Critical Dose of Lead Affecting δ-Aminolevulinic Acid Levels

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Abstract: Critical Dose of Lead Affecting δ-Aminolevulinic Acid Levels: Katsuyuki Murata, et al. Akita University School of Medicine —To estimate the critical dose of the association between the blood lead concentration (BPb) and δ-aminolevulinic acid (ALA) levels, ALA levels in plasma (ALA-P), blood (ALA-B), and urine (ALA-U), and the activity of δ-aminolevulinic acid dehydratase (ALAD) were determined in 186 Japanese lead workers, aged 18–62 yr, with BPb levels of 2.1–62.9 µg/dl. For this purpose, the benchmark dose (BMD) method, recently used in the environmental health field in place of the no-observed-adverse-effect level, was introduced into this study. The BMD was defined as the BPb level that resulted in an increased probability of abnormal change in ALA-related parameters by an excess risk (BMR) of 5% in exposed workers i.e., from P₀ (abnormal probability of 5% in unexposed workers) to P₀+BMR for exposed workers at the BMD. ALA-related parameters were significantly correlated with BPb. The BMDs computed from the 186 workers, after controlling for age, were 15.3–20.9 µg/dl for ALA levels, and 2.9 µg/dl for ALAD; likewise, the BMDs from the 154 workers with BPb levels of less than 40 µg/dl were 3.3–8.8 µg/dl for ALA levels, and 2.7 µg/dl for ALAD. Since the cutoff value of ALA-P, computed from the latter workers, seems to be closer to the upper normal limit in unexposed adults than does that from the former workers, it is suggested that the critical dose of BPb causing the increased levels of ALA is below 10 µg/dl. Such critical doses are necessary to promote preventive activities of adverse effects of lead.

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Key words: Lead, Critical dose, Adverse effect, δ-Aminolevulinic acid, Occupational exposure, δ-Aminolevulinic acid dehydratase

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workers, conducted under the Industrial Safety and Health Law in Japan, the nature of the procedure used in the present study was fully explained to all workers, and the study was carried out with their informed consent. Venous blood treated with heparin or EDTA-2K was taken from 186 male lead workers (18–62 yr old, mean 43). The lead workers were employed in a secondary smelter, in a glass factory, and in electrical appliance manufacture (soldering). Plasma was separated from whole blood immediately after sampling, stored at 4°C or –80°C, and used for the determination of ALA-P. Spot urine was also collected from the lead workers, and stored at –20°C until analysis.

**Measurement of ALA-related parameters**

ALA-P and ALA-B were determined by the methods previously reported 16–18). Briefly, 40 µl of 25% trichloroacetic acid (TCA) and 10 µl of 30 mM iodoacetamide were added to 100 µl of plasma or blood, and vigorously agitated with a vortex mixer. To determine ALA-P, iodoacetamide could be replaced by distilled water. After centrifugation at 13,000 rpm for 10 min in a microcentrifuge, 10 µl of the supernatant was used for the derivatization. The standard reaction mixture for the derivatization contained 10 µl of the supernatant, 240 µl of distilled water, 250 µl of 200 mM acetate buffer (pH 3.8), 1.25 ml of solution A (acetylacetone-ethanol-water 15: 10: 55 v/v/v), and 250 µl of solution B (8.5% w/v formaldehyde solution). The reaction was carried out at boiling point for 15 min. After cooling, the mixture was used for the HPLC analyses.

A liquid chromatograph (Shimadzu, Kyoto, Japan) consisting of a pump (LC-10A), an automatic sample injector (SIL-10A), a column oven (CTO-10A), a fluorescence detector (RF-550A) and a data processor (C-R4A) was used. The column (150 × 6 mm) was packed with reversed phase silica (Inertsil ODS-2, GL Science, Tokyo, Japan). The mobile phase was 50% methanol containing 0.1% acetic acid. The flow rate, oven temperature, and detector wavelength were set at 0.7 ml/min, 40°C, and 373 nm/463 nm (excitation/emission), respectively. Samples were cooled at 4°C during a series of analyses and 80 µl was automatically injected at 20-min intervals.

The method of ALA-U determination was basically the same as that used by Okayama et al. 19) ALA-U was corrected for the creatinine concentration (ALA-U, mg/g Cre). Creatinine was determined by the method of Jaffe with the “Creatinine Determination Kit” (Wako Pure Chemicals, Tokyo, Japan). Erythrocyte ALAD activity was determined by the Commission of European Communities (CEC) standard method, and the activity was expressed as units (u: µmol ALA/min/RBC) 20). BPb was determined by flameless atomic absorption spectrometry (Hitachi Z-8000, Tokyo, Japan).

