Urinary Excretion Levels of Osseous Collagen Metabolites in Cadmium Workers

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The toxic effect of a trace amount of inhaled cadmium oxide (CdO) dust on bone collagen metabolism in workers who were employed in a Ni-Cd battery plant (Cd-workers) was investigated by measuring the urinary excretion levels of hydroxylsine glycosides (Hyl-Gs) and hydroxylsylpyridinoline (Pyr) as bone collagen metabolites. Urinary Cd levels in the Cd-workers were apparently higher than control levels, but urinary \( \beta_1 \)-microglobulin levels were within control levels. There was no statistically significant difference between the urinary Hyl-Gs levels of the Cd-workers and those of age-adjusted controls who were not engaged in Ni-Cd battery making at the same plant. The urinary Pyr levels of the Cd-workers, however, were slightly higher than age-adjusted control levels, but statistical significance was not observed except for those in their forties. These results suggest that inhaled CdO dust is involved in osseous collagen metabolism in some way, especially in bone resorption.

Keywords —— cadmium oxide; bone metabolism; hydroxylsylpyridinoline; hydroxylsine glycoside; urinary excretion

Introduction

The development of advanced industrial technology often entails the use of numerous raw chemical materials containing heavy metals for making new and epochal products. The Ni-Cd battery has been instrumental in miniaturizing products. Workers employed in a Ni-Cd battery plant (Cd-Workers) inhale or are exposed to a trace amount of cadmium oxide (CdO) dust as a raw material. Chronic exposure to cadmium (Cd) has caused renal tubular dysfunction, resulting in an increase in the urinary excretion of protein, glucose, amino acids, and some enzymes. It is known that chronic occupational exposure to Cd, especially cadmium chloride (CdCl₂), can cause renal damage characterized by an increase in urinary \( \beta_1 \)-microglobulin. So far, few papers have been published on the direct action of Cd on bone. For example, Ouch-Ouch disease in Toyama Prefecture, Japan, caused by chronic Cd exposure was, at least partly, characterized by osteomalacia or osteoporosis, and increased urinary excretion levels of hydroxyproline (Hyp) were observed in the patients. However, the toxicity to human of CdO, a raw material in Ni-Cd batteries, is not well known. Consequently, this study has been undertaken because the health care of Cd-workers is the most important concern for an employer.

Recently, urinary excretion levels of hydroxylsine glycosides (Hyl-Gs) have been employed for the assessment of bone turnover. Two glycosides, \( \beta \)-D-galactopyranosylhydroxylsine (GHa) and 2-\( \alpha \)-D-glucopyranosyl-\( \alpha \)-D-galactopyranosylhydroxylsine (GGH), are contained only in a specific type of collagen. Moreover, the GGH/GH content ratio is varied according to the type of collagen; for example, the most common content ratio is about 2 in glomerular and/or tubular basement membranes and skin.
collagen, and less than 0.5 in bone collagen. Therefore, by observing the urinary excretion levels of Hyl-Gs and the urinary GGH/GH ratio, it can be determined which type of tissue is being degraded. The levels of urinary Hyl-Gs, instead of Hyp, are considered to be a more sensitive marker of bone resorption. On the other hand, urinary excretion levels of pyridinium crosslinks have been proved as a marker of bone resorption. Hydroxylsylypyridinoline (Pyr), a pyridinium crosslink, is specific to bone and articular cartilage. Both GH and Pyr are specific contents of bone collagen, but their biosynthesis processes are different. Hyl-Gs are formed during the extensive postribosomal process of collagen synthesis and, on the other hand, Pyr is formed nonenzymatically with maturation of the collagen fibrils. Nagai et al. reported that CdCl₂ injected s.c. to rat affected collagen metabolism, showing that urinary Hyp and Hyl-Gs excretion increased with dose, but there have been no appropriate studies concerning CdO and bone collagen metabolism until now.

In this paper, we investigated the effects of the chronic exposure to a trace amount of CdO on the kidney by observing urinary β₂-microglobulin and albumin, as well as the effects of exposure on bone collagen metabolism by observing the urinary excretion levels of Hyl-Gs and Pyr.

Methods

Subjects — All subjects were employees of a Ni-Cd battery factory plant run by M. E. Industrial Co., Ltd., Kanagawa, Japan. Thirty-nine male Cd-workers were investigated who were engaged in handling a powderd CdO compound, making a paste with glycerine. Then a Ni compound sheet (from which little dust was produced) was coated with the paste, automatically. As controls, 87 male workers who were not engaged in Ni-Cd battery making were also investigated. The concentration of CdO dust in the air of the Cd-working area ranged from 0.025 to 0.1 mg/m³. Prior to the determination of Cd using a polarized Zeeman atomic absorption spectrophotometer (Hitachi Z-8100), CdO dust in 20 l of air in the Cd-working area was collected onto a filter (QM-A, 37 mm i.d., Whatman Japan Ltd.) and was dissolved in 20 ml of a mixture of HNO₃, HCl, and H₂O (1:1:15, v/v). All the workers were good in health and had no abnormal data of serum enzymes or blood counts. Urine spot samples were collected from the workers before their work (around nine o’clock in the morning), thus avoiding contamination by their working clothes. After collection, the samples were immediately applied to the determination of albumin and β₂-microglobulin, and aliquots of the samples were stored at −20°C.

