Evaluation of δ -aminolaevulinic acid in blood of workers exposed to lead

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Abstract

Exposure-effect and exposure-response relation between exposure to lead and δ -aminolaevulinic acid concentration in blood (ALA-B) were examined in 238 male workers exposed to lead. Concentrations of ALA-B ranged from 26 to 352 $\mu\text{g/l}$ and lead concentrations in blood (Pb-B) from 7.1 to 86.0 $\mu\text{g/dl}$. Concentrations of ALA-B correlated closely with concentrations of Pb-B ($r = 0.74$), and increased ALA-B concentration occurred at Pb-B concentrations of around 30 $\mu\text{g/dl}$. Exposure-response curves indicated that the 50 percentile response doses were roughly 30, 40, and 50 $\mu\text{g/dl}$ Pb-B when cut off points of ALA-B were set at 50, 60, and 70 $\mu\text{g/l}$ respectively. The sensitivity and specificity of measurements of ALA-B concentrations for health screening were sufficiently high when the health based exposure limits of lead were set at 30-50 $\mu\text{g/dl}$. Moreover, a pronounced increase in ALA-B concentrations occurred when the inhibition rate of erythrocyte ALA dehydratase exceeded 85%. These findings suggest that ALA-B is a useful indicator for assessing the early effects of exposure to lead on haem biosynthesis.

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The effect of lead on haem biosynthesis has been recognised as the earliest response to exposure to lead. Inhibition of erythrocyte δ -aminolaevulinic acid dehydratase (ALAD), excessive excretion of δ -aminolaevulinic acid (ALA) and coproporphyrins (CPs) in urine, and increases in protoporphyrins (PPs) in red blood cells have been used to assess the effects of lead on health. The relation between blood

ALA (ALA-B) concentrations and exposure to lead has not been adequately assessed, however, although references to it have been made in some studies.¹⁻⁴

We performed a cross sectional study on workers exposed to lead to clarify the relations between blood lead (Pb-B) and ALA-B concentrations and to assess its validity as an indicator of the effects of lead on health.

Subjects and methods

The subjects of this study consisted of 240 male workers at a lead storage battery factory and a lead smelting works. They were 20-59 years old and had been exposed to lead for one to 32 years. Two workers had iron deficiency anaemia, and urinary concentrations for 22 workers were extraordinarily high or low on the examination day (urinary creatinine concentration >3 or <0.5 g/l). These workers were excluded. Thus the final number of subjects was 238 for blood analysis and 216 for urine analysis.

Concentration of ALA-B was measured by the method described in our previous report.⁵ Briefly, 2 ml of heparinised whole blood was treated with 0.15 ml 0.06 M iodoacetamide and 0.35 ml 35% (w/v) trichloroacetic acid to eliminate interfering thiol compounds and proteins, and centrifuged at 3000 rpm for 10 minutes. The ALA in the supernatant was condensed to pyrrol and acetylated by the addition of acetylacetone, formaldehyde, and acetic acid and heating at 110°C for 20 minutes. The solution was then subjected to high performance liquid chromatography (HPLC). Table 1 shows the conditions for HPLC. The standard ALA (purity roughly 98%) used for calibration was purchased from Sigma Chemical Co.

The urine samples were diluted with an equal volume of distilled water and condensed, acetylated, and subjected to HPLC in the same manner as that for ALA-B. Urinary ALA (ALA-U) concentrations were adjusted according to the concentration of creatinine in the urine.

For determination of Pb-B concentrations, 0.4 ml of whole blood was pretreated by the method of Stoeppeler and Brandt⁶ and introduced into a flameless atomic absorption spectrometer (Z-7000, Hitachi Ltd). Internal quality control was performed with

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Table 1 Analytical conditions for high performance liquid chromatography

Apparatus	HPLC Type 655A (Hitachi Co Ltd)
Column	TSK gel ODS-80Tm (TOSOH Co Ltd)
	Particle size : 5 μ m
	Column size : 2.6 mm $\times$ 15 cm
	Temperature : room temperature
Detector	Fluorescence type F-1000 (Hitachi Co Ltd)
	Excitation wave length : 373 nm
	Emission wave length : 463 nm
Mobile phase	Methanol 60% (w/v) and acetic acid 1% (w/v)
	Flow rate : 0.7 ml/min
Injection	100 μ l

pooled blood samples and commercially available control blood specimens (Control Blood for Metals 1, Behring Institut).