**Fig. 1.** Dose-effect relation of the blood lead concentration to hematocrit in lead workers for benchmark dose (BMD) calculation. P₀ and benchmark response (BMR) indicated an abnormal probability (5%) in unexposed workers and an excess risk (5%) above P₀ in exposed workers, respectively. The power parameter of K=1 was used in this figure to simplify the model.

**Statistical analysis**

The BMD was defined as the BPb level that resulted in an increased probability of abnormal change in ALA-related parameters by a benchmark response (BMR), i.e., from P₀ for unexposed subjects to P₀+BMR for exposed subjects at the BMD (Fig. 1) 9, 10). Previous applications of this method have used a P₀ (i.e., an abnormal probability in outcome data of unexposed subjects) of 5% and a BMR (i.e., an excess risk in exposed subjects) of 5% 8, 13, 14). Although observational studies have not included an unexposed group completely free of exposure, data of the group could be extrapolated from those of exposed subjects 19). And, the BMD and the cutoff value (C) of P₀ were calculated by using a statistical dose-response model based upon power functions for the dependence of ALA-related parameters on BPb (d) and the confounder: (1) µ (d)=β₀+β₁ · d+β₂ · [age], (2) P₀=Φ ((C-β₀) /σ), and (3) BMD=[Φ⁻¹(P₀+BMR)] · σ/ β₁ (Φ and σ indicate the standard cumulative normal distribution function and standard deviation of ALA-related parameters in unexposed subjects, respectively). A lower confidence limit (BMDL) for BMD was then calculated as the statistical 95% lower bound of the BMD 19). The power parameter K has been restricted to values equal to or above 1, thus allowing the dose-response curve to be nonlinear 14). We applied the K-power model in accordance with recent applications 13, 14). All analyses were performed by using the Statistical Package for the Biosciences (SPBS V9.5) with the BMD program 21).
Results

Table 1 summarizes the BPb, ALA-P, ALA-B, ALA-U, and ALAD of the lead workers. The BPb level ranged from 2.1 to 62.9 µg/dl. Since there was a negative exponential relationship between BPb and ALAD in the lead workers, the latter was logarithmically transformed when it was used as a dependent variable of the regression model. These ALA-related parameters were significantly correlated with BPb (Table 2). The BMDs for ALA levels, computed after controlling for age, were between 15.3 and 20.9 µg/dl (Table 3 and Fig. 2); ALA-U showed the highest BMD value among them. Also, the relation of BPb to log-transformed ALAD seemed to be almost linear (Fig. 2), and the BMD for log-transformed ALAD was 2.9 µg/dl (Table 3). In addition, according to the report by Sakai and Morita16, when BMDs were recalculated in 154 lead workers who had BPb levels of less than 40 µg/dl, those for ALA levels were between 3.3 and 8.8 µg/dl (Table 3).

Discussion

The purpose of the current study is the estimation of the "critical dose" of lead exposure causing increased levels of ALA. If we use a P0 of 5% and a BMR of 5% in BMD calculations, the cutoff value represents the upper limit (i.e., Pα=0.05) of a 90% confidence interval of ALA in unexposed subjects; and, since the proportion of exposed subjects with ALA levels above the cutoff value increases with elevated levels of lead, the BMD is the BPb level at which 10% of the exposed workers have such an abnormal ALA level on the dose-response curve. By using the K-power model, the critical dose for ALA levels was estimated to be 15.3–20.9 µg/dl in all subjects, and this value almost corresponded with the threshold for ALA-U estimated by Higashikawa et al.7 It is therefore suggested that the BMD method provides a promising approach for estimating the dose-response association with hazardous factors in the field of occupational health.

On the other hand, the cutoff value of ALA-P, specified from the 154 lead workers with BPb levels below 40 µg/dl by the BMD method, was 11.3 µg/l (Table 3). Morita et al.22 reported a 95% confidence interval of 6.0–12.5 µg/l as a reference value for ALA-P; similarly, the ALA-P level in 33 workers with BPb levels of 2.5–4.9 µg/dl ranged from 6.4 to 10.4 µg/l in another study16. In comparison, the cutoff value at BPb levels below 40 µg/dl seem to be closer to the upper normal limits of ALA-P than does that at BPb levels of 2.1–62.9 µg/dl. Moreover, when BPb exceeds approximately 40 µg/dl, ALA levels have been reported to be acceleratedly elevated7, 16,