Analytical Methods — Urinary albumin and β₂-microglobulin were determined by the methods based on latex agglutination immunoassay using clinical test kits (Eiken Chemical Co., Ltd., Tokyo, Japan). The concentrations of Cd in the urine spot samples were determined with a Hitachi Z-8100 polarized Zeeman atomic absorption spectrophotometer with a graphite furnace apparatus. The stored urine samples were gradually thawed and then prepared for Cd analysis as follows: 100 μl of each urine sample was added to a mixture of 100 μl of matrix modifier (mixture of HNO₃ and (NH₄)₂ SO₄ solution (1:1, v/v)) and 300 μl of water.

Urinary Hyl-Gs and Pyr were determined by HPLC following the procedures published previously. Urinary creatinine was measured by a colorimetric procedure based on the Jaffe method using clinical test kits (Creatinine-test Wako; Wako Pure Chemical Industries, Ltd., Tokyo, Japan) for the purpose of urine correction.

Statistical Methods — All results were expressed as the means ± standard deviation (S. D.). Tests of statistical significance were made using a paired or unpaired Student’s t-test, and significance levels (p values) were <0.05.

Results and Discussion

Urinary β₂-Microglobulin and Cd in
Cd-Workers

As basic information, the levels of urinary β2-microglobulin as an index of renal function, and Cd concentration in the urine in the Cd-workers are shown in Table I. Compared to control values, urinary Cd concentrations in the Cd-workers were higher, but not statistically significant, suggesting Cd exposure due to their working. The levels of urinary β2-microglobulin in the Cd-workers were within the control levels (below 250 µg/g creatinine), so no renal dysfunction was observed in spite of high urinary Cd levels. Generally, increased urinary excretion of β2-microglobulin indicates a defect in reabsorption by the renal proximal tubule, and is one of the earliest signs of Cd-induced proteinuria. In addition, the levels of urinary albumin of the Cd-workers (Table I) was also within the control levels (below 10 µg/g creatinine), except in subjects in their forties and fifties, suggesting that no marked renal dysfunction occurred.

Evaluation of the Situation of Bone Metabolism by Urinary Osseous Collagen Metabolites

The values of urinary Hyl-Gs and Pyr of each age group of Cd-workers are shown in Table II, together with those for the controls who were not engaged in Cd work. As for GGH, GH and the urinary GGH/GH ratio, the values of each age group of Cd-workers showed no appreciable difference compared to the age-adjusted controls. Urinary Pyr levels of all Cd-worker groups, however, tended to be slightly higher than the age-adjusted control levels, but not statistically significant by using the t-test. Next, we applied the Mann-Whitney test, another statistical way to conduct a non-parametrical test, and statisti-

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>20—29(15)a</th>
<th>30—39(10)</th>
<th>40—49(10)</th>
<th>50—59(4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working periods (year)</td>
<td>4.67±2.9b</td>
<td>11.90±6.1</td>
<td>15.3±9.1</td>
<td>24.25±5.9</td>
</tr>
<tr>
<td>Urinary Cd (µg/g cre.)</td>
<td>6.33±5.1</td>
<td>7.02±7.0</td>
<td>7.54±3.2</td>
<td>17.53±11.9</td>
</tr>
<tr>
<td>Controls</td>
<td>1.34±1.0(25)</td>
<td>1.90±1.6(39)</td>
<td>2.55±1.9(14)</td>
<td>2.72±2.2(9)</td>
</tr>
<tr>
<td>β2-Microglobulin (µg/g cre.)</td>
<td>71.14±28.1</td>
<td>88.37±71.1</td>
<td>145.2±103</td>
<td>182.1±119</td>
</tr>
<tr>
<td>Urinary albumin (µg/g cre.)</td>
<td>6.67±3.8</td>
<td>4.73±1.2</td>
<td>17.15±39</td>
<td>15.60±7.9</td>
</tr>
</tbody>
</table>

a) Brackets indicate the number of samples determined.
b) Values indicate the mean±S.D.
c) Male workers who were not exposed to Cd.
d) Normal level; below 250 µg/g creatinine.
e) Normal level; below 10 µg/g creatinine.

