Effects of Lead Exposure on the Activity of Erythrocyte Pyrimidine 5’-Nucleotidase and δ-Aminolevulinic Acid Dehydratase in Mice

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TOMOKUNI, K. Effects of Lead Exposure on the Activity of Erythrocyte Pyrimidine 5’-Nucleotidase and δ-Aminolevulinic Acid Dehydratase in Mice. Tohoku J. exp. Med., 1983, 139 (1), 27-32 — The effects of lead exposure on the activities of erythrocyte pyrimidine 5’-nucleotidase (Py5N) and δ-aminolevulinic acid dehydratase (ALA-D) were investigated in the mice given ad libitum the drinking water containing lead at 10 to 500 ppm for 30 days. The erythrocyte Py5N activity was significantly inhibited in the 250 and 500 ppm groups, and the activity in these groups was dropped to 62-81% of that in the control group. In addition, the erythrocyte Py5N activity was shown to have a significant negative correlation with the concentration of lead in blood (r = -0.79). The erythrocyte Py5N activity would be a better indicator than ALA-D in the exposure to relatively high lead concentrations, although the assay of Py5N is more time-consuming as compared with that of ALA-D, while, the erythrocyte ALA-D activity is useful in the evaluation of exposure to low levels of lead. The erythrocyte Py5N was less stable than ALA-D against heating at 60°C.

δ-Aminolevulinic acid dehydratase (ALA-D) catalyses the synthesis of porphobilinogen (PBG) from two molecules of δ-aminolevulinic acid (ALA) (Gibson et al. 1955). Pyrimidine 5’-nucleotidase (Py5N) catalyses the dephosphorylation of pyrimidine nucleotides, e.g., uridine and cytidine 5’-monophosphates (UMP and CMP) (Valentine et al. 1974; Paglia and Valentine 1975). Both enzyme are present in the erythrocyte cytosol of circulating blood.

There have been many papers (Hernberg and Nikkanen 1970; Hernberg et al. 1972; Tola et al. 1973; Tomokuni and Ogata 1976) reporting that the activity of erythrocyte ALA-D is sensitive to inhibition by inorganic lead and the degree of its inhibition as a close correlation with the concentration of lead in blood, in the persons exposed to lead. Thus the measurement of erythrocyte ALA-D activity has been used as a practical screening test for detecting the degree of lead exposure in workers occupationally exposed to lead.

The erythrocyte Py5N activity is also inhibited by lead in vivo and the degree of its inhibition is also closely related with the level of lead in blood (Paglia et al. 1975, 1977; Bue and Kaplan 1978; Angle and McIntire 1978; Satoh et al. 1979).

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suggesting that the erythrocyte Py5N test is also useful as an indicator of lead exposure or lead poisoning.

The present author also investigated the effects of lead on the activities of erythrocyte Py5N and ALA-D in the mice which were given ad libitum the drinking water containing lead at low and high levels, and found that the erythrocyte Py5N activity, as well as ALA-D activity, was negatively correlated with the blood lead concentrations. The present paper is a report of these results.

MATERIALS AND METHODS

Lead exposure. Male dd mice weighing 28 to 36 g were used in the experiment. They were divided at random into 5 groups and were fed a solid diet from the Oriental Yeast Company (Tokyo, Japan). One group was used as control and the other groups were given ad libitum the drinking water containing lead at 10, 50, 250 and 500 ppm, respectively. The drinking water containing lead was prepared with lead nitrate (reagent grade). After providing lead for 30 days, the mice were light anesthetized with ethyl ether and the blood was withdrawn into a glass test-tube through heparinized capillary tubes by an orbital bleeding technique.

Heat stability. The heparinized blood samples were obtained from normal and 500 ppm lead-exposed mice. Immediately before the enzyme assay, the hemolysate was heated for 5 to 20 min at 60°C. After cooling, the enzyme assay was carried out.

