Relation between urinary $\beta$-aminoisobutyric acid excretion and concentration of lead in the blood of workers occupationally exposed to lead

Katsumaro Tomokuni, Masayoshi Ichiba, Koji Mori

Abstract
Urinary $\beta$-aminoisobutyric acid (ABA) concentration was determined by fluorometric high performance liquid chromatography in 22 workers occupationally exposed to lead. The urinary excretion of ABA was increased with increasing exposure to lead. The excretion of urinary ABA had a significant correlation with concentration of lead in blood (Pb-B) ($r = 0.581$), similar to the correlation of Pb-B with urinary $\delta$-aminolevulinic acid (ALA) concentration ($r = 0.563$). The determination of urinary ABA concentration in workers exposed to lead, therefore, may offer a new approach for evaluating the health effect of lead.

$\beta$-Aminoisobutyric acid (ABA) is a normal degradation product of thymine, a constituent of DNA and tRNA. It is known that the urinary excretion of ABA is raised in many patients with urothelial tumours.

Recently, Farkas et al. have indicated that urinary excretion of ABA is increased in workers exposed to lead. This interesting finding may offer a new approach for evaluating the effects of exposure to lead on health. In their paper, however, a correlation between urinary ABA excretion and blood lead concentration (Pb-B) in workers exposed to lead was not shown.

The main purpose of the present study was to compare the relation between urinary excretion of ABA and Pb-B with that between urinary excretion of $\delta$-aminolevulinic acid (ALA) and Pb-B, in workers occupationally exposed to lead.

Materials and methods
SUBJECTS AND SAMPLE COLLECTION
Twenty two workers (19 men and three women) occupationally exposed to lead were selected. They were employed in secondary lead smelters. The mean age of the subjects was 47 (range 22-65). The mean occupational exposure of the workers to lead was five years (range 0.1-22 years). Heparinised venous blood and spot urine samples were collected. These samples were obtained on the same day at the end of a workshift. The blood samples were stored at $4^\circ$C and analysed as soon as possible. The urine samples were stored frozen at $-20^\circ$C. Normal spot urine samples were also collected from 28 healthy workers with no history of lead exposure, whose age ranged from 26 to 54 (mean 45). They were also stored frozen at $-20^\circ$C. Urinary ALA and ABA were stable for more than three months under the conditions used.

DETERMINATION OF URINARY ABA
The urinary ABA concentration (ABA-U) was determined by high performance liquid chromatography (HPLC) after preparation of a fluorescent derivative with o-phthalaldehyde reagent as follows: 27 mg of o-phthalaldehyde (Sigma Chemical Co) was dissolved in 0.5 ml of ethanol and to this solution were added 4.5 ml of sodium buffer (0.4 M, pH 9.5) and 24 $\mu$l of mercaptoethanol. This reagent was used within 24 hours of preparation.

The spot urine sample was diluted 100-fold with distilled water before analysis. A stock solution (100 mg/l) of ABA standard was prepared by dissolving 10 mg of ABA (Sigma Chemical Co) in distilled water and diluting it to 100 ml. This stock solution was stable for more than three months when stored in a refrigerator at $4^\circ$C. The working standard of ABA (1 mg/l) was made by 100-fold dilution of the stock solution.

To prepare the fluorescent derivative of ABA, 100 $\mu$l of the diluted sample and ABA standard (1 mg/l) were mixed with 100 $\mu$l of sodium borate buffer (0.4 M, pH 9.5) and 100 $\mu$l of the o-phthalalde-
hyde reagent. After exactly one minute the reaction was stopped by adding 100 μl of 0·1 M KH₂PO₄ (pH 4·0). The reaction mixture (10 μl) was promptly injected into the HPLC column. The conditions of HPLC analysis were as follows: apparatus, Shimadzu LC-6A; column, Shimpack CLC-ODS 150 × 4·6 mm; detector, Shimadzu RF-535 (330/460 nm); mobile phase, methanol/5 mM KH₂PO₄ (600/400); flow rate, 0·5 ml/min; column temperature, 40°C.

OTHER ANALYSES

The concentration of blood lead (Pb-B) was determined by flameless atomic absorption spectrophotometry after a 10-fold dilution of whole blood with 0·1 N HNO₃ containing 1% triton X-100. Haemoglobin concentration was measured by the standard method after conversion to cyanmethaemoglobin. Packed cell volumes were measured using a microcapillary centrifuge. Erythrocyte protoporphyrin concentration was determined fluorometrically according to the method of Piomelli. Values for ALA-U were determined by the colorimetric method of Tomokuni and Ogata after a threefold dilution with distilled water. Urinary coproporphyrin concentration was measured by the fluorometric HPLC method of Tomokuni and Hirai. Urinary creatinine was measured with a kit from Wako Pure Chemicals Ltd (Japan). The urinary concentrations of ABA, ALA, and coproporphyrin were adjusted to the concentration of urinary creatinine.

