Urinary N-acetyl-β-D-glucosaminidase isoenzyme measurement in lead-exposed workers

Ginji ENDO *1, Yoshitsugu KONISHI *1, Yoko Endo ICHIKAWA *2, Ikuko KITOTA *1 and Shun'ichi HORIZUCHI *1
(*1 Department of Preventive Medicine and Environmental Health, Osaka City University Medical School, 1-4-54 Asahi-machi, Abeno-ku, Osaka, 545 Japan, *2 Department of Public Health, Kansai Medical University, 1-, Fumizono-cho, Moriguchi, Osaka, 570 Japan)

(Received Sep. 7, 1992)

Urinary excretion of N-acetyl-β-D-glucosaminidase (NAG) is regarded as the most sensitive indicator of renal damage caused by occupational exposure to lead. In order to clarify the increase of NAG excretion caused by damage to the proximal renal tubule, we compared NAG isoenzyme patterns between a group of 17 male office workers and a group of 15 male lead-exposed workers using the electrofocusing-MCP-NAG method.

Blood levels of lead (PbB) in the office workers and the lead exposed workers were 9.7 ± 3.1 and 64.9 ± 9.8 μg/100g blood (mean ± S.D.), respectively. All the means of the total NAG, NAG-A and NAG-B activity of the lead-exposed workers (5.7 ± 4.9, 4.6 ± 4.1, 1.1 ± 0.8U/g creatinine, respectively) were significantly higher than those of the office workers (2.8 ± 1.4, 2.2 ± 1.0, 0.6 ± 0.4U/g creatinine, respectively). But no difference was observed in the mean percentages of the isoenzymes (NAG-A and NAG-B) between the two groups.

Since we found a significant increase in NAG excretion, but failed to find that the excess excretion was mainly due to NAG-B, there remained the possibilities that the lead exposure damaged the proximal renal tubular cell only very slightly, or stimulated the exocytosis of NAG, or stimulated renal activity of NAG.

Introduction

Kidneys are well known to be one of the target organs in both acute and chronic lead poisoning [1]. In occupational exposure to lead, prolonged, relatively high exposure causes chronic renal failure and contracted kidneys [2,3]. The most convincing evidence of the linkage between lead and chronic renal failure is a follow up study of lead poisoning in children which showed that acute lead poisoning led to chronic renal failure and contracted kidneys 10 to 40 years later [4]. Since the results from animal experiments on lead poisoned rats showed lead inclusion bodies in the proximal tubular nuclei [5], chronic nephrotoxicity caused by lead appeared in the form of proximal tubular abnormalities.

Determination of total urinary N-acetyl-β-D-glucosaminidase (NAG, EC 3.2.1.30) activity is used as an early indicator of renal dysfunction [6]. Among lead workers, an increase of urinary NAG activity [7] and a significant correlation between lead exposure indices and urinary NAG activity [8] were reported. But no researchers have reported an increase of β₂-microglobulin in urine, which indicates renal tubular impairment among lead workers, even in those who showed an increase in NAG in their urine [9,10].

Recently, NAG isoenzyme measurements have been applied to indicate that the renal dysfunction is due to proximal tubular damage [11-14].
NAG in normal urine is composed of two isoenzymes, A and B. Both isoenzymes exist in all nephron segments, such as glomerule, proximal convoluted tubule, pars recta, medullary thick ascending limb, and cortical collecting tubule (15). However, the distribution of isoenzyme B is abundant exclusively in the proximal convoluted tubule (15). NAG-A is mainly located in the soluble intralysosomal compartment and is normally excreted in small amounts by a physiological process of exocytosis, whereas NAG-B is essentially localized in the lysosomal membrane (16,17). When the proximal renal tubule is damaged, an increase of total urinary NAG with an increase in the proportion of NAG-B is found (18). Thus, it can be said that the increase of urinary excretion of NAG-B is caused by proximal tubular lesions. Therefore, the measurement of urinary NAG isoenzymes is expected to identify the affected lesions in the kidneys of lead workers who show an increase in urinary NAG activity.

