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Comparison between males and females with respect to the porphyrin metabolic disorders found in workers occupationally exposed to lead

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Abstract To elucidate the sex difference in porphyrin metabolic disorders induced by lead exposure, we determined plasma δ-aminolevulinic acid (ALA), urinary ALA, and urinary coproporphyrin (CP) in 298 lead-exposed workers (160 males and 138 females), and compared the data thus obtained. The use of fluorometric high-performance liquid chromatography (HPLC) method which is highly sensitive and specific made possible the measurement of ALA in a small volume (50 μl) of plasma. The concentrations (mean ± SD) of lead in blood (males: 55.1 ± 12.9 μg/dl; females: 54.7 ± 13.5 μg/dl) indicated that the intensity of occupational exposure to lead was almost equal in the two groups. However, the elevation of plasma ALA concentration and the increased urine ALA and CP excretion among these lead workers were much higher in females than in males, confirming the finding of a sex difference in the biological effect of human exposure. The difference in urine CP excretion was especially pronounced, the mean concentration of urinary CP in the female workers being 3.5–5 times higher than that in the male workers.

Key words Lead workers · Sex difference · Porphyrin metabolism · δ-Aminolevulinic acid · Coproporphyrin

Introduction

It is well known that exposure to lead causes a disturbance of porphyrin and heme metabolism in the bone marrow (Hammond 1977; Sassa 1978; Tsuchiya 1986). As a result, some findings reflecting this disturbance, e.g., elevation of δ-aminolevulinic acid (ALA) and coproporphyrin (CP) in urine, accumulation of free erythrocyte protoporphyrin (FEP), and inhibition of erythrocyte ALA dehydratase (ALAD) activity, are observed in humans exposed to lead (Lauwerys et al. 1973; Alessio et al. 1976; Tomokuni and Ogata 1976; Horiguchi et al. 1981; Ohmori et al. 1986). These biological indicators correlate closely with the concentration of lead in peripheral blood, indicating that they are useful in evaluating the health effect of lead in workers occupationally exposed to lead. Of these biological indicators, urinary ALA has been widely used in Germany and Japan and blood FEP in the United States as a measure of the biological effect of occupational exposure to lead.

Recently in Japan, a fluorometric high-performance liquid chromatography (HPLC) method for determining urinary ALA was developed and improved (Okayama et al. 1986, 1990; Tomokuni et al. 1987, 1992; Endo et al. 1994). This fluorometric HPLC method is more sensitive and specific than the conventional colorimetric methods based on the condensation of ALA-pyrrole with Ehrlich's reagent containing p-dimethylaminobenzaldehyde (Mauzerall and Granick 1956; Davis and Andelman 1967; Wada et al. 1969; Tomokuni and Ogata 1972; Tomokuni and Ichiba 1988). The use of this HPLC method makes it possible to determine ALA in small volumes of biological materials such as plasma and serum.

It has been reported that the amount of ALA in blood correlates highly with the concentration of lead in blood among lead workers (Takebayashi et al. 1993; Morita et al. 1993; Tomokuni et al. 1993). However, there are few reports on the differences between males and females in the responses of biological parameters for porphyrin metabolism caused by occupational exposure to lead. Only two reports have indicated that the responses of FEP and urinary ALA in workers...
moderately exposed to lead are higher in females than in males (Roels et al. 1975, 1979).

The main purpose of this study was to elucidate whether the response of biological parameters such as plasma ALA, urinary ALA, and urinary CP differs between male and female workers occupationally exposed to lead.

**Materials and methods**

**Subjects and sample collection**

The exposed subjects were 160 male and 138 female workers occupationally exposed to lead. They were employed in two factories processing lead glass and producing lead pigments. The mean age of the exposed subjects (± SD) was 36 (± 7) years for males and 28 (± 6) years for females. The mean occupational exposure of the subjects to lead was about 14 years with a range of 1 to 28 years.

Of the control subjects with no history of occupational exposure to lead, 70 were males and 68, females. The age (mean ± SD) of the control subjects was 34 ± 8 years for males and 25 ± 6 years for females. Heparinized venous blood and spot urine samples were collected from these subjects. The plasma sample was separated from an aliquot of whole blood by centrifugation. All biological samples obtained were kept cool or frozen at −20°C.

**Measurement of plasma and urinary ALA**

Plasma or urinary ALA was determined using a fluorometric HPLC method. A fluorescence derivatization of ALA was performed by the application of the Hantzsch reaction with acetylacetone and formaldehyde.