Results and discussion

The fluorometric HPLC method for determining ABA-U was designed to carry out this study. This HPLC analysis is simple but must be done promptly because the fluorescent derivative of ABA is unstable in the reaction mixture. When this procedure is used the large scale dilution (100-fold) of urine with distilled water is necessary for the analysis because the urinary concentration of ABA is high. The coefficient of variation, obtained from five repeated analyses, was about 6%.

Figure 1(A) shows the HPLC chromatogram of ABA-U obtained from a lead exposed worker and fig 1(B) the chromatogram from an ABA standard (1 mg/l). The fluorescence product of ABA was completely separated from other fluorescent substances in the reaction mixture. The retention time of the ABA derivative was about five minutes. This chromatogram corresponds to a concentration of ABA-U of 162 mg/g creatinine. Figure 2 shows the good linearity of a standard curve of ABA.

The table shows the mean, standard deviation (SD), and range of biological parameters obtained from 22 lead workers. The haemoglobin concentrations and packed cell volumes in these workers were within the normal range. The Pb-B ranged from 8 to 78 μg/100 ml with a mean of 43 μg/100 ml. The ABA-U in 22 lead-exposed workers averaged 104 mg/g creatinine with a range of 39 to 270 mg/g creatinine. On the other hand, the ABA-U obtained from 28 normal control workers averaged 41 mg/g.
Urinary $\beta$-aminoisobutyric acid and lead in the blood of workers exposed to lead

Concentrations of blood lead (Pb-B), erythrocyte protoporphyrin (FEP), urinary $\beta$-aminoisobutyric acid (ABA-U), urinary $\delta$-aminolaevulinic acid (ALA-U), and urinary coproporphyrin (CP-U) obtained from 22 lead workers

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb-B ($\mu$g/100 ml)</td>
<td>43 (19)</td>
<td>8–78</td>
</tr>
<tr>
<td>FEP ($\mu$g/100 ml)</td>
<td>153 (98)</td>
<td>43–343</td>
</tr>
<tr>
<td>ABA-U (mg/g creatinine)</td>
<td>104 (60)</td>
<td>39–270</td>
</tr>
<tr>
<td>ALA-U (mg/g creatinine)</td>
<td>5 (4)</td>
<td>2–18</td>
</tr>
<tr>
<td>CP-U (pg/g creatinine)</td>
<td>184 (501)</td>
<td>4–2405</td>
</tr>
</tbody>
</table>

![Figure 3](image3.png)

**Figure 3** Relation between urinary excretion of $\beta$-aminoisobutyric acid (ABA-U) and blood lead concentration (Pb-B) obtained from 22 lead workers.

creatinine with a range of 26 to 74 mg/g creatinine. Among these control workers, there was no correlation between ABA-U and age. These data indicated that ABA-U is higher in lead workers than in control workers.

Figure 3 shows the strong positive correlation between ABA-U and Pb-B in 22 lead workers ($r = 0.581$, $p < 0.01$). The finding of increased excretion of ABA-U in lead exposed workers suggests that the exposure to lead may not only affect the metabolism of thymine but may also damage DNA and tRNA. This raises the possibility of a new approach for evaluating the health effect of exposure to lead.

Figure 4 shows the ALA-U and Pb-B in 22 lead workers. The degree of urinary excretion of ALA is thought to be one of the most specific indicators in evaluating the health effect of occupational exposure to lead, and, therefore, its measurement is widely used for biological monitoring of the health effect in lead workers in Japan. The correlation between ALA-U and Pb-B was significant ($r = 0.563$), and the correlation coefficient was similar to that between ABA-U and Pb-B ($r = 0.581$). These results suggest that the urinary excretion of ABA is useful as a measure for evaluating the health effect of lead in workers occupationally exposed to lead. Contrary to expectation, however, the correlation between ABA-U and ALA-U was poor among the 22 lead workers ($r = 0.377$; fig 5). Because of this data obtained from large numbers of lead exposed workers need to be compared before a definitive statement is made.

No significant correlation between Pb-B and urin-
ary coproporphyrin concentration was found in the 22 lead workers. The highest correlation was obtained between Pb-B and erythrocyte protoporphyrin concentration ($r = 0.652$). In the present study, the correlation of Pb-B with other biological parameters was in the order erythrocyte protoporphyrin concentration > ABA-U > ALA-U.

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