In order to more easily determine the presence of NAG isoenzymes, we developed a new method which can use an ordinary spectrometer with a reagent of sodio-m-cresol-sulfonphthaleinyl-N-acetyl-β-D-glucosaminide (MCP-NAG), instead of using the fluorometer method with 4-methylumbellifery-N-acetyl-β-D-glucosaminide (4MU-NAG) (19). In this report, we determined the activity of urinary NAG isoenzymes among lead workers using the electrofocusing technique in order to learn if an increase of total NAG among lead workers is due to proximal renal tubular cell damage or not.

**Materials and methods**

The subjects were 15 male workers with moderate exposure to lead in a secondary lead refinery and 17 male office workers. The workers’ profiles are summarized in Table 1. Each person provided a medical and occupational history, and underwent physical examination and routine urinalysis. Any worker who reported a history of kidney disease in the past or who had diabetes was excluded from the study.

The exposure levels of workers were expressed as blood lead (PbB) which was determined according to the method explained in our previous report (20). Urine samples were obtained toward the end of the work-shift, immediately centrifuged at 1000 G for 10 minutes, and then the supernatants were stored at 4°C until analysis. The determination of total NAG activity was performed according to Noto’s MCP-NAG method using the NAG test Shionogi (21).

NAG isoenzyme determination was carried out using isoelectric focusing and the MCP-NAG method as described in our previous report (19). Briefly, 2.5 ml of urine sample was concentrated to about 200µl by Centricut (mini V-50, Kurabo) through centrifuge (2500 G x 60 min). Twenty µl of the aliquot was applied to an Ampholine PAG plate (pH rang of 3.5 to 9.5, Pharmacia LKB, Sweden) using a sample application pipet. The gel was put on a Flat Bed Apparatus (FBE-3000, Pharmacia, Sweden), and focused at 4-6°C by an electrophresis constant power supply (ECP 3000/150, Pharmacia, Sweden) at 1500 V, 50 mA and 30 W for 2.0 hr. Immediately after focusing, the pH gradient was measured by a surface pH electrode (IW216-BNC, Iwaki Glass, Japan). Then the gel was sliced over its entire length in 0.5 cm segments and the NAG activity of each segment was measured by the MCP-NAG method with a

<table>
<thead>
<tr>
<th>Table 1. The workers profiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indices</td>
</tr>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Duration (years)</td>
</tr>
<tr>
<td>PbB (µg/100g blood)</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Urinary N-acetyl-β-D-glucosaminidase isoenzyme measurement in lead-exposed workers

Modification of the prolonged reaction time to 60 min.

The two tailed t-test was used for statistical analysis for the difference between the values obtained from lead exposed workers and those from office workers.

Results

The blood lead values of 17 office workers and 15 lead exposed workers are shown in Table 1. There was a significant difference between the two groups (t=22.2, p<0.0001).

The mean age of the office workers was significantly lower than that of the lead workers (t=3.8, p<0.001). Total NAG activity of the lead exposed workers was significantly higher than that of the office workers (t=2.32, p<0.05).

All the urine used in the NAG isoenzyme determinations showed two isoenzymes of NAG-A and NAG-B. Typical zymograms of urinary NAG isoenzymes obtained from a lead worker and a office worker are shown in Fig. 1.

