SHORT COMMUNICATION

HUMAN ERYTHROCYTE GLUTATHIONE REDUCTASE AND GLUCOSE 6-PHOSPHATE DEHYDROGENASE ACTIVITIES IN NORMAL SUBJECTS AND IN PERSONS EXPOSED TO LEAD

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SUMMARY

1. Blood lead and erythrocyte glutathione reductase (GSSG-R) and glucose 6-phosphate dehydrogenase (G6P-D) activities were measured in normal subjects and in those with occupational exposure to lead.

2. With increasing blood lead concentration, GSSG-R activity increases and that of G6P-D decreases.

3. It is suggested that these changes represent part of a compensatory mechanism to overcome the reduction of sulphhydryl groups by lead ions.

Key words: glutathione reductase, glucose 6-phosphate dehydrogenase, lead, 5-aminolaevulinic acid dehydrase, sulphhydryl groups.

The enzyme 5-aminolaevulinic acid dehydrase (ALA-D) governs the condensation of two molecules of 5-aminolaevulinic acid (ALA) to form porphobilinogen (PBG) and requires the presence of reduced glutathione (GSH) for its activation (Gibson, Neuberger & Scott, 1955). Dresel & Falk (1956) showed that lead had a marked inhibitory effect on this enzyme system and this has been confirmed many times since, particularly in the erythrocytes of those exposed to lead (e.g. de Bruin & Hoolboom, 1967). It has been suggested that part of the depression of ALA-D induced by lead is not a direct effect on the enzyme, but is mediated by some secondary mechanism (Bonsignore, Calissano & Cartasegna, 1965). In view of the generally accepted postulate that lead affects enzyme systems by its ability to combine with sulphhydryl groups (Waldron, 1966) it was considered possible that the observed depression of ALA-D in the presence of lead might be due to the metal combining with GSH, thus preventing or reducing the activation of the enzyme.

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In the present work the activities of erythrocyte glutathione reductase (EC 1.6.4.2) (GSSG-R) and glucose 6-phosphate dehydrogenase (EC 1.1.1.49) (G6P-D) were measured in subjects with a wide range of blood lead concentrations on the hypothesis that if lead is combining with GSH then the enzyme systems responsible for the production of GSH are likely to be affected.

METHODS

Subjects

Blood samples were taken from eighty-two persons who had given their consent for the procedure. The group was made up of normal subjects with no occupational exposure to lead, workers from a lead battery factory with a wide range of lead exposure and patients referred to the Occupational Medicine Out-Patient Clinic at the Manchester Royal Infirmary with suspected or manifest lead poisoning. The eighty-two subjects were grouped solely by their blood lead concentrations into three groups: those with lead concentrations below 1.43 µmol/l (40 µg/100 ml) of blood (group I, containing thirty subjects), those with concentrations from 1.98 to 3.38 µmol/l (41-70 µg/l00 ml) of blood (group II, containing thirty-seven subjects) and those with lead concentrations 3.42 µmol/l (71 µg/100 ml) of blood or over (group III, containing fifteen subjects). These approximate to the generally accepted concentrations of normal, acceptable and excessive lead absorption (Lane, Hunter, Malcolm et al., 1968), although on the basis of a number of cases of clinical lead poisoning with blood concentrations below 3.86 µmol/l (80 µg/100 ml) (Torkington & Bhalla, 1974) it was considered that the lower concentration of excessive exposure should be reduced from 3.86 to 3.42 µmol/l.

Experimental

Venous blood samples (5 ml) were taken from each subject by normal venepuncture at approximately the same time in the morning (about 10.30 hours). The samples were taken into heparinized syringes and the haemolysate was prepared within 1 h of the sample being taken.

Erythrocyte GSSG-R. The activity of this enzyme was measured by the method of Glatzle, Körner, Christeller & Wiss (1970). Units are expressed in µmol of NADPH converted into NADP⁺ in 1 min/ml of packed erythrocytes.

Erythrocyte G6P-D. The method used was that of King (1965). Units are expressed as µmol of NADPH formed from NADP⁺ in 1 min/ml of packed erythrocytes.

For both enzymes the activity in each case was calculated from the mean rate of change of extinction of three runs on the same sample. In each case the variation in the rate of the reaction was less than 5%.

