

## IN VIVO INTERACTION OF LEAD WITH AMINOLEVULINIC ACID DEHYDRATASE AND INDUCTION OF A THERMOLABILE FACTOR: AN EXPERIMENTAL MODEL

(Aminolevulinic acid dehydratase; thermolabile factor; lead)

ANDREA TREVISAN, PAOLO CHIESURA, MASSIMO SABBATUCCI, MARCO RAIMONDI and AMEDEO BUZZO

*Istituto di Medicina del Lavoro, Università di Padova, Padova (Italy)*

(Received December 3rd, 1982)

(Revision received February 21st, 1983)

(Accepted March 1st, 1983)

---

### SUMMARY

Aminolevulinic acid dehydratase (ALA-D) activity of male albino Wistar rats was used as an experimental model for a study on the interaction of lead with biological systems. Lead at 1 mg/kg was administered i.p. and the rats were killed immediately, and at 30 min, 1, 2, 3, and 4 h after treatment. It was shown that lead (Pb) interacted directly with the enzyme molecule immediately after treatment, first on the active site of zinc (Zn) and then on the thiolic groups. Induction of the so-called thermolabile factor (TF) seemed to occur later, i.e., it may only be shown from the 2nd-3rd h after treatment. The long-term persistence of lead-induced TF in the acute phase of intoxication may be the key to the interpretation of some chronic toxic effects.

---

### INTRODUCTION

Many studies have been carried out to identify the relationships between Pb and biological systems. As yet there is no definite experimental evidence on how this metal exerts its toxic action in chronic conditions and in those situations in which effects still remain after exposure indexes have returned to normal after removal from risk.

The most interesting model for the study of the biological effects of lead seems to be the activity of the ALA-D (EC 4.2.1.24), commonly studied as a response test

---

Abbreviations: ALA-D, aminolevulinic acid dehydratase; GSH, reduced glutathione; PbB, lead in blood; TF, thermolabile factor; VC, variation coefficient.

to lead and considered an important index of subcritical effect [1]. This enzyme may be analysed easily and simply and, as its molecule contains an essential metal such as Zn and 56 thiolic residues [2], it is a biological target for lead. The direct *in vitro* interaction of lead with the enzyme can be seen experimentally, due to its chemico-physical features [3]: on the one hand, lead removes zinc competitively from the metal site [4], and on the other it bonds with the thiolic groups [5].

Our previous research [6] on chronic exposure hypothesized possible interaction of Pb with the  $-SH$  groups. Moreover, observing that, in chronically exposed subjects, heat treatment of the enzyme at  $60^{\circ}C$  for 5 min sufficed for complete enzyme reactivation, also advanced the hypothesis [7] of a different action by Pb: in these situations a probably protein-based TF inhibiting ALA-D activity is induced. This factor has recently been identified as bonded to fractions of hemoglobin [8] and is also present in small concentrations in unexposed subjects, in relation to the admittedly low degree of absorption of lead conditioned by environmental pollution.

The aim of this study was to verify how lead inhibits ALA-D activity *in vivo* and after a single injection. The reactivation capacity of the effectors with various mechanisms was studied: i.e., Zn which is able to remove competitively Pb from the metal site; GSH which reactivates the thiolic groups blocked by Pb; and heat reactivation, to establish how soon the TF begins to act after the first contact between Pb and the organism.

#### METHODS

14 male albino Wistar rats, initial weight 200 g (S. Morini, S. Polo d'Enza, RE, Italy) were subdivided into 7 groups each of 2 rats of which 6 were treated with  $Pb(NO_3)_2$  (C. Erba, Milan, Italy) dissolved in 0.5 ml deionized water with a dosage of 1 mg/kg *i.p.*, once. 2 animals were killed at time 0 and considered controls; the other 12 were killed in twos, at 30 min, 1, 2, 3 and 4 h after treatment. Blood was withdrawn from the heart under light anesthesia and preserved in ice in heparinized test-tubes.