Table 2. Urinary total NAG, NAG-A and NAG-B activities and the percentages of the isoenzymes in workers

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Office workers</th>
<th>Lead workers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total NAG activity (U/g creatinine)</td>
<td>2.8±1.4</td>
<td>5.7±4.9*</td>
</tr>
<tr>
<td>NAG-A (U/g creatinine)</td>
<td>2.2±1.0</td>
<td>4.6±4.1*</td>
</tr>
<tr>
<td>NAG-B (U/g creatinine)</td>
<td>0.6±0.4</td>
<td>1.1±0.8*</td>
</tr>
<tr>
<td>NAG-A (%)</td>
<td>68.8-87.1</td>
<td>79.1±3.5</td>
</tr>
<tr>
<td>NAG-B (%)</td>
<td>12.9-31.2</td>
<td>20.9±3.5</td>
</tr>
</tbody>
</table>

*: p<0.05

Table 2 shows total NAG activities and the percentages of the isoenzymes in the two groups. There was no statistically significant difference in percentages of NAG isoenzymes between the two groups.

Fig 1. Typical zymograms of urinary NAG isoenzymes obtained from a lead worker and a office worker
Discussion

Inglis et al. (4) reported that children who had earlier suffered from lead poisoning were later dying from renal failure. They inferred that lead had caused a chronic vascular sclerosis of the kidneys. Goyer (22) suggested that a blood lead level of 60 μg/dL was the threshold for proximal tubular cell injury. However, lead nephropathy in adults can not be detected by determination of low- or high-molecular weight proteins, such as β2-microglobulin or albumin, in urine (8,9,23).

The only marker that seems to respond at an early stage of lead nephropathy is urinary excretion of NAG (7-9). In our previous report, a significant positive correlation was found between total NAG activity and blood lead levels, and also a significant increase of the total NAG activity was found in lead workers with PbB levels higher than 80 μg/100g of blood (8). In this study, we found that the mean total NAG activity of lead exposed workers who had moderate exposure to lead (PbB ranged 54.5 to 88 μg/100g blood) was significantly higher than that of office workers.

The mean age of the lead exposed workers was higher than that of the office workers. In Japanese people, it is generalized that people older than 60 show higher NAG activity than younger people, but there is no aging effect on NAG activity among people under 60 (24). There were two individuals in the group of office workers and two in the lead exposed group who were over 60 years of age.

According to the generalized idea as mentioned above (24), the increase of total urinary NAG among our lead workers was considered to be the effect of lead exposure. However, the total NAG activity correlated significantly positive with age (r=0.652, p<0.005) among our office workers, and 73% of our lead workers were in their fifties. Therefore, though a small number of subjects give limited evidence, the increase of total NAG activity among lead workers may be the effect of both lead exposure and aging.

In our results, the mean of total NAG, the mean of NAG-A and the mean of NAG-B among lead workers, were significantly higher than among office workers (Table 2). But no difference was observed in the mean percentage of NAG-B between lead workers and office workers (Table 2), and no worker showed NAG-I. In normal subjects, NAG-I in urine is found only in trace amounts because the isoenzyme is normally present in the Golgi system and is then packed into the lysosomes as NAG-A (11). When a renal tubular cell is damaged, urinary excretions of NAG-B and NAG-I significantly increase (25).

The results reported from renal biopsy obtained from workers with heavy occupational exposure to lead was that the ultrastructural changes were localized to the proximal tubules and typical lead-induced nuclear inclusion bodies and increased lysosomes were found in workers with relatively short exposure (26). The researchers speculated that the decrease in inclusion body formation in chronic lead nephropathy may be due to an increased rate of renal cell turn over. Also Bernard and Lauwerys (10) suspected that while increased urinary leakage of NAG might result from cell damage and exfoliation, it might also reflect a stimulation by lead of the exocytosis or of the renal activity of NAG.

We found a significant increase of total NAG excretion but failed to find that the excess excretion was due to NAG-B or NAG-I. Therefore, there remains the possibilities that the increases of total NAG among lead workers may be caused by a slight degree of proximal renal tubular cell damage, or by stimulation of exocytosis, or by stimulation of renal activity of NAG.

References

1) Stokinger, H.E.; Lead, in "Patty’s industrial
Urinary N-acetyl-β-D-glucosaminidase isoenzyme measurement in lead-exposed workers


