Age Difference in Response of Erythrocyte $\delta$-Aminolevulinic Acid Dehydratase Amount to Lead Administration in Rats

Hiroyoshi FUJITA*, Kunihiko SATO** and Masayuki IKEDA*

*Department of Environmental Health, Tohoku University School of Medicine, Sendai, 980 Japan

**Kyoto Industrial Health Association, Kyoto, 604 Japan

(Received May 25, 1982 and in revised form July 5, 1982)

Abstract: Differences in the amount of $\delta$-aminolevulinic acid dehydratase (ALA-D) as determined by radioimmunoassay, hematology, and body weight increase were observed between infant and adult rats after lead treatment. Administration of the lead through drinking-water containing 25 mM lead acetate resulted in an increase in blood lead contents in both infant and adult rats to almost the same degree. The influence of lead on the body weight, however, was significant only in infant rats, with a reduction in body weight to 70% of the controls. The increase in ALA-D content of peripheral erythrocytes of infant rats (about 4 times that of the controls) was more marked than that of adult rats (about twice that of the controls). A possible explanation of this difference is that, in the case of adult rats, the increase in the amount of ALA-D is due to increased synthesis of the enzyme in bone marrow cells, while the increase of the enzyme in infant rats results from anemia with increased numbers of young erythrocytes in the peripheral blood in addition to the increased synthesis of ALA-D. The findings suggest that rapid growth during the infant stage prevents the hematological compensation for the effect of lead, resulting in anemia and marked increase in the amount of ALA-D. It was found in the present study that the European Standardized Method is not suitable for ALA-D assay in exposure of rats to lead.

Keywords: Age difference—$\delta$-Aminolevulinic acid dehydratase—European Standardized Method—Lead—Radioimmunoassay.

INTRODUCTION

There are clear differences between the effects of lead on infants and adults. In infants, lead exposure tends to produce acute serious symptoms, such as lead colic, lead encephalopathy, severe permanent neurologic sequelae, and lead nephropathy, while adults can be free of symptoms from chronic lead exposure or show relatively mild symptoms. Moreover, comparison between infants and adults indicates that infants are biologically more sensitive than adults
to the same blood level of lead. Thus lead poisoning of infants is still a serious public health problem despite many years of intensive investigation. The reasons why lead affects infants more severely than adults are believed to be as follows: the rapid absorption of lead from the intestinal tract, the greater degree of lead retention, and the rapid growth of infants\(^1\).\(^2\).

One of the most obvious sites of the toxic action of lead is the heme biosynthetic pathway, especially the inhibition of erythrocyte \(\delta\)-aminolevulinic acid dehydratase (ALA-D; 5-aminolevulinate hydro-lyase, EC 4.2.1.24), which is widely noted as a sensitive indicator\(^1\)-\(^4\). Recently, a radioimmunoassay (RIA) for rat ALA-D was developed, and its use for adult rats given lead revealed that the amount of ALA-D increased to twice that in the controls in contrast with its decreased activity\(^5\). It was also shown that the increase in the amount of ALA-D in bone marrow cells preceded that in peripheral erythrocytes by more than 5 days\(^5\). Under the conditions employed, no sign of anemia with increased numbers of reticulocytes was observed in either lead-treated or untreated groups. The increase in the amount of ALA-D in erythrocytes was therefore considered to be due to increased synthesis of the enzyme in bone marrow cells to compensate for the inhibition\(^5\). A RIA for human ALA-D was also developed, and its application to lead-exposed workers made it evident that the increase in the amount of ALA-D without anemia was one of the most sensitive parameters of lead exposure\(^6\). In addition, an increase in enzyme content coupled with decrease in its activity was observed in lead-exposed human subjects\(^6\) as in the case of rats\(^5\).

Although these reports indicate the importance of the amount of ALA-D in lead exposure, the amount in lead-poisoned infants was not studied and no comparison was made between lead-exposed infants and adults. In the present study, therefore, 3- and 16-week-old rats of the same (Wistar) strain were, given drinking water containing 25 mM lead acetate, under the same conditions, in order to compare the effect of lead on infants with that on adults. The ALA-D content was determined by RIA.

Maes and Gerber\(^7\) reported phenomenally increased activity of ALA-D as determined by the European Standardized Method in infant rats given a diet containing lead acetate at 1%. In our study, the significance of the activity revealed by the European Standardized Method was examined and the results of the present study was compared with those reported by Mas and Gerber\(^7\).