Table 1. Blood lead concentration (BPb), δ-aminolevulinic acid levels in plasma (ALA-P), blood (ALA-B), and urine (ALA-U), and δ-aminolevulinic acid dehydratase (ALAD) activity in 186 Japanese lead workers

<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range*</th>
</tr>
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<tbody>
<tr>
<td>BPb (µg/dl)</td>
<td>17.1</td>
<td>2.1 – 62.9</td>
</tr>
<tr>
<td>ALA-P (µg/l)</td>
<td>11.8</td>
<td>6.4 – 65.5</td>
</tr>
<tr>
<td>ALA-B (µg/l)</td>
<td>6.6</td>
<td>3.1 – 38.7</td>
</tr>
<tr>
<td>ALA-U (mg/g Cre)</td>
<td>0.81</td>
<td>0.22 – 3.97</td>
</tr>
<tr>
<td>ALAD (µ: µmol ALA/min/l RBC)</td>
<td>42.4</td>
<td>5.2 – 81.8</td>
</tr>
</tbody>
</table>

*Minimum and maximum.

Table 2. Pearson’s product-moment correlation coefficients (r) between blood lead (BPb) and δ-aminolevulinic acid (ALA) levels, and log-transformed δ-aminolevulinic acid (ALAD) activity in 186 lead workers

<table>
<thead>
<tr>
<th></th>
<th>N=186</th>
<th>N=154*</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALA in plasma</td>
<td>0.748*</td>
<td>0.793*</td>
</tr>
<tr>
<td>ALA in blood</td>
<td>0.743*</td>
<td>0.756*</td>
</tr>
<tr>
<td>ALA in urine</td>
<td>0.661*</td>
<td>0.458*</td>
</tr>
<tr>
<td>log-transformed ALAD</td>
<td>−0.908*</td>
<td>−0.844*</td>
</tr>
</tbody>
</table>

*: Workers with BPb levels of 2.1–39.4 µg/dl. *: p<0.001.
probably due to the increased activity of the ALA synthase\(^2, 4, 16, 23, 24\); consequently, distributions of the ALA were scattered at BPb levels above 40 \(\mu g/dl\) (Fig. 2). For that reason, we should discuss mainly the outcomes from the 154 lead workers with BPb levels below 40 \(\mu g/dl\), while consideration for such an inadequate population could occasionally be disregarded.

In the lead workers with BPb levels below 40 \(\mu g/dl\), the BMDs for ALA levels were estimated to be between 3.3 and 8.8 \(\mu g/dl\) (Table 3). These values seem to be considerably lower than those recognized in previous studies\(^1-6\), and the International Programme on Chemical
Safety\(^{10}\) has also mentioned that the BPb levels, above which effects are demonstrable with current techniques for parameters that may have clinical significance, are all greater than 20 \(\mu g/dl\). Nevertheless, since recent concern is directed to lead at “subclinical” low levels of exposure\(^{15}\), especially to lead affecting susceptible subpopulations such as young children, the range of BPb available for the study design should be considered in a future study. But, such a study would make no sense unless the previously estimated threshold were included within the BPb range.

The BMD for log-transformed ALAD was 2.7 or 2.9 \(\mu g/dl\) (Table 3), implying that the ALAD activity changes almost parallel to the BPb level. This finding agrees with other studies in the general population, which have confirmed the correlation and the apparent lack of a threshold for inhibition of ALAD in different age groups\(^{6, 25}\). The BMDs for ALA-P and ALA-B also followed that for log-transformed ALAD, and the BMD for ALA-U was the highest among ALA-related parameters; the latter finding may have been attributable to the fact that ALA-U is readily affected by urine volume\(^7\). The order of these BMDs is biochemically reasonable, and it is accordingly suggested that BMD calculations are biologically plausible, at least at low doses\(^{10, 13}\).

In conclusion, the inhibition of ALAD due to lead at low levels of less than 10 \(\mu g/dl\) is suggested to cause immediately increased levels of ALA-P and ALA-B. Although such subtle changes in ALA at low levels of exposure may hardly lead to direct impairment or disability in human life, this conclusion provides a notion of the discernible threshold of lead. On the other hand, the odds ratio, relative risk and the 95% confidence intervals have been frequently used in many epidemiological studies. These values imply only the strength of associations, but not a benchmark for preventive goals. For the development of effective public health policy, therefore, further research is necessary to identify the threshold, as noted above, of BPb affecting different target organs such as the brain, peripheral nerves and kidneys, possibly by using the same method.

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