<table>
<thead>
<tr>
<th>Age (year)</th>
<th>GGH (µmol/g cre.)</th>
<th>GH (µmol/g cre.)</th>
<th>GGH/GH ratio</th>
<th>Hydroxylysylpyridinoline (nmol/mmol cre.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>Cd-workers</td>
<td>Controls</td>
<td>Cd-workers</td>
<td>Controls</td>
</tr>
<tr>
<td>20—29</td>
<td>18.9±5.6(25)</td>
<td>13.1±7.4(15)</td>
<td>15.5±8.8</td>
<td>11.6±5.2</td>
</tr>
<tr>
<td>30—39</td>
<td>18.5±6.3(39)</td>
<td>12.5±6.3(10)</td>
<td>13.3±5.0</td>
<td>10.9±4.8</td>
</tr>
<tr>
<td>40—49</td>
<td>25.3±18(14)</td>
<td>10.3±3.4(10)</td>
<td>15.8±7.7</td>
<td>12.1±7.8</td>
</tr>
<tr>
<td>50—59</td>
<td>20.6±16(9)</td>
<td>12.8±12(4)</td>
<td>10.1±6.6</td>
<td>20.1±9.8</td>
</tr>
</tbody>
</table>

a) Values are mean±S.D. and number of samples is shown in the brackets.
b) Significantly different compared to the control (Mann-Whitney test, p<0.05).
cal significance was concluded between the levels of Pyr of Cd-worker group in their forties and the age-adjusted control. These results suggest that no apparent decrease in bone mass was evident in Cd-workers, but a trace amount of inhaled CdO dust is involved in osseous collagen metabolism in some way, especially in bone resorption. Hyl-Gs are a component of the α-chain of collagen molecules formed during the postribosomal processing of collagen biosynthesis, and Pyr is an intermolecular crosslinker of bone collagen. Therefore, it is considered that a trace amount of inhaled CdO dust does not affect the α-chain but affects the intermolecular crosslinker of mature collagen fibrils of bone. Urinary GGH levels of the Cd-worker groups which were a little bit less than those of the age-adjusted controls may suggest that inhaled CdO dust affects and reduces the collagen turnover or the metabolism of the renal glomerular and/or tubular basement membrane.

Relationship between Urinary Cd Concentration and Osseous Collagen Metabolites

Table III gives the correlation coefficients among the urinary levels of β₂-microglobulin, albumin, Cd, Hyl-Gs, Pyr and working periods for the Cd-workers. Urinary Cd levels of the Cd-workers tended to increase with their age (Table I), and were significantly correlated with the working periods. Urinary β₂-microglobulin excretion was also significantly correlated with their working periods, suggesting that inhaled CdO dust affected their renal functions.

Figure 1 shows the relationships between the urinary levels of Cd and GH, and of Cd and Pyr in the Cd-workers. The correlation coefficients between the urinary levels of Cd and those of GH (Fig.1a) or Pyr (Fig.1b) were not statistically significant. Thus, no apparent causality was observed between inhaled CdO dust and bone metabolism.

The urinary excretion levels of Hyl-Gs and Pyr are indirect information of bone resorption because they are excreted in urine without being metabolized by the liver or kidney. Especially, urinary Hyl-Gs follows the same physiological variation as urinary Hyp, but the levels are not influenced by dietary protein intake. Now, we first applied the determination of urinary Hyl-Gs and Pyr to Cd-workers, and then evaluated their health conditions.

To date, it has been demonstrated that urinary GH and Pyr excretion levels increase in patients with a metabolic bone disease such

<table>
<thead>
<tr>
<th>Working periods</th>
<th>Albumin (µg/g cre.)</th>
<th>β₂-MG (µg/g cre.)</th>
<th>Cd (µg/g cre.)</th>
<th>GGH (µmol/g cre.)</th>
<th>GH (µmol/g cre.)</th>
<th>GGH/GH (nmol/mmol cre.)</th>
<th>Pyr (nmol/mmol cre.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (µg/g cre.)</td>
<td>0.219</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>β₂-MG (µg/g cre.)</td>
<td>0.339*</td>
<td>0.098</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cd (µg/g cre.)</td>
<td>0.414**</td>
<td>0.121</td>
<td>0.138</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GGH (µmol/g cre.)</td>
<td>-0.209</td>
<td>-0.036</td>
<td>-0.119</td>
<td>-0.023</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GH (µmol/g cre.)</td>
<td>0.296</td>
<td>-0.069</td>
<td>0.089</td>
<td>-0.022</td>
<td>0.274</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GGH/GH</td>
<td>-0.299</td>
<td>0.070</td>
<td>-0.171</td>
<td>-0.043</td>
<td>0.653**</td>
<td>-0.471**</td>
<td>-</td>
</tr>
<tr>
<td>Pyr (nmol/mmol cre.)</td>
<td>-0.335*</td>
<td>0.022</td>
<td>0.057</td>
<td>-0.108</td>
<td>0.077</td>
<td>-0.250</td>
<td>0.229</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01.
as primary hyperparathyroidism,\textsuperscript{19} osteoarthritis,\textsuperscript{20} rheumatoid arthritis,\textsuperscript{19,20} Paget's disease,\textsuperscript{10,21,22} and osteoporosis.\textsuperscript{12,13,23} Hadley \textit{et al.}\textsuperscript{24} reported that inhaled CdO was first deposited on respiratory surfaces and then gradually distributed in the liver and kidney, causing pulmonary changes\textsuperscript{25} and renal\textsuperscript{26} and hepatic dysfunction,\textsuperscript{27} mainly.

In the present study, there was no clear relationship between urinary Cd levels and bone metabolism, but urinary Pyr tended to be higher in the Cd-workers compared to the controls. This result suggests that inhaled CdO dust affected osseous collagen metabolism in some way.

References