**Materials and Methods**

1. **Materials**

\(\delta\)-Aminolevulinic acid hydrochloride, rabbit anti-goat Ig-G serum, and normal goat serum were purchased from Nakarai Chemicals Ltd. (Kyoto, Japan). \(^{125}\)I was obtained from Radiochemical Centre Ltd. (Amersham, England). Other chemicals used were of analytical grade.
2. **Enzyme assays**

The activity of ALA-D without dithiothreitol (DTT) and zinc treatment was routinely determined by the modified European Standardized Method\(^5\) (the modified method), in which the lead-chelating phosphate buffer was replaced with Tris-acetate buffer, and will be called the non-restored activity. When necessary, the activity of ALA-D determined by the European Standardized Method\(^8\) and the activity after treatment with DTT and zinc\(^5,9\) were also determined. The activity after zinc and DTT treatment will be referred to as the restored activity. The determination of the enzyme activity was carried out within 1 hour after blood sampling. One unit of enzyme activity was defined as the activity which catalyzes the formation of 1 \(\mu\)mole of porphobilinogen formed per hour at 37°C. The protein concentration was determined by the method of Lowry et al.\(^10\). The absorption spectra were recorded with a Hitachi model 200-10 spectrophotometer.

3. **Radioimmunoassay**

(1) **Antigen:** ALA-D purified from rat erythrocytes\(^5\) was employed as the antigen. The preparation was precipitated with 55% saturated ammonium sulfate, stored at 4°C until use, and activated immediately before use as described by Tsukamoto et al.\(^9\) and Fujita et al.\(^5\). The purified enzyme was homogeneous on gel filtration chromatography and in analytical polyacrilamide gel electrophoresis both in the absence and presence of sodium dodecyl sulfate. The specific activity of the purified enzyme was 26.0 units/mg of protein.

(2) **Radioiodination of the antigen:** The activated ALA-D was labeled with \(^{125}\text{I}\) using chloramine T according to the method of Hunter and Greenwood\(^11\). After the labeling, the antigen was further purified by chromatography on a Dowex I \(\times\) 8 column. The specific radioactivity was approximately 6.80 \(\mu\)Ci/\(\mu\)g.

(3) **Antisera:** An antisera against rat erythrocyte ALA-D was produced in a goat by inoculation of the activated enzyme in Freund's complete adjuvant\(^5\). The antisera was used at a dilution of 1:85,000, which resulted in approximately 50% of the maximal binding of the labeled antigen.

(4) **Radioimmunoassay procedure:** The RIA for rat ALA-D was performed by the method of Fujita et al.\(^5\), and the results will be referred to as the RIA-based amount.

4. **Determination of lead**

For the determination of the lead in blood, the samples were digested with \(\text{HNO}_3\) and analyzed with a flameless atomic absorption spectrophotometer (Nippon Jarrell Ash AA-8200, FLA-100). All glassware was washed with \(\text{HNO}_3/\text{HCl}\) and rinsed thoroughly with metal-free distilled water.
5. Administration of lead to rats

Male Wistar rats, 3 and 16 weeks old, were housed individually in stainless steel wire cages and kept in a temperature-controlled and light-regulated animal room. The 3-week-old rats were divided into a control group (6 rats with a mean body weight of 55.3 g) and a lead-treated group (6 rats with a mean body weight of 54.8 g). The animals in the latter group were given 25 mM lead acetate in deionized water ad libitum. Body weight, hematocrit, reticulocyte counts and typing, erythrocyte ALA-D activity and its RIA-based amount, and the blood lead content were determined 3 and 4 weeks after the initiation of lead administration. The 16-week-old rats were also divided into a control group (4 rats with a mean body weight of 308 g) and a lead-treated group (4 rats with a mean body weight of 314 g), and the test group were also given 25 mM lead acetate-containing water ad libitum. After 4 weeks of lead treatment, hematological examinations and body weight measurements were carried out.

RESULTS AND DISCUSSION

1. Selection of the enzyme assay method

It was previously reported that sodium phosphate buffer used in the European Standardized Method removes lead from the system partially, and that the DTT and zinc treatment of the samples almost quantitatively restores the activity of the enzyme inhibited by lead.

In the present study, in order to define the best method for assaying erythrocyte ALA-D activity, the restored activity, the non-restored activity, and the European Standardized Method-based activity were compared. When samples containing 145.8±38.3 µg lead/100 ml of whole blood (mean ±SD) were obtained from 12 lead-treated rats and assayed for ALA-D, the restored activity, the non-restored activity, and the European Standardized Method-based activity of ALA-D were 3.06±1.13, 0.05±0.01, and 1.36±0.59 units/ml of packed cells, respectively. It is therefore evident that, when assayed by the European Standardized Method,

\[ \text{55\% (i.e., } \left(1 - \frac{1.36}{3.06}\right) \times 100 \text{) of the enzyme was apparently inhibited, while the modified method showed inhibition of 98\% (i.e., } \left(1 - \frac{0.05}{3.06}\right) \times 100 \text{).} \]

