Lead and zinc concentrations in plasma, erythrocytes, and urine in relation to ALA-D activity after intravenous infusion of Ca-EDTA

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ABSTRACT Lead and zinc concentrations in plasma, erythrocytes, and urine, urinary ALA concentration, and ALA-D activity in blood were studied for four hours in two male lead workers during and after a one hour infusion of Ca-EDTA 2Na. Urinary and plasma lead concentrations increased as a result of administering Ca-EDTA 2Na, and the ratios of lead concentrations in plasma to those in urine were greatly increased. The increase of plasma lead concentration was not due to the haemolytic effect of Ca-EDTA 2Na but was mobilised lead, rapidly excreted in the urine. ALA-D activity in blood increased at the end of the experiment with a transient decrease during the infusion of Ca-EDTA 2Na. As zinc concentrations in erythrocytes and plasma did not decrease during the infusion despite an increase in the urinary excretion of zinc, the transient decrease of ALA-D activity was not due to a loss of zinc caused by Ca-EDTA 2Na. From the results of additional experiments *in vitro*, this transient decrease could be related neither to Ca-EDTA 2Na nor to lead in the blood.

The effects of intravenous infusion of calcium disodium ethylene diamine tetra-acetate (Ca-EDTA 2Na) on the elimination of lead from the body have been well studied.¹⁻⁸ In most studies^{1-5,7,8} the lead concentration in the whole blood has been determined, and attention has been given mainly to the changes reflecting lead absorption. The selective determination of plasma lead concentrations is of great importance for the study of the urinary excretion of lead but is found in few reports.^{6,9} McRoberts observed that plasma lead concentrations decreased 2.5 hours after the administration of Ca-EDTA 2Na with a clear increase in urinary lead excretion,⁶ but he did not study the time course of the lead concentrations in the early stages after the initiation of the Ca-EDTA 2Na infusion. Few detailed data for the urinary excretion of lead in the early phase of Ca-EDTA 2Na infusion are available. Therefore, we have examined the time course of lead concentrations in plasma, erythrocytes, and urine in the early stages of the lead mobilisation test by Ca-EDTA 2Na.

The inhibition of delta aminolaevulinic acid

dehydratase (ALA-D) activity in erythrocytes is a useful indicator of lead absorption, and the inhibition is correlated with the blood lead concentration.^{10,11} It is reasonable, therefore, to consider what effects the administration of Ca-EDTA 2Na might have on ALA-D activity. Chisolm observed in the treatment of lead intoxicated children with Ca-EDTA 2Na that the return to normal levels of delta aminolaevulinic acid (ALA) in urine and plasma was preceded by a transient rise that was maximal between eight and 16 hours after the institution of treatment.¹² Hammond considered that this transient rise in ALA concentrations in urine and plasma might be due to a transient increase in ALA-D inhibition.¹³ Thomasino *et al* observed the fall of ALA-D activity in human erythrocytes during the infusion of Ca-EDTA 2Na despite the clear reduction in the body burden of lead,¹⁴ and they considered that the fall of ALA-D activity during the infusion of Ca-EDTA 2Na resulted from the loss of zinc caused by Ca-EDTA 2Na. Nevertheless, they did not present the data relating to ALA-D activity and zinc concentrations in the early phase of Ca-EDTA 2Na infusion, and did not determine the zinc concentration in the erythrocytes. Therefore, we have examined the time course of ALA-D activity in

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Results of the medical examination of two Japanese male workers

	Subject 1	Subject 2
Blood:		
Erythrocytes ($\times 10^6/\text{mm}^3$)	473	514
Haemoglobin (g/100 ml)	15.4	15.4
Haematocrit (%)	43.3	47.8
Reticulocytes (0/00)	16	14
Lead (nmoles pb/100 ml of whole blood)	270	300
Urine:		
Coproporphyrin ($\mu\text{g/g}$ creatinine)	30.8	25.3
ALA (mg/g creatinine)	4.3	3.1

Examined in February 1981; the experiments took place in March 1981.

erythrocytes and the zinc concentrations in the plasma, erythrocytes, and urine in order to study the effect of Ca-EDTA 2Na administration on ALA-D activity.

