Interrelation Between Urinary δ-Aminolevulinic Acid (ALA), Serum ALA, and Blood Lead in Workers Exposed to Lead

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Abstract: Using a fluorometric HPLC method, we determined δ-aminolevulinic acid (ALA) in sera and urine samples from 16 lead workers with blood lead levels ranging from 19 to 107 µG/100 ml. The concentration of ALA in serum correlated highly with the urinary ALA concentration (γ=0.957 for ALA mg/l; γ=0.967 for ALA mg/g creatinine). The ALA concentrations in the serum of lead workers ranged from 11 to 151 µg/l with a mean of 51 µg/l. In addition, the concentrations of urinary ALA (mg/g creatinine) and serum ALA (µg/l) had a strong correlation with blood lead concentrations (γ=0.838 and 0.892, respectively).

These data indicate that the measurement of serum ALA, as well as urinary ALA, is very useful for the biological monitoring of occupational lead exposure.

Key words: Lead exposure-δ-Aminolevulinic acid-Urine-Serum-Blood lead

INTRODUCTION

δ-Aminolevulinic acid (ALA) is a product of the first and rate-limiting step of heme biosynthesis, and is formed by condensation of succinyl CoA with glycine. It is known that ALA accumulates in certain diseases such as acute hepatic porphyrias and lead poisoning. Increasing excretion of urinary ALA has been considered one of the most specific indices for evaluating the health effects of occupational exposure to lead. Therefore, the urinary ALA has widely been used as a measure of the biological effect of lead, in workers occupationally exposed to lead. In Germany, the biological tolerance value, i.e., Biologischer Arbeitsstoff-Toleranzwert (BAT) level, of urinary ALA for biological monitoring has been recommended to be 15 mg/l (6 mg/l for women younger than 45 years)1). In Japan, the urinary ALA test was introduced into the health examination of lead

Colorimetric methods based on the color reaction of ALA-pyrrole with Ehrlich’s reagent have widely been used for determining ALA in urine \(^2-^7\). Because of its low sensitivity, however, it is impossible to determine ALA in a small volume of human serum or plasma using this colorimetric method. On the other hand, a high-performance liquid chromatographic (HPLC) method with fluorometry has recently been developed by several investigators\(^8-^10\). This fluorometric HPLC method is highly sensitive and specific for measuring urinary ALA. If this fluorometric HPLC method is adopted, it will be possible to determine ALA in human serum and plasma.

In this study we determined ALA in the serum and urine of lead workers, compared the ALA values obtained and discussed these findings with reference to the concentration of lead in blood.

SUBJECTS AND METHODS

Subjects and Sample Collection

Sixteen workers (14 men and 2 women) occupationally exposed to lead were selected. They were employed in a secondary lead smelter factory. The mean age of the subjects was 41 years old (range 18–64). The mean occupational exposure period to lead was 7 years (range; 0.1–23 years). Venous blood samples, with and without anticoagulant (heparin), and spot urine specimens were collected. These samples were obtained at the end of a workshift, on the same day. Heparinized blood was used for blood lead analysis. Unheparinized blood was used for serum separation. These blood samples were stored at 4°C and analysed as quickly as possible. The urine samples were stored at —20°C until analysis.

Determination of Urinary and Serum ALA

The determination of ALA in urine was performed using a recently described fluorometric HPLC method\(^11\). The serum ALA was determined by a modification of this HPLC method. The fluorescent derivatization of serum ALA was the same as that of urinary ALA. After derivatization, the reaction mixture was filtered through a disposable HPLC filter (DISMIC-25cs, 1.8 μm) (Advantec-Toyo, Ltd., Tokyo), and 20 μl of the filtrate was injected into the HPLC column. The analytical conditions for HPLC were the same as those used for urinary ALA. The concentration of a working ALA standard used for serum analysis was 100 μg/l. Using this procedure, deproteinization of the serum sample could be carried out by both heating and addition of ethanol during the fluorescent derivatization, without trichloroacetic acid.
Other Analyses

The concentration of lead in blood was determined by flameless atomic absorption spectrophotometry after the whole blood had been diluted 10-fold with 0.1 N HNO₃ containing 1% triton X-100. The flameless atomic absorption spectrophotometer used was a Hitachi Z-9000 (Hitachi, Ltd., Tokyo). Urinary creatinine (Cr) was measured with a kit from Wako Pure Chemicals Ltd. (Osaka). Haemoglobin concentration was measured by the standard method after conversion to cyanmethaemoglobin. Packed cell volumes were measured using a microcapillary centrifuge.

