SOME METABOLIC INTERRELATIONSHIPS AMONG CADMIUM, LEAD, COPPER AND ZINC: RESULTS FROM A FIELD SURVEY IN CD-POLLUTED AREAS IN JAPAN
PART FOUR: HEAVY METAL RATIOS AND CORRELATIONS

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ABSTRACT

A series of analytical studies on heavy metal distributions in the foods, feces and urinary samples had been presented in this Journal. In the present paper, further statistical analyses on the interrelationship of each heavy metal were done with special reference to the heavy metal ratios and correlation coefficient matrices of these metals. Positive correlation coefficients between Cd and Cu in the fecal and urinary samples, which were observed among the inhabitants of Cd-polluted area, dissapeared among the inhabitants of the control area. This indicated the possible depression of Cu intake due to the higher dietary intake of Cd. At these levels of Pb intake observed in the both polluted and control area inhabitants, correlation coefficients of Pb to the other three metals among the former group showed similar values to those of the latter group. The ratios of all pairs of heavy metals in feces were similar to those ratios in rice and the other food samples, but different from those of the urinary metals. This suggests the different metabolism in human organs results the different excretion in the urine and in the feces.

INTRODUCTION

Determination of the heavy metal ratio in rice and the other foods is helpful not only to estimate the contribution of mining or industrial activity to the pollution of agricultural land, but also to investigate the possible mechanism
of the geochemical behavior of the metals. The Zn to Cd ratio, for instance, varies widely in the lithosphere, depending on the type of rocks.\(^1\) The median Zn/Cd ratio for normal soil is reported 1400, ranging from 800 to 12000. Marine sediment in the Japan Sea off Toyama showed 1300 to 1400 of the Zn/Cd ratio.\(^2\) With reference to such data and the fact in the Jinzu River Basin where Itai-itai disease occurred the Zn/Cd ratio of the polluted area (142 to 364) in contrast to the larger values of that ratio in non-polluted areas upstream from the mine (430 to 1600) suggest the possible use of this ratio as an indicator of Cd-pollution.

In the previous reports, basic results of the four heavy metals (Cd, Pb, Cu and Zn) in the foods, feces and the urine were discussed.\(^3\)--\(^5\) This final report estimates the possible interrelationships between the heavy metals in the foods, which reflect the local heavy metal distribution in nature, and the fecal or urinary heavy metals which are in part metabolized and excreted from the human body. Also the correlation coefficient matrix tables were obtained to determine whether these in the both Cd-polluted and control area inhabitants are consistent with the report by the author.\(^9\) These correlation coefficient tables as well as the metal ratios might be considered useful for the better understanding of the interrelationship of the heavy metals.

**MATERIALS AND METHODS**

As described in the previous reports,\(^3\)--\(^5\) ten of each rice sample and the other foods from the both Cd-polluted and the control area inhabitants, 216 fecal and urine samples from the inhabitants of the both areas were collected and heavy metals (Cd, Pb, Cu and Zn) in those samples were analyzed. Association of the metals were statistically obtained from the correlation coefficient matrix tables together with the tables of ratios of the four heavy metal concentrations grouped by the two areas.

**RESULTS**

Significant correlation coefficients were obtained from the Cd-Cu relationship in rice samples (\(r=0.387\); \(p<0.05\)) as well as the Pb-Zn relationship (\(r=0.427\); \(p<0.05\)) in polluted areas, as shown in Table 1-1. In control areas, such was not the case, in stead, the Cd-Pb and Cu-Zn correlations were significant (\(r=0.412\); \(p<0.05\), \(r=0.453\); \(p<0.05\), respectively).

In foods excluding rice, a positive correlation was found for all pairs of the four heavy metals among the polluted area inhabitants. Among them, statistical
evident correlations were found between Cd and Pb, between Pb and Cu, and between Cd and Zn \( (r=0.553, 0.641, 0.416; p<0.01, 0.01, 0.05, \text{respectively}) \). However, no favorable correlation coefficients between any two metals in the control area inhabitants were found (Table 1-2).