Subjects and methods

SUBJECTS

Two male Japanese workers, who have been exposed to inorganic lead (lead oxides) at a concentration of 0.01–3.16 mg Pb/m³ (8 hours/day, 44 hours/week) consented to take part in the experiment after being informed of the procedure and expected sequelae. Subject 1 was 46, weighed 50 kg, and had been engaged for 10 years in a plant manufacturing rubber belts, hose, and containers. Subject 2 was 45, weighed 65 kg, and had worked in the same plant for 11 years. The table shows the results of their most recent periodical medical examination. They have had no complaints or symptoms suggestive of inorganic lead poisoning.

ADMINISTRATION OF Ca-EDTA 2Na AND COLLECTION OF SAMPLES

Ca-EDTA 2Na was administered intravenously for one hour in a dose of 20 mg/kg in 250 ml of 5% glucose solution. Urine was collected immediately before the intravenous infusion was started and was then collected every hour for four hours. Five millilitres of blood was collected into heparin from the cubital vein at 0, 0.5, 1, 2, 3, and 4 hours after the start of the infusion.

LEAD DETERMINATIONS

Twenty millilitres of urine were digested by the addition of an acid mixture (5 ml of 14.9 N nitric acid, 1 ml of 11.7 N perchloric acid, and 1 ml of 36 N sulfuric acid). The digested samples were diluted to 10 ml by doubly distilled water, and taking 10 μl of the diluted samples, the urinary lead concentration was determined by flameless atomic absorption spectrophotometry (Jarrel-Ash, AA

8200 and FLA 100). The minimum detectable amount of lead in our laboratory was 2.4 pmoles of lead, and the coefficient of variation was 2.9%. Blood samples were centrifuged at 3000 rpm for 15 minutes immediately after sampling. After removing the plasma, doubly distilled water was added to the erythrocytes to obtain a haemolysate equal to that of the sampling volume. Two millilitres of plasma or 5 ml of haemolysate were digested by the same procedure as that used for the urine and the digested samples were diluted to 10 ml by doubly distilled water; lead concentrations in 20 μl of the diluted sample were determined by the method used for the urine.

ZINC DETERMINATIONS

Using the digested and diluted samples of urine, plasma, and erythrocytes, zinc concentrations were determined by flame atomic absorption spectrophotometry (Perkin-Elmer, Model 370). The minimum detectable concentration of zinc in our laboratory was 9 nmoles of Zn/ml of digested and diluted sample.

ASSAY OF ALA-D ACTIVITY

The activity of ALA-D was assayed by the European standard method¹⁵ using 0.1 ml of heparinised blood.

EFFECTS OF Ca-EDTA 2Na ON ALA-D ACTIVITY IN BLOOD

Blood was obtained from a Japanese man, age 44, with no occupational exposure to inorganic lead, and heparinised. ALA-D activity was measured in whole blood or in erythrocytes suspended in physiological saline by the European standard method¹⁵ in the presence of various concentrations of Ca-EDTA 2Na (0.5×10^{-6} M– 1.0×10^{-3} M in the assay system). The number of erythrocytes in the suspension was equal to that in the whole blood. The effect of the preincubation of whole blood or erythrocyte suspensions in physiological saline with Ca-EDTA 2Na was studied as follows; 1.0 ml of whole blood or erythrocyte suspension was preincubated at 37°C with various concentrations of Ca-EDTA 2Na (10^{-6} M– 10^{-3} M in the preincubation mixture) for 30, 60, 120, and 180 minutes. The erythrocytes were then collected by centrifugation at 3000 rpm for 20 minutes, washed twice, and suspended in physiological saline to a volume 1.0 ml. ALA-D activity in this suspension was measured by the European standard method.¹⁵

EFFECT OF INORGANIC LEAD ON ALA-D ACTIVITY

Using whole blood from a Japanese man with no

occupational exposure to inorganic lead, the effect of inorganic lead on ALA-D activity was examined. A neutralised aqueous solution of lead nitrate was added to the reaction mixture for the assay of ALA-D activity up to a final concentration of 1.0×10^{-5} M.

HAEMOLYTIC EFFECT OF Ca-EDTA 2Na

To 5 ml of heparinised blood, obtained from a Japanese man with no occupational exposure to inorganic lead was added 0.005 ml of Ca-EDTA 2Na solution (0.12, 0.24, and 0.47 M in water or physiological saline). The blood samples were incubated at 37°C for 30, 60, and 90 minutes, and plasma was separated by centrifugation at 3000 rpm for 20 minutes. The haemoglobin concentration in the plasma was determined spectrophotometrically.