Fig. 1 indicates that lead inhibits the purified enzyme from rat erythrocytes non-competitively with a Ki of 0.7×10⁻⁶ M. This Ki value is similar to that of purified bovine liver (1.1×10⁻⁶ M) or the human erythrocyte (1.7×10⁻⁶ M) enzyme. Calculating from the Ki value, 92% of the enzyme should be inhibited in the presence of 150 µg of lead/100 ml. This calculated inhibition is almost equal to the inhibition obtained by the modified method (98%), and the inhibition obtained by the European Standardized Method (55%) was much less. Moreover, it is generally accepted that ALA-D in peripheral erythrocytes is almost
completely inhibited by lead when the blood lead concentration is more than 100μg/100 ml (see the report of the National Academy of Science¹). The activity determined by the European Standardized Method is, therefore, probably higher than the presumed activity in the body, while the non-restored activity represents the in vivo activity. This partial restoration of the activity detected by the European Standardized Method is probably due to lead-chelation by the phosphate, resulting in remove of the lead from the enzyme.

On the other hand, comparison of the European Standardized Method-based activity with the restored activity indicates that the restoration of the lead-inhibited activity is only 44.2±13.1%, when assayed by the European Standardized Method. This rate indicates that full restoration of the enzyme activity is not detected by the European Standardized Method. Fig. 2 shows the poor correlation (r=0.392, n=12) between the RIA-based ALA-D content and the European Standardized Method-based activity. It is hence obvious that the activity determined by the European Standardized Method does not reflect the amount of enzyme.

These observations indicate that the European Standardized Method is not suitable for determining the enzyme activity in lead-exposed rats. Accordingly, the modified method was selected as an appropriate method for assaying ALA-D in the present study.
2. Comparison of the hematological effect of lead in adult and infant rats

The results of administration of lead to rats are summarized in Tables 1 and 2. Blood lead levels of infant rats after 3 or 4 weeks of lead treatment were almost equal to those of the adult rats after 4 weeks of lead administration.

After lead administration, non-restored activity of ALA-D in infant rats de-

Fig. 2. Correlation between the amount of ALA-D and its activity in lead-exposed rats.

○: restored activity of ALA-D as assayed in the presence of DTT and zinc. ●: activity as determined by the European Standardized Method. The restored activity and the amounts of ALA-D show a good correlation ($r=0.923$, $n=12$, $p<0.001$) with a linear regression line of $y=12.57x+0.068$, where $x$ is the amount of ALA-D (mg/ml of packed cells) and $y$ is the restored activity (units/ml of packed cells). The correlation between the European Standardized Method-based activity and the amount of ALA-D is poor ($r=0.392$, $n=12$).

2. Comparison of the hematological effect of lead in adult and infant rats

The results of administration of lead to rats are summarized in Tables 1 and 2. Blood lead levels of infant rats after 3 or 4 weeks of lead treatment were almost equal to those of the adult rats after 4 weeks of lead administration.

After lead administration, non-restored activity of ALA-D in infant rats de-
## Table 1. Behavior of ALA-D, Body Weight, Hematocrit, Reticulocyte Count, and Blood Lead Level in Lead-treated and Non-treated Rats.

<table>
<thead>
<tr>
<th>Duration of lead treatment (weeks)</th>
<th>3</th>
<th>4</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>55.3±2.9</td>
<td>148.1±1.5</td>
<td>167.7±2.9</td>
</tr>
<tr>
<td>lead-treated</td>
<td>54.8±2.9</td>
<td>104.7±1.5</td>
<td>118.3±1.5</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>40.1±6.7**</td>
<td>39.4±6.7*</td>
<td>40.5±6.7*</td>
</tr>
<tr>
<td>lead-treated</td>
<td>41.2±6.7*</td>
<td>32.0±6.7*</td>
<td>30.0±6.7*</td>
</tr>
<tr>
<td>Reticulocyte count (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>5.7±1.2</td>
<td>5.3±1.2</td>
<td>6.2±1.2</td>
</tr>
<tr>
<td>lead-treated</td>
<td>5.6±2.0</td>
<td>13.0±2.0</td>
<td>13.3±2.0</td>
</tr>
<tr>
<td>ALA-D activity (unit/ml of packed cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>1.46±0.36</td>
<td>0.75±0.27</td>
<td>0.68±0.11</td>
</tr>
<tr>
<td>lead-treated</td>
<td>1.39±0.04</td>
<td>0.06±0.04</td>
<td>0.04±0.04</td>
</tr>
<tr>
<td>ALA-D amount (µg/ml of packed cells)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>131±32</td>
<td>81±32</td>
<td>61±16</td>
</tr>
<tr>
<td>lead-treated</td>
<td>125±37</td>
<td>230±88**</td>
<td>237±75**</td>
</tr>
<tr>
<td>Blood lead (µg/100 ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-treated</td>
<td>7.8±6.7</td>
<td>8.2±5.9</td>
<td>7.3±6.3</td>
</tr>
<tr>
<td>lead-treated</td>
<td>7.2±5.6</td>
<td>138.6±36.3**</td>
<td>153.6±41.5**</td>
</tr>
</tbody>
</table>

Male Wistar rats, 3 weeks old, were divided into a lead-treated and a non-treated group. Body weight, hematocrit, reticulocyte count, ALA-D activity, ALA-D amount, and blood lead level were determined 3 and 4 weeks after the initiation of lead administration. The results with 16-week old male Wistar rats given the same treatment for 4 weeks are also shown. The numbers in the table are mean±SDs.  