ALA-D activity in blood was assayed immediately using the standardized European method [9]; the pH of the substrate (ALA HCl 0.01 M, Merck, Darmstadt, FRG) being brought to 7.0 with phosphate buffer. This pH was necessary to obtain maximum activation from the GSH. The blood was then subdivided, placed in 4 test tubes, and treated as follows: (1) considered as a control; (2) heat-treated at  $60^{\circ}C$  for 5 min in a thermostable bath according to the method of Gerhart and Pardee [10]; (3) treated with GSH (Merck, Darmstadt, FRG) at a final concentration of  $1.0 \cdot 10^{-2}$  mol/l; (4) treated with Zn (C. Erba standard, Milan, Italy) at a final concentration of  $1.5 \cdot 10^{-4}$  mol/l. Incubation with GSH and Zn lasted 10 min at room temperature.

In our laboratory, the VC for assaying ALA-D activity is  $\pm 5\%$ . Spectrophotometer determination was carried out using a Perkin-Elmer model 550.

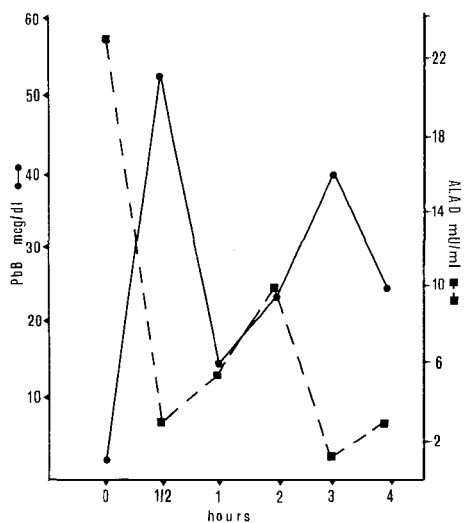


Fig. 1. Trend of lead in blood (PbB) and ALA-D activity at different times after treatment.

PbB was determined using the direct method of Fernandez [11] after dilution in Triton X 0.1%, using an atomic absorption spectrophotometer Perkin-Elmer model 305 with an HGA76B graphite furnace and background corrector. The VC of the method was  $\pm 5\%$ ; accuracy was 92–95%.

## RESULTS

Fig. 1 shows the trend of PbB after a single i.p. injection of lead. The rapid increase in blood concentration after 30 min and later settling due to distribution are both evident. ALA-D activity is also shown.

Fig. 2 shows inhibition of ALA-D activity and its behavior after treatment with the activators at different times after injection. The most significant results are: (1) Zn (with only a small activating effect under basic conditions: time 0 + 12%), is constantly able to reactivate enzyme activity for the whole length of the experiments, from 30 min to 4 h after injection; (2) GSH inhibits enzyme activity in untreated rats (–18%): strong activation is observed in the blood sampled 1 h after treatment and maximum activation occurs 3–4 h later; (3) heat treatment (60°C for 5 min) causes a slight reduction in enzyme activity in untreated rats (–6%); this inhibiting effect is even more evident and accentuated 30 min and 1 h after treatment; at 2 h activation is moderate, and is further accentuated at 3 h.

## DISCUSSION

The sequence of interactions of Pb with the active sites of the enzyme ALA-D may be deduced by comparing the effects produced by the different activating

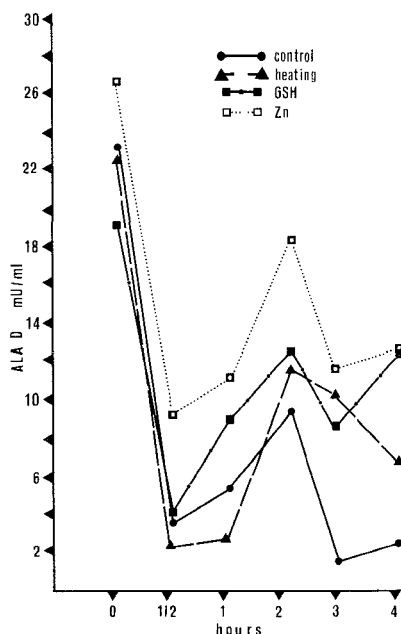


Fig. 2. Effect of activating systems (heat treatment at 60°C for 5 min, GSH at final concentration of  $1.0 \cdot 10^{-2}$  mol/l and Zn at final concentration of  $1.5 \cdot 10^{-4}$  mol/l) at time 0 and at various moments after single injection of lead.

systems. The first target appears to be the metal site of Zn, which Pb is able to replace and from which it is displaced (at least partially) with relative enzyme reactivation, by adding a suitable concentration of zinc. Blocking of the thiolic groups comes later, as shown by reactivation on adding GSH. Lastly, heat treatment is capable of developing reactivation from the 2nd h onwards, and shows the presence of the so-called TF, which therefore should be induced early.