Measurement of erythrocyte Py5N activity. Plasma and erythrocytes were separated by centrifugation at 3,000 rpm for 5 min, and erythrocytes were washed twice with pre-cooled 0.9% saline. The washed erythrocytes were hemolyzed with 3-fold volumes of deionized water and the hemolysate was dialysed for 20 hr at 4°C against 150 volumes of isotonic saline buffered to pH 8.0 by 0.01 M Tris-HCl. Assay of erythrocyte Py5N was performed according to the method of Paglia and Valentine (1975), using CMP (Sigma Chemical Co., St. Louis, USA) as a substrate. The amount of orthophosphate (Pi) produced during incubation for 2 hr at 37°C was determined by the method of Takahashi (1957). The enzyme activity was expressed as μmoles of Pi formed per hr per g of hemoglobin (Hb).

Measurement of erythrocyte ALA-D activity. The hemolysate was prepared by diluting 0.1 ml of whole blood with 1.4 ml of deionized water. Assay of ALA-D was performed according to the method of Nikkanen et al. (1972). The enzyme activity was expressed as μmoles of PBG formed per hr per 1 red blood cell (RBC).

Other analyses. Hb was determined by the standard method after conversion to cyanmethemoglobin. Hematocrit levels were measured by the use of a microcapillary centrifuge. The blood lead was determined by flameless atomic absorption spectrophotometry with a deuterium background corrector. A sample for lead analysis was prepared by hemolyzing 0.1 ml of whole blood with 0.4 ml of deionized water followed by freezing.

RESULTS

Table 1 summarizes several parameters obtained from the groups of control and lead-exposed mice. The concentration of lead in blood increased significantly in the groups exposed to lead at 50, 250 and 500 ppm, when compared with that in the control blood (p<0.001). There was no decrease in Hb level in any group of lead exposure. The inhibition of erythrocyte Py5N activity was found in the 250 and 500 ppm groups. The level of erythrocyte Py5N activity in the 250 ppm group significantly (p<0.01) decreased to 81% of that in the control group and the level in the 500 ppm group also decreased to 62% of the control value (p<0.001).
The ALA-D activity significantly ($p<0.05$) decreased in the 50 ppm group. The most significant ($p<0.001$) inhibition of erythrocyte ALA-D activity was found in both the 250 ppm group and the 500 ppm group and the activity level in those two groups was dropped to 10–11% of the control value.

Fig. 1 shows the relationship between the erythrocyte Py5N activity and the concentration of lead in the blood of 48 mice. The erythrocyte Py5N activity negatively correlated with the concentration of lead in blood ($r=-0.78$, $p<0.001$).

The relation of the erythrocyte ALA-D activity with the blood lead concentration in 48 mice is shown in Fig. 2. The negative correlation between the logarithmic plot of erythrocyte ALA-D activity and the concentration of lead in blood was very high ($r=-0.89$, $p<0.001$).

Fig. 3 shows the data on heat stability of both erythrocyte Py5N and ALA-D in normal and lead-exposed mice. The lead concentration of a blood sample

<table>
<thead>
<tr>
<th>Group</th>
<th>No.</th>
<th>Hemoglobin (g/100 ml)</th>
<th>Blood lead (μg/100 ml)</th>
<th>Py5N activity (μmol Pi/h/g Hb)</th>
<th>ALA-D activity (μmol PBG/h/1 RBC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>14.5±0.7</td>
<td>6.5±2.0</td>
<td>21.5±3.0</td>
<td>992±238</td>
</tr>
<tr>
<td>Pb, 10 ppm/&quot;</td>
<td>7</td>
<td>14.6±0.5</td>
<td>6.0±1.3</td>
<td>20.2±2.2</td>
<td>980±133</td>
</tr>
<tr>
<td>Pb, 50 ppm/&quot;</td>
<td>7</td>
<td>14.3±0.4</td>
<td>17.9±3.5</td>
<td>18.8±2.6</td>
<td>734±59*</td>
</tr>
<tr>
<td>Pb, 250 ppm/&quot;</td>
<td>8</td>
<td>14.4±0.5</td>
<td>32.3±11.1</td>
<td>17.5±2.1†</td>
<td>114±38†</td>
</tr>
<tr>
<td>Pb, 500 ppm/&quot;</td>
<td>14</td>
<td>14.0±1.1</td>
<td>52.4±15.2</td>
<td>13.3±5.0‡</td>
<td>97±84‡</td>
</tr>
</tbody>
</table>

The values in the Table are mean±s.d.