OTHER TESTS

Urinary creatinine was determined by Jaffe's reaction. The ALA concentration in the urine was

Fig 1 Time course of lead concentrations in plasma, erythrocytes, whole blood and urine, and ALA concentration in urine. Hours after start of infusion are plotted horizontally. Ordinate indicates lead concentration in plasma (nmoles pb/ml, ○), erythrocytes (nmoles pb/ml, ●), whole blood (nmoles pb/ml, △), and urine (nmoles, pb/mg creatinine, ■), and ALA concentration in urine (nmoles ALA/mg creatinine, ▲).

Fig 2 ALA-D activity in blood. Hours after start of infusion are plotted horizontally. Ordinate indicates ALA-D activity in blood (units: ○—subject 1, ●—subject 2).

determined by the method of Wada *et al.*¹⁶ The haematocrit was determined using the capillary method.

Results

Figure 1 indicates the change of the lead concentrations in the plasma, erythrocytes, urine, and whole blood with time. The urinary ALA concentration is also indicated. The urinary lead concentration shows a sharp rise during the Ca-EDTA 2Na infusion in both subjects, and stays at a raised level thereafter. In both subjects the plasma lead concentration begins to increase at 0.5 hours, and reaches a maximum at the end of the infusion. The lead concentration in the erythrocytes, on the other hand, decreases in the first half hour, by contrast with the increase of plasma lead concentration. The urinary ALA concentration does not decrease during the course of the experiment.

As shown in fig 2, the ALA-D activity in the blood continues to decrease during the infusion, but increases after the infusion is finished. The decrement in the ALA-D activity at the end of the infusion is 42.5% or 38.7% of the activity at zero time.

Figure 3 indicates that the zinc concentrations in the erythrocytes and the plasma do not decrease despite the clear increase in the urinary excretion of zinc after the start of the Ca-EDTA 2Na infusion.

The results of the in vitro experiments are shown in fig 4. The ALA-D activity in the blood is

Fig 3 Time course of zinc concentrations in plasma, erythrocytes, and urine. Hours after start of infusion are plotted horizontally. Ordinate indicates zinc concentration in plasma (nmol Zn/ml, ○), erythrocytes (nmol Zn/ml, ●) and urine (nmol Zn/mg creatinine, ■).

decreased by the addition of Ca-EDTA 2Na to a final concentration of 2.5×10^{-5} M or more, but the decrement of ALA-D activity is about 10–20% of the control activity. Another experiment in vitro (fig 5) indicates that the inhibition of ALA-D activity by added inorganic lead is about 10% at the final concentration between 5.0×10^{-8} M and 10^{-6} M; 50% inhibition is achieved at 5.0×10^{-5} M. Pretreatment of the whole blood or erythrocytes suspension with Ca-EDTA 2Na at a final concentration between 10^{-6} M to 10^{-3} M did not reduce ALA-D activity.

Fig 5 Effect of inorganic lead on ALA-D activity in erythrocytes. Final concentration of lead in the assay system is plotted horizontally. Ordinate indicates ALA-D activity (% of the activity without added inorganic lead).

Discussion

Our experiments in vivo indicate that the intravenous administration of Ca-EDTA 2Na increases the concentrations of lead in the plasma and in the urine, and that ALA-D activity in blood is depressed during the infusion. It is also clear that zinc concentrations in the plasma and erythrocytes are not decreased by the infusion of Ca-EDTA 2Na despite the clear increase in urinary zinc excretion. Experiments in vitro do not present evidence for a clear relationship between the transient decrease in ALA-D activity and the blood concentrations of lead or Ca-EDTA 2Na.