TF induction is evident when the enzyme reaches its maximum inhibition (3rd h). It is interesting to note how in this phase, complete reactivation of the enzyme activity is obtained by adding the effects of heat treatment, GSH and Zn. The percentages show how, between the 3rd and 4th h after treatment, about 70% of enzyme inhibition is due to the direct interaction of Pb with the molecule (approx. 37% with the thiolic groups, approx. 43% with the metal site); the remaining 30% is probably due to induction of the TF.

The mechanism of early though not immediate induction of TF is not yet known. However, it is clear that direct inhibition caused by the interaction of lead with the enzyme molecule is added to partial early inhibition due to TF. Data exist for believing that the TF is the sole factor responsible for inhibition of ALA-D activity encountered in cases of chronic and past intoxication: in effect, in cases, heat treatment causes complete reactivation, while the removal of lead by chelating therapy with  $\text{Na}_2\text{Ca EDTA}$  has no effect [6, 7, 12]. The effects that other activators such

as GSH and Zn exert on ALA-D activity in these types of intoxications should therefore be due to an aspecific positive allosteric effect, and not to removal of Pb by the enzyme molecule. This further suggests that TF acts by modifying the structure of the enzyme which regains its catalytic capacity in the presence of positive allosteric effectors.

#### REFERENCES

- 1 G.F. Nordberg, Effects and dose-response relationships of toxic metals. A report from an international meeting, *Scand. J. Work Environ. Health*, 2 (1976) 37.
- 2 P.E. Gurba, R.E. Sennet and R.D. Kobes, Studies on the mechanism of action of delta-aminolevulinic acid dehydratase from bovine and rat liver, *Arch. Biochem. Biophys.*, 150 (1972) 130.
- 3 J.R. Davis and M.J. Avram, Correlation of the physicochemical properties of metal ions with their activation and inhibition of human erythrocytic delta-aminolevulinic acid dehydratase (ALA D) in vitro, *Toxicol. Appl. Pharmacol.*, 55 (1980) 281.
- 4 A. Trevisan, G.P. Gori, A. Zangirolami, C. Benevento, A. Rosa and P. Chiesura, Site of action of metals on the aminolevulinic acid dehydratase of human erythrocytes, *Enzyme*, 25 (1980) 33.
- 5 P. Calissano, C. Cartasegna e D. Bonsignore, Azione di alcuni metalli sull' ALA-deidratasi eritrocitaria purificata da sangue umano, *Lav. Umano*, 17 (1965) 493.
- 6 A. Trevisan, V. Chinello, A. Buzzo and G.P. Gori, Postulated mode of action of lead on aminolevulinic acid dehydratase in chronic exposure, *Int. Arch. Occup. Environ. Health*, 48 (1981) 295.
- 7 D. Bonsignore, C. Cartasegna, C. Vergnano e V. Ardoino, Studi sulla intossicazione umana e sperimentale da piombo. Sul meccanismo inibitorio dell' ALA-deidratasi eritrocitaria, *Med. Lavoro*, 59 (1968) 419.
- 8 T. Sakai, S. Yanagihara and K. Ushio, Erythrocyte factors concerned in the inhibition of ALA-D by lead, *Br. J. Ind. Med.*, 38 (1981) 268.
- 9 A. Berlin and K.H. Schaller, European standardized method for the determination of delta aminolevulinic acid dehydratase activity in blood, *Z. Klin. Chem. Klin. Biochem.*, 12 (1974) 389.
- 10 J.C. Gerhart and A.B. Pardee, The enzymology of control by feedback inhibition, *J. Biol. Chem.*, 237 (1962) 891.
- 11 F.J. Fernandez, Micromethod for lead determination in whole blood by atomic-absorption, with use of the graphite furnace, *Clin. Chem.*, 21 (1975) 558.
- 12 D. Bonsignore and F. Ottenga, Different comportamento della ALA D eritrocitaria nel saturnismo florido e cronico sotto trattamento col sale disodico monocalcico di EDTA, *Atti del XXXVIII Congresso Nazionale di Medicina del Lavoro. Padova-Verona, 20-22 ottobre 1975. Parte I*, pp. 81-84.