Erythrocyte ALAD activity was measured by the European standardised method.⁷ The effect of lead on ALAD activity was expressed as the % of non-activated to activated ALAD activity (%ALAD-I); ALAD was activated using the method of Kajimoto *et al.*⁸

Concentration of zinc protoporphyrin IX (ZPP) in packed red blood cells was measured by the method of Sakai *et al.*⁹

Results

Concentrations of Pb-B in the subjects of this study ranged from 7.1 to 86.0 μ g/dl with an arithmetic mean of 37.0 μ g/dl. Figure 1 shows a typical ALA-B chromatogram; ALA was sufficiently separated, and 5 μ g/l could be determined at a signal/noise ratio of 5. Retention time of ALA was around 9.2 minutes. Concentrations of ALA-B ranged from 26 to 352 μ g/l with an arithmetic mean and a geometric mean of 67.5 and 60.8 μ g/l respectively. The arithmetic mean and geometric mean of ALA-U concentrations were 1.6 and 1.2 mg/g creatinine, ranging from 0.3 to 11.0 mg/g creatinine. The range of ZPP concentrations was from 74 to 1960 μ g/dl red blood cells (arithmetic mean 361 and geometric mean 292 μ g/dl red blood cells), and that of %ALAD-I was from 15.0 to 98.0% (arithmetic mean 74.5%).

Table 2 shows the Spearman rank correlation coefficients between Pb-B concentrations and four indices of effects of lead on haem synthesis. All four indices were closely correlated with Pb-B concentrations.

Figures 2 and 3 show the exposure-effect relations between Pb-B and ALA-B concentrations and between Pb-B and ALA-U concentrations. As Pb-B concentration increased, both ALA-B and ALA-U concentrations increased exponentially. Table 3 shows the arithmetic mean and standard deviation of ALA-B and ALA-U by Pb-B concentrations. Significant increases in ALA-B concentration occurred in the group in which Pb-B concentration was from

Figure 1 A typical ALA-B chromatogram.

Table 2 Spearman rank correlation coefficients between Pb-B concentration and biological effect parameters of lead

	Pb-B
ALA-B	0.74
ALA-U	0.59
%ALAD-I	0.89
ZPP	0.71

25 to 34 $\mu\text{g}/\text{dl}$ compared with the group in which Pb-B concentration was 14 $\mu\text{g}/\text{dl}$ or less. That of ALA-U was in the group of 35–44 $\mu\text{g}/\text{dl}$; ALA-B concentration started to increase at a lower Pb-B

Figure 2 Exposure-effect relation between Pb-B and ALA-B concentrations.

Figure 3 Exposure-effect relation between Pb-B and ALA-U concentrations.

concentration, (roughly 30 $\mu\text{g}/\text{dl}$) than did ALA-U.

Figure 4 shows the relation between %ALAD-I and ALA-B. Concentration of ALA-B increased when %ALAD-I rose to 85% or above. A similar relation was found between %ALAD-I and ALA-U concentration.

Figure 5 shows the exposure-response relations between Pb-B concentration and indices of the effect of lead on haem synthesis. When cut off points of ALA-B concentration were set at 50 or 60 $\mu\text{g}/\text{l}$, 50 percentile responses occurred around 30 or 40 $\mu\text{g}/\text{dl}$ of Pb-B respectively. Furthermore, when the cut off point was set at 70 $\mu\text{g}/\text{l}$, ALA-B concentration responded with most sensitivity at around 50 $\mu\text{g}/\text{dl}$ of Pb-B, the biological exposure index of the American Conference of Governmental and Industrial Hygienists.¹⁰ Compared with other indices, the ALA-B responses occurred at lower Pb-B concentrations than those of ALA-U (cut off values 2 or 5 mg/g creatinine), and similar to %ALAD-I (cut off values 70 or 85%) and ZPP (cut off values 200 or 300 $\mu\text{g}/\text{dl}$ red blood cells).