*, ** Difference from non-treated is significant at p<0.05 (*) or p<0.01 (**).

## Table 2. Distribution Profile of Reticulocytes after 4 Weeks of Lead Administration.

<table>
<thead>
<tr>
<th>Group</th>
<th>Type I (%)</th>
<th>Type II (%)</th>
<th>Type III (%)</th>
<th>Type IV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infant rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>1.60±0.33</td>
<td>1.60±0.69</td>
<td>2.08±0.81</td>
<td>0.92±0.29</td>
</tr>
<tr>
<td>Lead-treated</td>
<td>4.26±2.21*</td>
<td>4.15±2.18*</td>
<td>2.88±1.19</td>
<td>2.10±0.76*</td>
</tr>
<tr>
<td>Adult rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-treated</td>
<td>0.65±0.29</td>
<td>1.03±0.32</td>
<td>1.04±0.52</td>
<td>1.13±0.37</td>
</tr>
<tr>
<td>Lead-treated</td>
<td>0.58±0.22</td>
<td>1.16±0.38</td>
<td>1.01±0.56</td>
<td>1.09±0.41</td>
</tr>
</tbody>
</table>

Distribution profile of reticulocytes was determined according to Heilmeyer's classification after 4 weeks from the initiation of lead administration. The numbers in the table are mean±SDs.  

* Difference from non-treated is significant at p<0.05.
creased to below 10% of the controls. When the ratio of ALA-D activity in the lead-treated animals to that in the control animals was compared after 4 weeks of treatment, it was found to be somewhat higher in infant rats (ca. 6.4%) than in adult rats (ca. 4.2%). In the preceding section we suggested that the apparent increase in ALA-D activity in infant rats as reported by Maes and Gerber\textsuperscript{7)} is probably due to the unsuitable selection of the European Standardized Method for the assay. In contrast with the decrease in non-restored activity, a remarkable increase in the RIA-based ALA-D amount was observed in infants after 3 or 4 weeks of lead treatment. The ratio of the increase (lead-treated/control) in infants (3 to 4 times) was higher than that in adults (about 2 times).

Other hematological findings in infant rats were also different from those in adult. The administration of lead at the dose employed did not affect the hematocrit or the number and type of reticulocytes in adult rats, while the same treatment of infant rats decreased the hematocrit to 74% of that of the controls and increased the reticulocyte counts to twice that of the controls. Reticulocytes in the peripheral blood were counted and classified according to Heilmeyer\textsuperscript{14).} The increase in reticulocyte counts of lead-treated infant rats was more marked (about 3 times that of the controls) in Type I and II than Type III and IV (about 1.5~2 times that of the controls) (Table 2). In serious hemolytic anemia after phenylhydrazine treatment, the increase in the restored activity of ALA-D strongly correlated with the counts of Type I and II reticulocytes (manuscript in preparation). The findings with lead and phenylhydrazine therefore indicate that, in the case of lead-exposed infant rats, the greater increase in the amount of ALA-D in erythrocytes than that in adult rats was due not only to the increased synthesis of ALA-D in bone marrow cells but also to anemia with increased numbers of young erythrocytes. It is also possible to infer that the increase in the amount of ALA-D in peripheral erythrocytes does not always mean an increased capacity to compensate for the inhibition, especially when the anemia is severe.

The body weight in the lead-treated group only doubled during the period of the 4 weeks' study in contrast with a more than three fold increase in the control group, showing that lead administration marked impaired the growth of the infant rats. In the adult rats, the body weights of the lead-treated rats were almost the same as those of the control animals. It is generally accepted that the rapid growth of infants limits their capacity to respond to lead exposure\textsuperscript{11).} The anemia observed is very probably a result of this reduced capacity.

The present experiments clearly demonstrated that a marked difference in response to lead load does exist between adult and infant rats; at a blood lead level of 150–160 \( \mu g/100 \text{ ml} \), only a reticulocytopenia-free increase in ALA-D synthesis to compensate for the enzyme inhibition was observed in the adult rats, while reticulocytemia also plays some role in the increase in the amount of ALA-D in erythrocytes of infant rats.
REFERENCES