Evidence of the induction of de novo synthesis of δ-aminolaevulinate dehydratase by lead

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ABSTRACT Inhibition of δ-aminolaevulinate (ALA) dehydratase (porphobilinogen synthase: EC 4.2.1.24) coupled with an increase in the enzyme concentration was observed in the liver of rats exposed to lead by mouth for 150 days. ALA dehydratase concentration increased by 25% in rats exposed to lead and cell free translation also showed an increase in de novo synthesis of ALA dehydratase by 20% in the liver of rats exposed to lead. The addition of lead in vitro to the cell free translation system had no effect on the de novo synthesis of ALA dehydratase. These results suggest that exposure to lead caused a transcription dependent induction of ALA dehydratase which might compensate for the enzyme inhibition by lead.

Delta-aminolaevulinate (ALA) dehydratase (porphobilinogen synthase:EC4.2.1.24) is well known to be inhibited by lead via mercaptide formation,¹ and the inhibition of ALA dehydratase activity in peripheral erythrocytes is well recognised as one of the most sensitive indicators of exposure to lead.²⁻⁵ The enzyme plays a limiting part in haem metabolism under some conditions⁶⁻⁷ and the de novo synthesis of the enzyme protein may occur to compensate the enzyme inhibition by lead. In 1978 Maes and Geber reported a phenomenal increase in ALA dehydratase activity in the peripheral erythrocytes of lead treated animals⁸ which was considered to be due to the chelation of lead by phosphate used in the enzyme assay and to an increase in the dehydratase concentration,⁹ which seems to result from an increase in “young erythrocytes.”¹⁰

We developed a radioimmunoassay of ALA dehydratase in 1980 to look for an increase in the enzyme protein in men⁵ and animals¹⁰⁻¹¹ exposed to lead. There was evidence to suggest that exposure to lead could induce ALA dehydratase⁵⁻¹¹ but the effects of anaemia on the enzyme concentration could not be completely excluded.

The aim of the present study was to try to detect an induction of de novo synthesis of ALA dehydratase in the liver after exposure to lead, since the hepatic enzyme is not affected by anaemia. Lead has a Janus faced effect on ALA dehydratase; it both inhibits its activity and induces its de novo synthesis.

Materials and methods

Ten male Wister rats, weighing 254 ± 28 g (mean ± SD) were kept in a temperature and light controlled room (24–26°C; 12 hour light/12 hour dark). They were fed ad libitum with commercial laboratory food and deionised water. The animals were divided into the control (n = 5) and lead treated (n = 5) groups. The lead treated group daily received 25 mM lead acetate in deionised water ad libitum as described in a previous report.¹⁰ After 150 days of exposure to lead the rats were killed and the livers collected after perfusion with isotonic KC1 via the abdominal aorta. Body weight and liver wet weight were recorded. The activity and concentration of ALA dehydratase, total protein concentration, and the lead concentration were estimated in the liver homogenate. A portion of the liver (5 g of each) was supplied to prepare polysomes for cell free translation.¹²

ALA dehydratase was purified from rat liver as described previously.¹² The purified preparation is homogenous by analytical polyacrylamide gel electrophoresis both in the presence and absence of sodium dodecyl sulphate,¹⁰ and has a specific activity to form 23.8 μmoles of porphobilinogen/mg.protein/hour at 37°C. ALA dehydratase was labelled with 125-I (Amersham International Ltd, Bucks, UK) according to the previous report,¹⁰ and had a specific radioactivity of 6.5 μCi/μg.

The assay of the enzyme activity was carried out with or without dithiothreitol (DTT) and zinc.¹⁰ One unit of enzyme activity was defined as the activity
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which catalysed the formation of 1 μmole of por-
phobilinogen/hour at 37°C. The inhibition ratio of the
dehydratase (%)—that is, the ratio of inhibited
enzyme to total enzyme—was calculated as follows:

\[
100 - \left( \frac{\text{the activity without DTT and zinc}}{\text{the activity with DTT and zinc}} \right) \times 100
\]

Since an antiserum against rat erythrocyte ALA
dehydratase produced in rabbits showed complete
cross reaction with the rat liver enzyme, the rabbit
IgG fraction against rat erythrocyte enzyme was used
in the present study for the determination of ALA
dehydratase concentration by radioimmunoassay.