Tables 4 and 5 show the sensitivity and specificity¹¹ of ALA-B and ALA-U concentrations for screening at various Pb-B biological exposure limits and at various cut off values. Judged on the basis of the J-index (sensitivity + specificity - 1),¹² acceptable ALA-B cut off values were 50 $\mu\text{g}/\text{l}$ for 30 $\mu\text{g}/\text{dl}$ Pb-B, 60 $\mu\text{g}/\text{l}$ for 40 $\mu\text{g}/\text{dl}$, and 70 $\mu\text{g}/\text{l}$ for 50 $\mu\text{g}/\text{dl}$. Concentrations of ALA-B had a higher J-index than concentrations of ALA-U at 30 and 40 $\mu\text{g}/\text{dl}$ Pb-B.

Discussion

Activity of ALAD and concentration of PP in erythrocytes, and ALA and CP concentrations in urine have been used as major indices of the effects of lead on haematopoietic systems. Furthermore, an increase in PP concentration has been reported to occur at lower Pb-B concentrations than the increase in ALA-U.¹³ The newly developed HPLC for determination of ALA made it possible to detect slight increases in ALA-U at Pb-B concentrations of 40 $\mu\text{g}/\text{dl}$ or below without the interference from urinary components as found in colorimetric determinations.¹⁴ The role of ALA-B, however, had not yet been evaluated. In this study, concentration

Table 3 Arithmetic mean (SD) of ALA-B and ALA-U by Pb-B concentrations

	Pb-B ($\mu\text{g}/\text{dl}$)					
	≤ 14	15–24	25–34	35–44	45–54	≥ 55
ALA-B ($\mu\text{g}/\text{l}$)	43.0 (3.3) (n = 5)	42.3 (7.3) (n = 38)	52.8 (11.8)** (n = 69)	65.2 (16.0)** (n = 58)	84.8 (42.4)** (n = 49)	146.2 (74.0)** (n = 18)
ALA-U (mg/g creatinine)	0.8 (0.2) (n = 5)	0.8 (0.3) (n = 30)	0.9 (0.4) (n = 61)	1.2 (0.5)** (n = 55)	2.3 (2.1)** (n = 46)	4.5 (2.9)** (n = 18)

* $p < 0.05$; ** $p < 0.01$) compared with the ≤ 14 $\mu\text{g}/\text{dl}$ Pb-B group.

Figure 4 Relations between %ALAD-I and ALA-B concentration.

of ALA-B correlated closely with Pb-B concentration, and increase in ALA-B occurred at Pb-B concentrations of around 30 $\mu\text{g}/\text{dl}$; ALA-B also responded with sensitivity at 30, 40, or 50 $\mu\text{g}/\text{dl}$ Pb-B, when cut off values were set at 50, 60 or 70 $\mu\text{g}/\text{l}$ ALA-B, respectively. Concentration of ALA-B is therefore a useful indicator for assessing the effects of lead on haem synthesis at Pb-B concentrations of around 30–50 $\mu\text{g}/\text{dl}$.

Measurement of ALA-U concentration has been used as a reliable screening item to detect the health effects of lead. In Japan, the Ordinance on Prevention of Lead Poisoning requires measurement of ALA-U concentration as one of the essential health screening items in all workers exposed to lead. The advantage of using urine is that samples can be obtained easily. The disadvantage is the need to adjust measured values because of wide intra and interindividual

Figure 5 Exposure-%response relations between Pb-B concentration and the biological effect parameters of lead. Numbers are set cut off concentrations (see text).

