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Lipid peroxidation and concentration of glutathione in erythrocytes from workers exposed to lead

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Abstract

Lipoperoxide concentration in erythrocytes from workers occupationally exposed to lead (mean blood lead concentration 57·1 (SD 17·6) μg/dl) was significantly higher than that in controls. It was not different in plasma from the two groups. The activity of superoxide dismutase (SOD) and catalase in erythrocytes from workers exposed to lead was significantly lower than that of control subjects. The effect of lead was also seen in the glutathione concentration of erythrocytes from lead exposed workers, which was reduced to 69% of that found in erythrocytes from control workers. The increase in methaemoglobin content of erythrocytes from workers exposed to lead was less than expected and not significantly different from that of controls. A positive correlation between lipoperoxide concentration in erythrocytes and lead concentration in blood and a negative correlation between glutathione concentration in erythrocytes and blood lead concentration were found. Incubation of erythrocytes for 24 hours at 37°C in the presence of lead (100 μg/dl) produced no changes in glutathione and lipoperoxide concentrations, although there was inhibition of activity of SOD (14·3%), catalase (10·1%), and glutathione peroxidase (35·1%). A similar experiment with heparinised whole blood showed increased haemolysis with no changes in membrane lipid peroxidation of erythrocytes. It is postulated that the lowered concentration of glutathione and decreased activity of SOD, catalase, and glutathione peroxidase in erythrocytes from workers exposed to lead may play a part in the increased membrane lipid peroxidation. Furthermore, the results suggest the possibility that leucocytes, or platelets, or both, may induce haemolysis in the presence of lead.

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Among heavy metals copper[1] and lead[2] are known to have an oxidising action. In lead induced anaemia production of Heinz bodies in erythrocytes has often been found,[3] suggesting that oxidative damage to haemoglobin may be induced by lead. Ribarov *et al*[2] showed that lead induced haemolysis is associated with peroxidation of erythrocyte membranes. Although lead itself cannot initiate the peroxidation by direct action on the membrane lipids, it stimulates haemoglobin catalysed lipid peroxidation.[4] Here superoxide radicals and hydrogen peroxide are likely to be implicated. The erythrocytes have a variety of mechanisms to protect proteins such as haemoglobin and also membrane function from superoxide radicals and hydrogen peroxide. For instance, these compounds, produced in erythrocytes, may be enzymatically removed by SOD,[5] glutathione peroxidase,[6] or catalase.[7] Thus the nature of oxidative stress induced by lead in erythrocytes remains unclear.

The present study was undertaken to examine membrane lipid peroxidation and the activity of some enzymes concerned with the removal of superoxide radicals and hydrogen peroxide of erythrocytes from workers exposed to lead.

Although much remains to be clarified in this regard, another point emphasised in our study is the participation of leucocytes and platelets in the increased haemolysis found in the presence of lead. To determine whether leucocytes and platelets make some difference to the oxidative stress of erythrocytes in the presence of lead, heparinised whole blood was incubated with lead, and the membrane lipid peroxidation of erythrocytes and the extent of haemolysis were compared with those of red blood cells alone.

Materials and methods

Heparinised venous blood was obtained from workers occupationally exposed to lead and normal subjects with no history of exposure. About 1 ml of blood was used for analysis of blood lead by the standard addition technique with a carbon rod atomic absorption spectrophotometer (Z-9000,

Table 1 Lipoperoxide, SOD, and catalase activities and glutathione and methaemoglobin concentrations in erythrocytes and plasma from workers exposed to lead

	Erythrocytes		*Plasma*	
	Control (mean (SD))	*Lead exposed workers (mean (SD))*	*Control (mean (SD))*	*Lead exposed workers (mean (SD))*
Lipoperoxide	15·5 (2·4)	25·2 (5·5)**	4·45 (0·76)	5·11 (0·61)
	(pmole/10^7 cells)		(nmole/ml)	
SOD (%)	25·0 (3·6)	10·7 (3·1)**	9·4 (2·9)	16·9 (2·5)**
Catalase (nmole/10^7 cells)	3·65 (0·43)	2·08 (0·25)**	—	—
Glutathione (mg/100 ml cells)	83·1 (7·9)	57·3 (11·8)**	—	—
Methaemoglobin (%)	1·4 (0·6)	3·5 (2·4)	—	—

**The difference between controls and lead exposed workers was statistically significant ($p < 0·01$; Student's *t* test).