The statistical significance from the control (by t-test) is shown as follows: * $p<0.05$; † $p<0.01$; ‡ $p<0.001$.

" The concentration of lead in the drinking water.

Fig. 1. Correlation between the erythrocyte pyrimidine 5'-nucleotidase (Py5N) activity and the concentration of lead in blood. 48 mice were examined; o, control and . Pb-exposed.
pooled from several normal mice was 6 μg/100 ml and the counterpart value for the blood from the lead-exposed mice was about 50 μg/100 ml. When the hemolysate from lead-exposed mice was heated for 5 min at 60°C, the erythrocyte ALA-D was activated and the activity obtained was 3.3 times higher than the initial one. On the contrary, the erythrocyte Py5N was markedly inactivated and the activity dropped to 9% of the initial one. When the hemolysate of normal blood was heated at 60°C, both Py5N and ALA-D was inactivated; the degree of the
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inactivation of Py5N was more remarkable. These data suggest that the erythrocyte Py5N is less stable than ALA-D against heating.

DISCUSSION

Angle and McIntire (1978) demonstrated that there was a significant negative linear correlation \((r = -0.85)\) between the blood lead concentration and the erythrocyte Py5N activity in 17 each of rats in the control group and the lead-exposed group with blood lead of 7 to 36 \(\mu g/100\) ml. The simultaneous measurement of erythrocyte Py5N and ALA-D was, however, not performed in their studies. It was found, however, that the erythrocyte Py5N activity was not reduced, although the activity of ALA-D was remarkably decreased, in lead-exposed mice with the blood lead concentrations of 11 to 43 \(\mu g/100\) ml due to oral administration of a smaller amount of lead nitrate (Tomokuni and Ogata 1980).

In the present study with animals exposed to either low or high level of lead, the results obtained indicated that the erythrocyte Py5N was inhibited in the group of mice with blood lead levels of 25 to 77 \(\mu g/100\) ml and that the activity showed a significant negative correlation with the blood lead concentration. These findings suggest that the erythrocyte Py5N activity would be a better indicator of lead exposure when animals were exposed to relatively high lead concentrations, although the assay of erythrocyte Py5N is more time-consuming as compared with that of erythrocyte ALA-D. While, the erythrocyte ALA-D activity seems to be a useful indicator under conditions of exposure to low levels of lead because of its higher sensitivity. Therefore, the monitoring in the wide range of lead exposure can be expected to be possible by means of the simultaneous measurement of erythrocyte ALA-D and Py5N activities.

Valentine et al. (1974) reported that unidentified pyrimidine nucleotides were accumulated in human subjects with Py5N deficient hemolytic anemia, while no pyrimidine nucleotides were present in normal erythrocytes. Paglia et al. (1977) demonstrated that lead intoxication in humans resulted in an inhibition of erythrocyte Py5N which was accompanied by an intracellular accumulation of pyrimidines. Swanson et al. (1980) demonstrated the accumulation of significant amounts of uridine 5'-triphosphate (UTP) and cytidine 5'-triphosphate (CTP), which are normally present in only trace amount, in erythrocytes of the rabbits after 60 days of oral administration of lead acetate at a dose of 30 mg/kg/day, whereas the accumulation of UMP and CMP was insignificant. In addition, his co-investigators (Angle et al. 1980) reported that the logarithm of erythrocyte CTP correlated negatively with the erythrocyte Py5N activity and positively with erythrocyte zinc protoporphyrin, in the rabbits given orally the same dose of lead acetate. These reports suggest that the accumulation of erythrocyte UTP and CTP in chronic lead exposure may be accounted for by the combination of inhibition of Py5N and the functioning of nucleoside diphosphokinase.
Acknowledgment

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References