Table 4 Sensitivity and specificity of ALA-B by biological exposure limits for Pb-B

Biological exposure limit for Pb-B ($\mu\text{g/dl}$)	ALA-B cut off point ($\mu\text{g/l}$)					
	50		60		70	
	Sens	Spec	Sens	Spec	Sens	Spec
30	0.85	0.81	0.58	0.97	0.43	1.00
40	0.94	0.55	0.75	0.82	0.57	0.89
50	0.98	0.42	0.93	0.69	0.80	0.80

Sens = sensitivity; Spec = specificity.

Table 5 Sensitivity and specificity of ALA-U by biological exposure limits for Pb-B

Biological exposure limit for Pb-B ($\mu\text{g/dl}$)	ALA-U cut off point (mg/g creatinine)					
	1.0		1.5		2.0	
	Sens	Spec	Sens	Spec	Sens	Spec
30	0.77	0.55	0.46	0.89	0.27	0.97
40	0.84	0.45	0.61	0.83	0.42	0.96
50	0.95	0.39	0.80	0.73	0.70	0.90

variations in urine concentration. The results of this study showed that the variation of ALA-B in the exposure-effect curve of Pb-B is smaller than that of ALA-U concentration corrected for urinary creatinine concentrations, and that the response of ALA-B is more sensitive than that of ALA-U at Pb-B concentrations of 30–40 $\mu\text{g/dl}$. Consequently, ALA-B is superior to ALA-U concentration in terms of smaller intra and interindividual variations in measurements and the lack of any need to adjust concentration. Furthermore, because the Ordinance on Prevention of Lead Poisoning requires determination of Pb-B concentration at intervals of six or 12 months, it is not a critical advantage to use urine samples for health screening of workers exposed to lead in Japan.

In this study, a pronounced increase in ALA-B concentration occurred at a %ALAD-I of around 85%. This suggests that %ALAD-I is a good predictor of overflow of ALA from the haematopoietic organ. The most sensitive indicator of lead exposure has been reported to be ALAD activity,¹⁵ but its biological significance remains controversial. One opinion is that it is an effect index of lead above a Pb-B concentration of 40 $\mu\text{g/dl}$ because it is unaccompanied by any detectable effects on the biological function of humans below 40 $\mu\text{g/dl}$ of Pb-B.¹⁵ A contrasting opinion is that it is only an exposure index of lead because it is strongly influenced by Pb-B concentration and ALAD activity in peripheral blood never contributes to haem synthesis in the haematopoietic organ.¹⁶ As an exposure index, Pb-B concentration is superior to %ALAD-I

because Pb-B concentration is a direct exposure indicator and chemically stable without any treatment of blood until determination. From the view of the effect index, %ALAD-I correlated closely with Pb-B concentration, and responded with sensitivity at Pb-B concentrations of 30 or 50 $\mu\text{g/dl}$ when cut off points of %ALAD-I were set at 70% or 85% respectively in this study. If the health based exposure limit for lead is set at 30–50 $\mu\text{g/dl}$ Pb-B, however, ALA-B is superior to %ALAD-I because the measuring method for %ALAD-I is more complicated than that of ALA-B in terms of analytical procedure and preservation without loss of enzymatic activity.

To determine ALA-B concentration by HPLC, elimination of proteins and thiol compounds is required. Measurement of ZPP by HPLC requires an extraction procedure,⁹ and CP measurement requires protection of urine samples from light.¹⁷ Consequently, the technical procedures for measurement of ALA-B do not seem as complicated as measurement of other parameters of haem synthesis, and measurements of both Pb-B and ALA-B concentration are useful for health screening for the haematological effects of lead if blood specimens are collected.

In conclusion, the results of this study indicate that ALA-B concentration, measured by HPLC, is a useful effect index of exposure to lead on haem synthesis, and measurement of Pb-B and ALA-B concentrations may be the best combination for health screening of workers exposed to lead when the health based exposure limit for lead has been set at 30–50 $\mu\text{g/dl}$ Pb-B. The inhibition rate of ALAD activity in erythrocytes in the presence of appreciable increases in ALA-B concentration is presumed to be 85% or more. Further studies are necessary to evaluate the differences in concentration of ALA-B in males and females and in whole blood and serum.

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