McRoberts reported a fall in plasma lead concentrations after Ca-EDTA 2Na administration to lead workers,⁶ but he did not determine the plasma lead concentration before the Ca-EDTA 2Na treatment was begun nor did he examine the time course of lead concentrations in the early stages of the treat-

Fig 4 Effect of Ca-EDTA 2Na on ALA-D activity. Final concentration of Ca-EDTA 2Na in assay system is plotted horizontally. Ordinate indicates the ALA-D activity (% of the activity without Ca-EDTA 2Na; ○—whole blood, ●—erythrocytes). Dotted line indicates calculated maximum concentration of Ca-EDTA 2Na in assay system.

ment. As the half life of Ca-EDTA 2Na after intravenous infusion in man is 65 minutes,¹⁷ the amount remaining at the end of treatment and the concentration in the circulating blood can be calculated (the circulating blood volume for male adult Japanese is 90 ml/kg). The maximum concentration thus obtained at the end of the infusion is 0.42 mM (subject 1) or 0.43 mM (subject 2). In the in vitro experiment no release of haemoglobin from the erythrocytes into the plasma was observed in the presence of 0.47 mM of Ca-EDTA 2Na. Therefore, the rise in plasma lead concentrations is not the result of haemolysis, but a real increase in plasma lead concentration due to the mobilisation of lead from the tissues by Ca-EDTA 2Na. The rapid rise of the lead concentration in the urine suggests that the mobilised lead is readily eliminated.

Chisolm, Hammond, and Thomasino *et al* reported a fall in ALA-D activity in human erythrocytes during the infusion of Ca-EDTA 2Na, and we observed the same phenomenon.¹²⁻¹⁴ As shown in fig 2, ALA-D activity continues to decrease during the infusion of Ca-EDTA 2Na without an increase in erythrocyte lead concentration, but increases when the infusion ends. Thomasino *et al* considered that the transient fall in ALA-D activity during the infusion resulted from a loss of zinc caused by Ca-EDTA 2Na.¹⁴ If so the zinc concentration in erythrocytes should decrease during the infusion; this is not the case in the present study. As shown in fig 3, zinc concentrations in the plasma and the erythrocytes do not decrease during the infusion of Ca-EDTA 2Na, notwithstanding the clear increase in the urinary excretion of zinc. Therefore, the fall of ALA-D activity during the infusion does not result from a loss of zinc.

The interaction between the erythrocytes and the Ca-EDTA 2Na in the circulating blood might have an effect on the ALA-D activity in the erythrocytes. But after the preincubation of whole blood or erythrocyte suspensions with Ca-EDTA 2Na for 180 minutes at a final concentration of 10^{-3} M (which is greater than the calculated maximum concentration) the ALA-D activity was not affected. Therefore, the fall in ALA-D activity during the infusion does not result from an interaction between Ca-EDTA 2Na and the erythrocytes.

As whole blood is used as the enzyme source in the experiments shown in fig 2, the inhibition of the ALA-D activity by the carry-in of Ca-EDTA 2Na or plasma lead is possible. The carry-in of Ca-EDTA 2Na into the assay system is about 3.4×10^{-5} M (final concentration), because the calculated maximum concentration of Ca-EDTA 2Na in the circulating blood is 0.43 mM. As shown in fig 4, the decrement of ALA-D activity is less than 20% of

the control activity even in the presence of Ca-EDTA 2Na at a final concentration of 1×10^{-4} M, and is smaller than that shown in fig 2. Therefore, the inhibitory effect of Ca-EDTA 2Na in the blood can only partly explain the reduction of ALA-D activity during the infusion of Ca-EDTA 2Na.

The possibility of the inhibition by the carry-in of lead in plasma should be examined. The maximum lead concentration in the plasma in subject 1 is 0.16 nmoles/ml, and his haematocrit value is 45%. Therefore, the maximum increment of lead concentration in the assay system for the ALA-D activity due to carry-in is about 4×10^{-9} M. Figure 5 indicates that the decrement of the ALA-D activity is about 10% in the presence of added inorganic lead at a final concentration of 10^{-7} M. Therefore, the decrease of ALA-D activity during the infusion seems not to result from the carry-in of lead in the plasma. The mechanism of the reduction of ALA-D activity during the infusion cannot be explained completely at present and is under further investigation.

Chisolm observed a transient increase in the ALA concentration in urine between eight and 16 hours after the institution of Ca-EDTA 2Na treatment to lead intoxicated children,¹² but he presented no data from an earlier phase after the start of treatment. Haeger-Aronsen reported a clear decrease in the ALA concentration in the urine in man four hours after Ca-EDTA 2Na administration.¹⁸ In our experiment, however (fig 1), the urinary ALA concentration does not decrease four hours after treatment. This discrepancy between our observation and others might be dependent on the difference of the exposure level for the subject.

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