Correlation coefficients of two out of the four fecal heavy metals in each polluted and control area residents are shown in Table 1-3. In all the polluted areas, the following significant correlation coefficients were found; Pb to Cu \( (r=0.201, p<0.05) \), Pb to Zn \( (r=0.267, p<0.01) \), Cd to Zn \( (r=0.362, p<0.01) \) and Cu to Zn \( (r=0.204, p<0.05) \). In the control areas, the relationships between Cd and Cu, between Cd and Zn, and between Cu to Zn showed the statistical significance \( (r=0.213; p<0.05, r=0.422; p<0.01, r=0.231; p<0.05, \text{respectively}) \).

Correlation coefficients of each pair of the urinary four metals in each polluted and control area are shown in Table 1-4. For the polluted area, significant correlations were found between Pb and Cu, between Zn and Cu, and between Cd and Zn \( (r=0.361, 0.401 \text{ and } 0.472, \text{respectively with } p<0.01) \). On the other hand, correlation coefficients between Pb and Cu, between Cd and Cu, and between Cu and Zn in the control area were statistically significant \( (r=0.297, 0.217 \text{ and } 0.380, \text{respectively with } p<0.01, 0.05 \text{ and } 0.01, \text{respectively}) \).

To sum above results, following observations were made; (1) Cd-Cu corre-
Correlation in the polluted area was only significant in food samples, whereas in the control area, that was significant in fecal and urinary samples. (2) Cd-Zn correlation in the polluted area inhabitants showed positive relation in the food samples excluding rice, and in the fecal and urinary samples. Control area inhabitants only showed positive correlation coefficient between Cd and Zn in the fecal samples. (3) Pb-Cd relationship was not observed in all the biological samples except food in the polluted area. (4) Pb-Cu relationship was found significant in the urine of both groups, and also significant in the feces as well as foods other than rice of the polluted area residents. (5) The correlation coefficient between Pb and Zn was significant in the rice and the feces among the polluted area inhabitants. (6) The Cu-Zn relationship was significant in the excreted samples from the both areas.

The overall ratio of the Cu concentration to Cd (Cu/Cd) in the polluted areas was 15.17; this was different from the control areas with the ratio of Cu/Cd being 44.44 (F=20.84; p=0.001). A statistically significant difference was found when 88.50 as Zn/Cd in the polluted areas was compared to 238.1 as that in the control areas (F=19.49; p=0.001). However, there was no statistical significance found when Cu/Pb and Zn/Pb ratios between both areas were tested, as shown in Table 2-1.
Since the Cu concentration in foods showed differences between the polluted areas and the control areas, the ratios of Cd, Pb and Zn to Cu indicated statistical significance as shown in Table 2-2 (Cu/Cd: polluted 13.66, control 23.42; F=7.56, p<0.01, Cu/Pb: polluted 10.75, control 18.62; F=11.50, p<0.01, Zn/Cu: polluted 11.63, control 8.40; F=5.92, p<0.05).

All possible ratios of the two heavy metals among fecal Pb, Cd, Cu and Zn are presented in Table 2-3; all ratios except Zn/Cu showed the statistical differences between the polluted and the control areas. Among those, Zn/Cd showed the greatest difference (F=84.52, p<0.01). Cu/Cd also showed the great difference between the two areas (F=58.46, p<0.01).

