean	Dispersion factor
Blood lead ($\mu\text{g}/\text{dl}$)	41.6 $\pm$	1.26**	5.89 $\pm$	1.41
Urine lead ($\mu\text{g}/\text{l}$)	119.8 $\pm$	1.74**	10.8 $\pm$	1.42
Urine δ -ALA (mg/l)	9.13 $\pm$	3.34**	2.25 $\pm$	1.33
Urine copro ($\mu\text{g}/\text{l}$)	66.0 $\pm$	3.85**	12.3 $\pm$	1.80

ALA = Aminolevulinic acid; copro, coproporphyrin.

**Significant difference at $p < 0.01$.

Osmotic resistance and lead

Fig 2 Relationship between blood lead and osmotic resistance in lead workers.

Fig 3 Relationship between lecithin-cholesterol acyltransferase activity and osmotic resistance in lead workers.

Fig 4 Relationship between cholesterol of red cell membrane and osmotic resistance in lead workers.

phyrin ($p < 0.01$), and urine δ -ALA ($p < 0.01$), but not in red cell count, haematocrit, LCAT activity, and cholesterol-to-phospholipid ratio of red cell membrane.

There were significant correlations in the lead workers between blood lead and osmotic resistance (HMP) ($r = -0.515$, $p < 0.05$), LCAT activity and osmotic resistance (HMP) ($r = -0.596$, $p < 0.01$) and cholesterol of the red cell membrane and osmotic resistance (HMP) ($r = -0.492$, $p < 0.05$) (figs 2-4).

Discussion

Aub *et al*¹⁵ showed an increased osmotic resistance in red cells treated with lead in vivo and in vitro. These changes result from alterations in the structure of the red cell membrane, but the exact mechanisms of the increased osmotic resistance are not completely understood. For the in-vitro conditions the following hypothesis has been proposed. Lead causes a leakage of potassium but not sodium from red blood cells; a diminution in cell volume follows and hence an increased ratio of cell surface area to cell volume, allowing more water to enter the cell before it takes the critical spherical form that leads to haemolysis.¹⁶ The same hypothesis cannot be applied to the in-vivo condition, however, because a decrease in intracellular potassium has not been observed in lead workers.¹⁷

Cooper *et al*⁵⁻⁷ reported an increased osmotic resistance in patients with obstructive jaundice and summarised their results as follows: (1) An increased amount of bile salts in patients with obstructive jaundice inactivates LCAT. (2) Inactivation of LCAT causes accumulation of cholesterol and the cholesterol-to-phospholipid ratio of the red cell membrane. (3) Accumulation of cholesterol increases the surface-to-volume ratio of red blood cells. (4) Affected cells have a normal volume but an increased surface area, which gives them a broad, flat appearance described by the term "target cell." (5) The increased surface-to-volume ratio of red blood cells is definitely related to the osmotic resistance.

LCAT is an enzyme controlling cholesterol esterification in plasma and is derived from liver parenchymal cells. Therefore, inactivation of LCAT increases free cholesterol in serum, which is followed by free cholesterol accumulation in the red cell membrane.

We found a higher cholesterol-to-phospholipid ratio of the red cell membrane in lead workers than in controls in this study, but the difference between the two groups was not significant. Therefore, we assume that the increased osmotic resistance occurs through the following mechanism: a raised concentration of blood lead inactivates LCAT indirectly,

through impairment of the liver parenchyma, or directly. This is followed by the accumulation of cholesterol in the red cell membrane and an increment in the red cell surface, which leads to increased osmotic resistance. This hypothesis is almost the same as that for the formation of the target cell in a patient with obstructive jaundice.

This study has thus clarified the close relationships between increased osmotic resistance and increased cholesterol of the red cell membrane. From these observations, we assume that the major factor of the increased osmotic resistance in lead workers is the increase of cholesterol in the cell membrane.

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