Hitachi, Japan). The rest was centrifuged for 10 minutes at 3000 rpm and plasma was pipetted off. After removing white cells, erythrocytes were washed with 20 volumes of 0·15 M NaCl solution, and then suspended in the same solution.

Lipoperoxide concentration was determined by the method of Yagi,[8] and SOD activity by the nitro blue tetrazorium (NBT) method[9] using assay kits. Catalase activity was measured by the method of Beers and Sizer,[10] and glutathione peroxidase activity by the improved method of Gross,[11] in which 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB) was used for the measurement of glutathione. Glutathione concentration was determined by the method of Beutler *et al*,[12] haemoglobin concentration by the cyanogenmethaemoglobin method,[13] and methaemoglobin content was estimated according to Dubowski.[14]

For in vitro studies on incubation with lead, blood was withdrawn by venepuncture and placed in heparinised collecting tubes. After removing white cells, erythrocytes were mixed with two volumes of plasma. Erythrocytes and also heparinised whole blood were incubated for 24 hours at 37°C in the presence of lead (100 μg/dl). Because plasma glucose concentration was decreased below 20 mg/dl after 24 hours, glucose was added to give a concentration of 10 mM at the start of incubation.

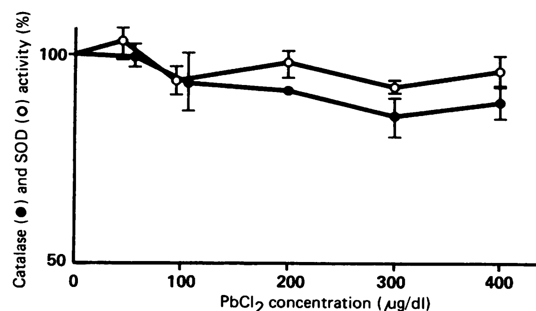

Figure 1 Effect of incubation with lead on activities of purified SOD and catalase.

Purified SOD (from erythrocytes) and catalase (from bovine liver) were purchased from Wako Pure Chemical Industries Ltd, Japan. These (SOD 0·12 mg/ml or catalase 0·1 mg/ml) were incubated for 30 minutes at 37°C with various lead concentrations in 0·05 M phosphate buffer, pH 7·4 and the final activities were then assayed as described.

Results and discussion

Lipoperoxide concentration was significantly higher in erythrocytes but not in plasma from workers exposed to lead (mean blood lead concentration 57·1 (SD 17·6) μg/dl) compared with controls (table 1). This shows that peroxidation of membrane lipids is stimulated in lead loaded erythrocytes. The activities of SOD and catalase in erythrocytes from workers exposed to lead were 42·8% (SOD) and 57·0% (catalase) of control subjects. When we tested a direct effect of lead on both purified enzymes, SOD activity was inhibited by about 10% and catalase activity by about 20% at a lead concentration of 400 μg/dl (fig 1). Thus the extent of decrease in SOD and catalase activities in erythrocytes from workers exposed to lead was much larger than the inhibition of purified enzymes. Because the decrease in activity of these enzymes in lead loaded erythrocytes, in which the glutathione concentration was maintained (table 2), was comparable with the direct inhibition of purified enzymes by lead, this difference may be due to the lowered concentration of glutathione in erythrocytes from lead exposed workers, which was only about 69% of the control value. As reduced glutathione serves to maintain the cysteine residues of haemoglobin and other proteins in the reduced state, the low concentration of glutathione would affect various enzymes and haemoglobin. Under oxidative stress, an increased tendency toward methaemoglobin formation has been found; the increase of methaemoglobin content in erythrocytes from lead exposed workers, however, was small.

