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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 P_0 (abnormal probability of 5% in unexposed workers) to P_0+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

The immediate effect of the inhibition of δ-aminolevulinic acid dehydratase (ALAD) due to lead exposure is an increased level of δ-aminolevulinic acid (ALA) in the plasma (ALA-P) and blood (ALA-B), which then leads to increased urinary excretion (ALA-U)[1, 2]. Selander and Cramer[3] showed a clear threshold effect at about 40 μg/dl in occupational subjects, regarding the correlation between the blood lead concentration (BPb) and ALA-U. Based upon several studies on the associations between BPb and ALA levels[3–6], lead has been suggested to have discernible effects on the ALA-U at a BPb level of around 35 μg/dl[1]. Likewise, the threshold of BPb for decreased ALAD activity has been estimated to be approximately 10 μg/dl[1]. However, since these values may differ according to the used data (e.g., those including an inadequate population) or the mathematical model used for the estimation[7], they should be reconfirmed by using the latest technique; because, an allowable exposure dose computed from such values has been used as a measure of the "acceptability" of occupational exposure levels.

The no-observed-adverse-effect level (NOAEL) is the highest dose at which no statistically or biologically significant adverse effects are identified[8]. On the other hand, the benchmark procedure is a statistical tool used to determine an allowable exposure to a toxic chemical[9, 10]. The benchmark dose (BMD) is a dose that causes a prescribed adverse change in response. Recently, the BMD has been used in the field of environmental health in place of the NOAEL[11–14], because many shortcomings of the NOAEL have been identified, including not adequately reflecting the shape of the dose response and not appropriately accounting for study size[8, 10].

Lead at low levels of exposure has been recognized to be toxic[15], and it would be important to discern a threshold for lead toxicity in populations exposed to lead. For this reason, the benchmark procedure was introduced into this study to identify the BPb level at which abnormal changes in ALA-related parameters emerged in lead workers.

Methods

At the time of the special health checkup for lead

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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 m*l* 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 m*l*/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/*l* RBC) [20]. BPb was determined by flameless atomic absorption spectrometry (Hitachi Z-8000, Tokyo, Japan).

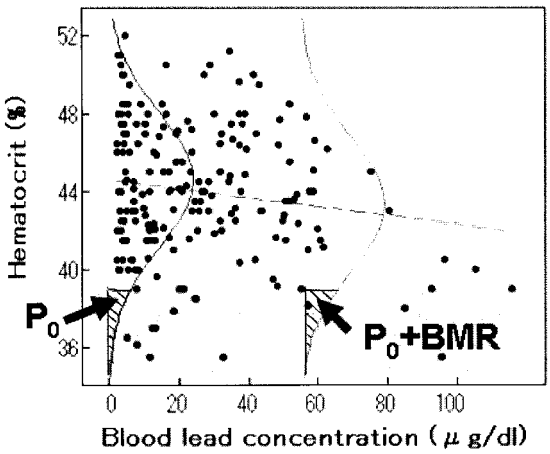

Fig. 1. Dose-effect relation of the blood lead concentration to hematocrit in lead workers for benchmark dose (BMD) calculation. P_0 and benchmark response (BMR) indicated an abnormal probability (5%) in unexposed workers and an excess risk (5%) above P_0 in exposed workers, respectively. The power parameter 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 P_0 for unexposed subjects to P_0+BMR for exposed subjects at the BMD (Fig. 1)[9, 10]. Previous applications of this method have used a P_0 (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[13]. And, the BMD and the cutoff value (C) of P_0 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)=$\beta_0+\beta_1 \cdot d^K+\beta_2 \cdot$ [age], (2) $P_0=\Phi((C-\beta_0)/\sigma)$, and (3) $BMD=[\Phi^{-1}(P_0)-\Phi^{-1}(P_0+BMR)] \cdot \sigma/\beta_1$ (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[13]. 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/d*l*. 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/d*l* (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/d*l* (Table 3). In addition, according to the report by Sakai and Morita[16], when BMDs were recalculated in 154 lead workers who had BPb levels of less than 40 μg/d*l*, those for ALA levels were between 3.3 and 8.8 μg/d*l* (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 P_0 of 5% and a BMR of 5% in BMD calculations, the cutoff value represents the upper limit (i.e., P_0=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/d*l* 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/d*l* 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/d*l* ranged from 6.4 to 10.4 μg/*l* in another study[16]. In comparison, the cutoff value at BPb levels below 40 μg/d*l* seem to be closer to the upper normal limits of ALA-P than does that at BPb levels of 2.1–62.9 μg/d*l*. Moreover, when BPb exceeds approximately 40 μg/d*l*, ALA levels have been reported to be acceleratedly elevated[7, 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

	Median	Range*
BPb (μg/d*l*)	17.1	2.1 – 62.9
ALA-P (μg/*l*)	11.8	6.4 – 65.5
ALA-B (μg/*l*)	6.6	3.1 – 38.7
ALA-U (mg/g Cre)	0.81	0.22 – 3.97
ALAD (u: μmol ALA/min/l RBC)	42.4	5.2 – 81.8

*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

	Correlation coefficient	
	N=186	N=154[a]
ALA in plasma	0.748*	0.793*
ALA in blood	0.743*	0.756*
ALA in urine	0.661*	0.458*
log-transformed ALAD	–0.908*	–0.844*

[a]: Workers with BPb levels of 2.1–39.4 μg/d*l*. *: p<0.001.