RESULTS AND DISCUSSION

The table shows the mean, standard deviation (SD), and the range of biological parameters obtained from 16 lead workers. The haemoglobin concentrations and packed cell volumes in these workers were within the normal range.

Figure 1 shows the relationship between the concentration of lead in blood and the amount of urinary ALA as determined with the fluorometric HPLC method. The urinary concentration of ALA was corrected with the concentration of urinary creatinine. A good correlation between the two values was found ($\tau = 0.838$).

Figure 2 shows the correlation between the concentrations of ALA in serum and blood lead levels obtained from 16 lead workers. The concentration of ALA in serum had a strong correlation with that of blood lead in these workers ($\tau = 0.892$). This finding indicates that the measurement of serum ALA in lead workers is available as a tool for biological monitoring of occupational lead exposure. When the fluorometric HPLC method is used, the concentration of ALA in serum and plasma can be measured using small sample volumes (50µl).

Figure 3 shows the correlation between the concentration of ALA in serum and

| Table 1. Concentrations of blood lead, serum δ-aminolevulinic acid (ALA), urinary δ-aminolevulinic acid (ALA), and urinary creatinine in 16 lead workers |
|---------------------------------|------|--------|
| Blood lead (µg/100 ml)         | 62   | 26     |
| Serum ALA (µg/l)               | 51   | 40     |
| Urinary ALA (mg/l)             | 7.7  | 9.3    |
| Urinary ALA (mg/g creatinine)  | 4.8  | 4.7    |
| Urinary creatinine (g/l)       | 1.36 | 0.62   |
| Mean                           | (SD) | Range  |
| 19 - 107                       |      |        |
| 11 - 151                       |      |        |
| 0.7 - 35.5                     |      |        |
| 0.8 - 17.8                     |      |        |
| 0.26 - 2.25                    |      |        |
Fig. 1. Relation between urinary ALA concentrations (mg/g creatinine) and blood lead levels in 16 lead workers.

Fig. 2. Relation between serum ALA concentrations and blood lead levels obtained from 16 lead workers.
Fig. 3. Relation between serum ALA concentrations (µg/l) and the corrected urinary ALA concentrations (mg/g creatinine) obtained from 16 lead workers.

Fig. 4. Relation between serum ALA concentrations and the uncorrected urinary ALA concentrations (mg/l) obtained from 16 lead workers.
the corrected concentration of urinary ALA (mg/g creatinine) in 16 lead workers. The correlation coefficient (r) was 0.967.

Figure 4 shows the correlation between the concentrations of ALA in serum and the uncorrected concentrations of urinary ALA (mg/l) in the same workers. A significant correlation was found (r=0.957). The regression equation between serum ALA (µg/l)(Y) and corrected urinary ALA (mg/g Cr)(X) was Y = 8.13X + 12.4. On the other hand, the regression equation between serum ALA (µg/l)(Y) and uncorrected urinary ALA (mg/l)(X) was Y = 4.09X + 19.8. The concentration of serum ALA corresponding to 10 µg/l of urinary ALA (screening level in Japan) was calculated to be 61 µg/l from these regression equations, and its value corresponded to 6.0 mg/g creatinine of urinary ALA.

The above results demonstrate that in the biological monitoring of occupational lead exposure, serum ALA measurement yields nearly the same results as the measurement of urinary ALA.

In this study, the number of biological samples was limited to 16. However, we suggest that the present results would be confirmed in studies involving a greater number of lead workers.

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REFERENCES