Figure 2 shows the relation between lipoperoxide concentration in erythrocytes and blood lead concentration (regression equation $y = 0·13x + 13·8$,

Table 2 Effect of lead on SOD, catalase, and glutathione peroxidase activities, glutathione and lipoperoxide concentrations of erythrocytes, and haemolysis

	Red blood cells		*Whole blood*	
	Without lead (mean (SD))	*With lead (mean (SD))*	*Without lead (mean (SD))*	*With lead (mean (SD))*
SOD (%)	21·0 (1·0)	18·0 (2·0)	20·0 (1·0)	18·0 (2·5)
Catalase (nmole/10^7 cells)	2·67 (0·18)	2·40 (0·27)	2·04 (0·16)	2·03 (0·31)
Glutathione peroxidase	23·1 (5·2)	15·0 (1·9)	19·9 (7·8)	15·0 (4·8)
Glutathione (mg/100 ml cells)	96·4 (3·0)	93·1 (2·7)	91·9 (0·9)	96·3 (0·2)
Lipoperoxide (pmole/10^7 cells)	17·9 (0·9)	14·1 (1·1)	19·2 (5·6)	16·6 (1·0)
Haemoglobin in plasma (%)	0·033 (0·005)	0·030 (0·003)	0·070 (0·008)	0·187 (0·048)

r = 0·656). Figure 3 shows the relation between glutathione concentration in erythrocytes and blood lead concentration (regression equation y = −0·438x + 85·5, r = −0·631). Thus the present study shows a positive correlation between concentration of lipoperoxide in erythrocytes and blood lead concentration, and a negative correlation between the glutathione concentration of erythrocytes and concentration of lead in blood.

Although we have found that lymphocytes from lead exposed workers also contained lead (data not shown), it is unclear whether lead affects leucocyte function. We attempted to examine whether leucocytes and platelets alter the oxidative stress of erythrocytes in the presence of lead. When only erythrocytes were mixed with two volumes of plasma and incubated in the presence of lead (100 μg/dl) for 24 hours at 37°C with glucose, there was a decrease in activity of SOD (14·3%), catalase (10·1%), and glutathione peroxidase (35·1%) (table 2). The decrease of glutathione concentration in these erythrocytes was much less, and the lipoperoxide concentration did not show any increase. These results suggest that membrane lipid peroxidation may be difficult to produce if the glutathione concentration in erythrocytes is maintained. We noted that haemolysis did not occur in this situation. Furthermore, we have performed similar experiments with heparinised whole blood with lead added at a final concentration of 100 μg/dl. In comparison with red blood cells alone, the presence of leucocytes and platelets did not show any additional effects on the lipoperoxide and glutathione concentration of erythrocytes or on activities of SOD, catalase, and glutathione peroxidase (table 2). Thus leucocytes and platelets do not produce a noticeable additional effect on the oxidative stress of erythrocytes induced by lead. Interestingly, however, we noted an increased haemolysis in heparinised whole blood incubated with lead.

Discussion

We have shown a lowered glutathione concentration and decreased activity of SOD, catalase, and glutathione peroxidase, as well as increased membrane lipid peroxidation in erythrocytes from workers exposed to lead compared with controls. The study by Ribarov *et al*[4] using liposomes has shown that lead greatly stimulates lipid peroxidation in the presence of haemoglobin, but lead alone does not. As most lead in blood exists in erythrocytes[15] in protein bound forms,[16] lead protein complexes are likely to induce membrane lipid peroxidation. Ribarov *et al* also showed, based on the use of SOD and catalase, that superoxide radicals and hydrogen peroxide are partly

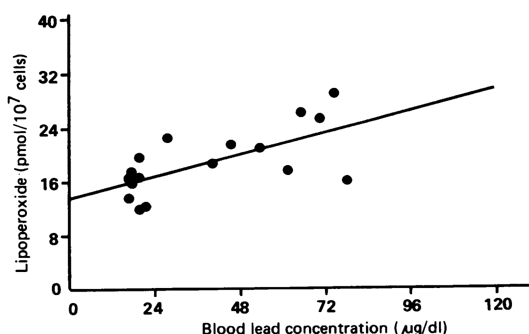

Figure 2 Relation between concentration of lipoperoxide in erythrocytes and blood lead concentration.