Protein concentration was estimated by the method
of Lowry et al. Determination of lead in tissues
was carried out as described elsewhere and the tech-
ique for cell free translation of ALA dehydratase has
also been reported. The results of cell free transla-
tion was expressed as a ratio of neosynthesised ALA
dehydratase to the neosynthesised total protein (%).

Student’s t test (two tailed) was applied for the
examination of significant differences.

ALA hydrochloride was purchased from Sigma
Chemical Co (St Louis, Mo) and other chemicals used
were of analytical grade. All glassware was washed
with HNO₃/HCl and rinsed thoroughly with metal free
distilled water. Distilled water and all reagents used in
the experiments were routinely checked with a flame-
less atomic absorption spectrophotometry to prevent
contamination by divalent metals, including lead and
zinc.

Results

After 150 days the body weight of rats in the control
group was 434 ± 27 g and in the exposed group 370 ±
32 g, showing that the administration of lead reduced
the rate of increase in body weight (p < 0·05). Table 1
shows that the packed cell volume was slightly reduced in the rats exposed to lead (p < 0·01). The

<table>
<thead>
<tr>
<th>Packed cell volume value (%)</th>
<th>Control group (n = 5)</th>
<th>Lead exposed group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group (n = 5)</td>
<td>44.1 (1.81)</td>
<td>38.7 (1.29)*</td>
</tr>
<tr>
<td>Lead concentration (µg Pb/100 ml of blood)</td>
<td>8.5 (2.8)</td>
<td>151.0 (26.6)*</td>
</tr>
<tr>
<td>ALA dehydratase activity without dithiothreitol and zinc (× 10⁻³ unit/ml of RBCs)</td>
<td>18.3 (26)</td>
<td>8.7 (1.7)*</td>
</tr>
<tr>
<td>Inhibition ratios of ALA dehydratase (%)</td>
<td>17.7 (2.0)</td>
<td>99.0 (0.17)*</td>
</tr>
<tr>
<td>ALA dehydratase concentration (µg/ml of RBCs)</td>
<td>9.3 (0.9)</td>
<td>39.7 (3.2)</td>
</tr>
</tbody>
</table>

*Difference from the control group is significant at p < 0·01.

Table 2 Effects of lead administration on hepatic ALA dehydratase

<table>
<thead>
<tr>
<th>Control group (n = 5)</th>
<th>Lead exposed group (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver wet weight (g)</td>
<td>13.5 (2.1)</td>
</tr>
<tr>
<td>Liver total protein (g)</td>
<td>1.17 (0.07)</td>
</tr>
<tr>
<td>ALA dehydratase activity without dithiothreitol and zinc (× 10⁻³ unit/mg protein)</td>
<td>8.29 (0.76)</td>
</tr>
<tr>
<td>Inhibition ratio of ALA dehydratase (%)</td>
<td>3.9 (5.2)</td>
</tr>
<tr>
<td>ALA dehydratase concentration (µg/mg protein)</td>
<td>0.81 (0.064)</td>
</tr>
<tr>
<td>Neosynthesised ALA dehydratase concentration (µg/mg protein)</td>
<td>0.813 (0.028)</td>
</tr>
</tbody>
</table>

Difference from the control is significant at *p < 0·05 or **p < 0·01.
+Three of five rats in each group were selected at random for cell
free translation. ALA dehydratase concentration of the selected rats
in the control and lead exposed groups were 0·82 (0·065) and 0·97
(0·074) µg/mg protein.

blood lead concentration increased to 151 ± 26·6 µg/
100 ml of blood; accordingly, 99·0 ± 0·17% of the
zyme activity in the erythrocytes was inhibited. As is
clearly shown in table 1, however, the ALA
dehydratase concentration in the erythrocytes
increased to four times that of the control value after
the administration of lead.