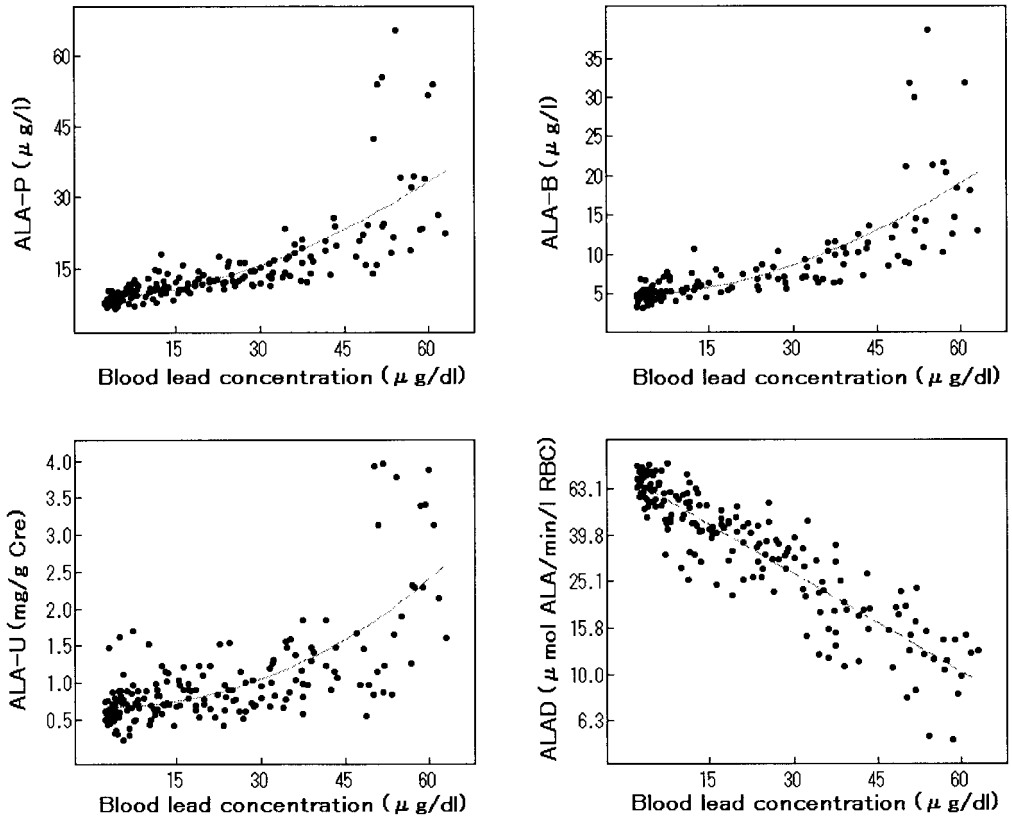

Fig. 2. Dose-effect relations of the blood lead concentration to δ-aminolevulinic acid levels in plasma (ALA-P), blood (ALA-B), and urine (ALA-U) and δ-aminolevulinic acid dehydratase (ALAD) activity in 186 lead workers for benchmark dose (BMD) calculation.

Table 3. Benchmark doses (BMD, μg/d*l*) of blood lead (BPb) and the lower 95% confidence limits (BMDL, μg/d*l*), set at the P_0 of 5% and BMR of 5% after controlling for age, for δ-aminolevulinic acid levels in plasma (ALA-P), blood (ALA-B), and urine (ALA-U), and log-transformed δ-aminolevulinic acid dehydratase (ALAD) activity in two groups (Group A: 186 lead workers with BPb levels of 2.1–62.9 μg/d*l*; Group B: 154 lead workers with BPb levels of 2.1–39.4 μg/d*l* of Group A)[a]

	Group A			Group B		
	Cutoff*	BMD	BMDL	Cutoff*	BMD	BMDL
ALA-P (μg/*l*)	17.9	15.3	14.3	11.3	3.3	2.9
ALA-B (μg/*l*)	10.7	16.6	15.3	6.6	4.2	3.5
ALA-U (mg/g Cre)	1.43	20.9	19.6	1.1	8.8	6.8
log-transformed ALAD (u)	46.9	2.9	2.6	48.8	2.7	2.3

[a]: P_0 and BMR indicate an abnormal probability in unexposed workers and an excess risk in exposed workers, respectively (see *Methods*). *: Cutoff value, specified by the BMD method, of each of the ALA-related parameters.

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 μg/d*l* (Fig. 2). For that reason, we should discuss mainly the outcomes from the 154 lead workers with BPb levels below 40 μg/d*l*, while consideration for such an inadequate population could occasionally be disregarded.

In the lead workers with BPb levels below 40 μg/d*l*, the BMDs for ALA levels were estimated to be between 3.3 and 8.8 μg/d*l* (Table 3). These values seem to be considerably lower than those recognized in previous studies[3–6], and the International Programme on Chemical

Safety[1)] 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 μg/d*l*. 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 μg/d*l* (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 μg/d*l* 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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