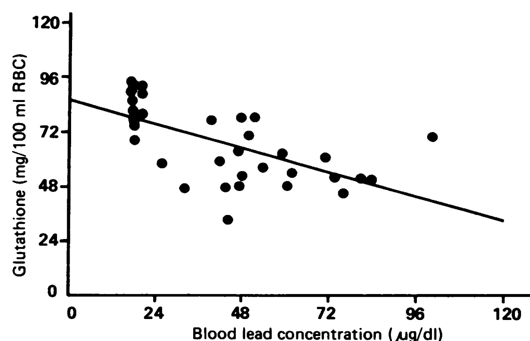

Figure 3 Relation between concentration of glutathione in erythrocytes (RBC) and blood lead concentration.

concerned in lipid peroxidation. Therefore, in lead loaded erythrocytes the increased production of oxidising intermediates, including superoxide radicals and hydrogen peroxide, is presumed. Erythrocytes have a variety of mechanisms to protect haemoglobin, other cytosolic proteins, and membrane function from superoxide radicals and hydrogen peroxide. For example, SOD, catalase, and glutathione peroxidase work to remove superoxide radicals and hydrogen peroxide produced in erythrocytes. In erythrocytes from workers exposed to lead, the activity of SOD and catalase was significantly decreased. The effect of lead was also reflected in reduced concentrations of glutathione peroxidase during the incubation of erythrocytes in the presence of lead. Therefore, the increased membrane lipid peroxidation in erythrocytes in lead exposed workers can be explained in part by the decreased activity of SOD, catalase, and glutathione peroxidase in these erythrocytes.

The hexose monophosphate shunt is important for maintaining glutathione concentration and is especially important in removal of hydrogen peroxide by glutathione peroxidase. The lowered level of glutathione in erythrocytes from lead exposed workers suggests two possibilities: either the utilisation of glutathione and NADPH is increased, or the flux of glucose through the hexose monophosphate shunt is inhibited in these erythrocytes. When red blood cells were incubated for 24 hours at 37°C in the presence of lead, the decrease in glutathione concentration, however, was much less. Interestingly, lipoperoxide concentration in these erythrocytes did not show any increase, despite the appreciable decrease in activity of SOD, catalase, and glutathione peroxidase. This may be because a supply of NADPH through the hexose monophosphate shunt is sufficient to maintain glutathione concentration and therefore protect membrane lipid peroxidation. Although we do not know yet whether the flux of glucose through the hexose monophosphate shunt is affected by lead, our results indicate a decreased efficiency of mechanisms for detoxification of oxidising intermediates, including superoxide radicals and hydrogen peroxide in erythrocytes from workers exposed to lead.

The degree of haemolysis found in heparinised whole blood containing lead is intriguing. Because the extent of haemolysis of heparinised whole blood without lead is much less, it is difficult to imagine that heparin, leucocytes, or platelets are alone responsible for the increased haemolysis. In the presence of lead, leucocytes, or platelets, or both are much more likely to induce haemolysis by hitherto unknown mechanisms rather than through membrane lipid peroxidation because the peroxidation of membrane lipids should be sufficiently protected in these erythrocytes. Although shortening the life span of circulating erythrocytes is one of the causes of lead induced anaemia,[17] the mechanism by which lead does this is not well understood.

We postulate that the shortened life span of circulating erythrocytes in lead poisoning may be attributed to the combined effect of (1) the decreased efficiency of the mechanisms to protect various cytosolic proteins and membrane lipid from superoxide radicals and hydrogen peroxide and (2) the increased susceptibility to haemolysis induced by leucocytes, or platelets, or both.

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