**Reagents**

All chemicals were of analytical grade. Acetylacetone reagent was prepared by mixing 15 ml of acetylacetone, 10 ml of ethanol, and 75 ml of distilled water. Formaldehyde solution (10%) was prepared by 3.7-fold dilution of the chemical reagent (37%) with distilled water and stored in the dark. The stock solution of ALA (100 mg/l) was made by dissolving 12.8 mg of ALA hydrochloride in 100 ml of distilled water, and it was stored in a refrigerator at 4°C.

**Fluorescence derivatization of ALA**

To a test tube, 3.5 ml of acetylacetone reagent, 50 μl of plasma or urine sample, and 0.45 ml of formaldehyde solution (10%) were added and mixed with a vibrator mixer for about 3 s. This mixture (total 4 ml) was heated for 10 min at 100°C with an aluminum-block heater (Taitec Co., Japan). The test tube was cooled in an ice-bath. For plasma samples only, the reaction mixture was filtered after derivatization through a disposable HPLC filter (φ25 mm, 0.8 μm). These samples were allowed to stand in the dark until termination of analysis. The concentration of a working ALA standard used was 100 μg/l for plasma and 1 mg/l for urine.

**Analytical conditions for HPLC**

Apparatus and conditions were as follows: apparatus, LC-6A HPLC (Shimadzu Ltd., Japan); column, Shim-pack CLC-ODS 150 × 4.6 mm (Shimadzu), at 40°C; detector, Shimadzu RF-535, excitation/emission wavelength (370/460 nm), range × 4, sensitivity high; mobile phase, methanol/water/acetetic acid (500/500/10 by vol); flow rate, 0.7 ml/min; data processor, chromatopac C-R3A (Shimadzu); inj. volume, 20 μl for plasma sample, 10 μl for urine sample. The injection was performed in the dark using an automatic sampler-injector (Gilson 231-401, USA). The detection limit for ALA in plasma and urine was 1.2 μg/l and 3 μg/l, respectively. The within-run variation (CV) of the used HPLC method was about 3%.

**Other analyses**

Urinary CP was determined according to the fluorometric HPLC method (Tomokuni and Hirai 1986). The sample preparation for urinary CP analysis was performed by mixing 0.1 ml of urine and 0.1 ml of acetic acid. The detection limit for urinary CP in this method was 5 μg/l, and the CV of the method was about 7%.

The concentration of blood lead was determined by flameless atomic absorption spectrometry after tenfold dilution of whole blood with 0.1 N nitric acid containing 1% Triton X-100. Urine creatinine (Cr) was measured colorimetrically with a kit from Wako Pure Chemicals Ltd., Japan. The urinary concentrations of all the measurements were standardized with the urinary concentration of Cr.

**Results**

In the measurement of plasma and urinary ALA by the HPLC method, the retention time of the fluorescent ALA derivative was 6.1 min. This fluorescent ALA derivative was very stable for more than 20 h in the dark, but was comparatively unstable in the light. Therefore, the sample tube containing the reaction mixture after derivatization must be placed in the dark until termination of HPLC analysis.

Table 1 summarizes the blood lead, plasma ALA, and urinary ALA concentrations obtained from male and female workers occupationally exposed to lead. The range of concentrations observed was higher in females than in males (Roels et al. 1975, 1979).

**Table 1** Concentration of blood lead, plasma ALA, and urinary ALA obtained from control subjects

<table>
<thead>
<tr>
<th>Controls</th>
<th>(n)</th>
<th>Blood lead (μg/100 ml)</th>
<th>Plasma ALA (μg/l)</th>
<th>Urinary ALA (mg/l)</th>
<th>(mg/g Cr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>(70)</td>
<td>11.0 ± 2.3*</td>
<td>3.9 ± 2.5*</td>
<td>0.86 ± 0.37</td>
<td>0.76 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.0–17.2</td>
<td>1.5–14.2</td>
<td>0.20–1.95</td>
<td>0.37–1.50</td>
</tr>
<tr>
<td>Females</td>
<td>(68)</td>
<td>6.4 ± 1.6</td>
<td>2.4 ± 0.8</td>
<td>1.00 ± 0.46**</td>
<td>1.08 ± 0.21*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.8–11.4</td>
<td>ND–5.9</td>
<td>0.21–2.23</td>
<td>0.66–1.55</td>
</tr>
</tbody>
</table>

*P < 0.001, ** P < 0.053