Blood lead. This was measured at the National Occupational Hygiene Service in Manchester by using a modification of the dithizone method. The method used is reported and validated in the data from the European inter-laboratory comparison of methods for measuring blood and urine lead concentrations (Hoschek & Schittke, 1973).

RESULTS

The mean blood lead value for the thirty members of group I was 1.28 µmol/l (26.7 µg/100 ml), range: 0.48–1.84 µmol/l (10.0–38.0 µg/100 ml), that for the thirty-seven members of group II was 2.57 µmol/l (53.2 µg/100 ml), range: 1.18–3.38 µmol/l (21.0–70.0 µg/100 ml), and for the
fifteen members of group III it was 4.5 μmol/l (93.5 μg/100 ml), range: 3.47–5.99 μmol/l (72.0–124.0 μg/100 ml). The mean GSSG-R activity was 0.69 unit/ml (SD 0.14) in group I, 0.83 unit/ml (SD 0.21) in group II and 1.05 units/ml (SD 0.32) in group III. The mean G6P-D activity for group I was 2.10 units/ml (SD 0.33), that for group II was 1.87 units/ml (SD 0.32) and for group III it was 1.58 units/ml (SD 0.29).

Fig. 1. Changes in GSSG-R activity with rising concentrations of lead in blood. Abscissa values in parentheses are in μg/100 ml. Enzyme units are defined in the Methods section.

The relationship between blood lead concentration and activities of the two enzyme systems is shown in the scatter diagrams (Figs. 1 and 2). As the differences in mean values in the three
groups would suggest, there is a distinct increase of GSSG-R activity with rising amounts of blood lead \( (r = 0.538) \) and a concomitant decrease in the activity of G6P-D \( (r = -0.567) \). There is a good correlation when the activities of the two enzymes are plotted against one another \( (r = -0.501) \). The differences between the mean values for GSSG-R activity in the three groups was highly significant (groups I and II: \( t = 3.16, \text{df} = 65, P<0.005 \); groups II and III: \( t = 2.92, \text{df} = 50, P<0.005 \); groups I and III: \( t = 5.27, \text{df} = 43, P<0.001 \)). The same was also true of the differences between the mean values for G6P-D activity in the three groups (group I and II: \( t = 2.81, \text{df} = 65, P<0.005 \); groups II and III: \( t = 2.96, \text{df} = 50, P<0.005 \); groups I and III: \( t = 5.08, \text{df} = 43, P<0.001 \)).

**DISCUSSION**

The significance of free sulphydryl groups for the normal activity of ALA-D is underlined by the

![Fig. 2. Changes in G6P-D activity with rising concentrations of lead in blood. Abscissa values in parentheses are in μg/100 ml. Enzyme units are defined in the Methods section.](image)
necessity of GSH for its activation together with the fact that the inhibitory effect of lead on the system can be reversed in vitro by the addition of GSH to the reaction mixtures (Lichtman & Feldman, 1963). Similar results have since been reported by De Barreiro (1969, 1971). It therefore seems likely that the inhibitory effect of lead on ALA-D is thus the result of the metal combining with GSH. It has been known for a long time that lead decreases the amounts of GSH in the blood (Albahary, 1944), and more recently it has been shown that the decrease in plasma concentrations of GSH roughly corresponds to the length or degree of exposure to lead (Batolska & Marinova, 1970). The production of GSH is governed by the enzyme GSSG-R, which converts the double molecule of oxidized glutathione (GSSG) into two molecules of the reduced form (GSH). The reducing equivalents required for this transformation are derived from the reduced form of triphosphopyridine nucleotide (NADPH), which is thus converted into the oxidized form (NADP⁺). This NADP⁺ is reduced in turn by the action of G6P-D, thus enabling the action of GSSG-R to continue.

If GSH is being removed by lead then it might be expected that the enzymes responsible for its production would be stimulated by a mass-action type of effect. The results of this study show that this appears to be happening and the rise in GSSG-R activity with increases in blood lead accords with the suggestion that lead may be binding with GSH. Greenberg (1961) has suggested that there may be a biological control mechanism of enzymes through their sulphydryl groups and regulated through GSH. The rise in GSSG-R activity in association with increased blood lead appears to be part of such a control mechanism, designed, in this case, to compensate for the decrease in available sulphhydryl groups by lead ions. These relationships may be shown schematically:

The fall in G6P-D activities with increasing blood lead cannot be explained easily, however, on the above hypothesis.

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REFERENCES