Liver weight and liver total protein were not affected
by exposure to lead (table 2). Lead concentration in
the liver of the lead exposed group was nine times that
of the controls. The inhibition ratio of ALA
dehydratase in the liver increased significantly (p <
0·01) from 4% (control animals) to 65% (exposed
animals). The data in table 2 also indicate that the liver
zyme concentration increased by 25% after treatment
with lead (significant at p < 0·01), and this value
agrees well with the results of the cell free translation,
which shows approximately a 20% increase in ALA
dehydratase synthesis in the rats exposed to lead (p <
0·05).

The in vitro addition of lead to the cell free
translation system had no effect on the ratio of
neosynthesised ALA dehydratase to total protein,
about 0·79% for 150 µg of lead/100 ml of assay
mixture and 0·86% for 15 µg of lead/100 ml of assay
mixture.

Discussion

The fact that lead might induce ALA dehydratase was
made first in 1958 but after it was found that ALA
dehydratase is inhibited by lead, little attention was
given to the induction of the enzyme by lead. The first
observation of an increase in ALA dehydratase caused
by lead was reported by Maes and Geber and was
subsequently confirmed in a series of studies using a radioimmunoassay technique.5-11 Some of these studies suggested an increase in ALA dehydratase without any sign of anaemia or any increase in young erythrocytes.5-10,11 It had also been reported that there is an increase in the concentration in the bone marrow cells which precedes that in the peripheral erythrocytes by five to seven days.11 A weak correlation was observed between ALA dehydratase concentration and the number of young erythrocytes in the peripheral blood.10,11 Probably, therefore, ALA dehydratase is induced by lead to compensate for the enzyme inhibition.10,11 Immunochemical studies, however, do not indicate whether lead increases ALA dehydratase via an induction in m-RNA (the translational level), an increase in the turnover rate of m-RNA (the transcriptional level) or a decreased degradation of the enzyme protein (the post-translational level).5-11 The possible effects of anaemia on the increase in ALA dehydratase due to the appearance of premature erythrocytes in the peripheral blood has not been completely ruled out, since in some conditions, as in the present case (table 1), lead anaemia seems to have additional effects on the enzyme concentration.8,9

The present study deals with the hepatic enzyme to eliminate any possible effect of anaemia on the enzyme concentration. On the last day of any exposure, the enzyme concentration in the liver increased significantly (p < 0.05) by 25% notwithstanding the clear reduction in its activity (p < 0.01) (table 2). According to the calibration curves observed in workers exposed to lead,4 the dehydratase concentration in the erythrocytes increases to 127% of control values when the inhibition ratio is around 65%. The present results are, therefore, essentially identical not only to the previous report on rat hepatic enzyme11 but also to that on the human erythrocyte enzyme.5 Cell free translation showed that exposure to lead increased hepatic ALA dehydratase synthesis by 20% (p < 0.05) (table 2). This is the same as the increase in the hepatic enzyme and shows a significant induction of de novo synthesis of the enzyme by exposure to lead. The in vitro addition of lead failed to indicate an effect on enzyme induction at the translational and in the post-translational levels. It is concluded, therefore, that lead induces the de novo synthesis of ALA dehydratase at the transcriptional level. In other words, lead seems to have a Janus faced effect on ALA dehydratase; it inhibits the activity of the enzyme and also induces its de novo synthesis. A recent molecular biological study shows that there is no difference between liver and erythrocytic ALA dehydratase,18 so the erythrocytic enzyme is probably also induced by lead to compensate for the inhibition caused by the metal.

References
1 Tsukamoto I, Yoshinaga T, Sano S. The role of zinc with special reference to the essential thiol groups in δ-aminolevulinic acid dehydratase of bovine liver. Biochim Biophys Acta 1979;570:167-78.
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