Table 2-4 indicates the ratio of the combination of the four heavy metals in the urine. The highest statistical difference was found when the average
Table 2-3

The ratios of the heavy metals in the feces

<table>
<thead>
<tr>
<th></th>
<th>Polluted area</th>
<th>Control area</th>
<th>signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>N</td>
</tr>
<tr>
<td>Cu/Cd</td>
<td>11.55</td>
<td>2.04</td>
<td>(102)</td>
</tr>
<tr>
<td>Zn/Cd</td>
<td>99.01</td>
<td>1.77</td>
<td>(106)</td>
</tr>
<tr>
<td>Cu/Pb</td>
<td>21.88</td>
<td>2.38</td>
<td>(103)</td>
</tr>
<tr>
<td>Zn/Pb</td>
<td>190.48</td>
<td>2.13</td>
<td>(107)</td>
</tr>
<tr>
<td>Pb/Cd</td>
<td>0.521</td>
<td>2.60</td>
<td>(106)</td>
</tr>
<tr>
<td>Zn/Cu</td>
<td>8.55</td>
<td>1.74</td>
<td>(103)</td>
</tr>
</tbody>
</table>

Expressed in geometric mean and s.d., **: p<0.01

Table 2-4

The ratios of the heavy metals in the urine

<table>
<thead>
<tr>
<th></th>
<th>Polluted area</th>
<th>Control area</th>
<th>signif.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>s.d.</td>
<td>N</td>
</tr>
<tr>
<td>Cu/Cd</td>
<td>1.81</td>
<td>2.46</td>
<td>(88)</td>
</tr>
<tr>
<td>Zn/Cd</td>
<td>36.23</td>
<td>2.24</td>
<td>(93)</td>
</tr>
<tr>
<td>Cu/Pb</td>
<td>1.14</td>
<td>1.91</td>
<td>(65)</td>
</tr>
<tr>
<td>Zn/Pb</td>
<td>18.45</td>
<td>2.67</td>
<td>(72)</td>
</tr>
<tr>
<td>Pb/Cd</td>
<td>1.87</td>
<td>2.46</td>
<td>(83)</td>
</tr>
<tr>
<td>Zn/Cu</td>
<td>20.20</td>
<td>2.35</td>
<td>(77)</td>
</tr>
</tbody>
</table>

Expressed in geometric mean and s.d., **: p<0.01

urinary Zn/Cd ratio among residents in the polluted area was compared to that of the control area residents (polluted: 36.23; N=93, control: 109.05; N=102, F=54.75, p=0.001).

DISCUSSION

Significance of a correlation coefficient generally depends on the number of samples. Several correlation coefficients between the two metals out of the four in this study were significant although the correlation showed relatively low, between r=0.2 to r=0.4. However, these figures are consistent with those reported by the author.6

The positive relationship between Cd and Cu in the feces and the urine did not appear among the polluted area inhabitants. Higher dietary levels of Cd reportedly depress Cu uptake.7 It also says, a relatively small increase in Cd
intake can adversely affect Cu metabolism when Cu intake is marginal. From the correlation tables in this study, the high exposure group excreted Cd without accompanying Cu since there was no significant correlation of urinary Cd with Cu. This may suggest increased Cd depresses Cu excretion in the same manner.

Pb has a different metabolic pathways from the other three heavy metals that it primarily binds to the surface of erythrocytes when absorbed. Interactions of Pb with Zn are less well described than those between calcium and iron. Since a Zn-dependant enzyme is found in the hem synthesis pathway, the inhibition of the enzyme by Pb is apparently alleviated by Zn. In this study, the Cd-polluted area was also polluted by Pb, based upon the fecal analysis. However, at these levels of Pb intake observed in the both polluted and control area inhabitants, correlation coefficients of Pb to the other three metals among the former group showed similar values to those of the latter group. Thus, there seems no interaction of Pb with Zn at this level of exposure.

It is reported that renal cortex contains both Cd and Zn at concentrations about 60 µg/g wet weight for the age between 50 and 70 years. And the correlation coefficient between Cd and Zn in the renal cortex at that study showed \( r = 0.37 \) \( (N = 85) \), which was significant at \( p < 0.01 \). In the present study, if the urinary Cd and Zn concentration reflect the renal Cd and Zn accumulation, correlation coefficient of these two metals in the urine is very much consistent with the reported figure. Good correlation coefficient between Cd and Zn in urine was found in the Cd-polluted area population, however, that was not found in the control area population. Therefore, whether the urinary heavy metal excretion reflects the human body metabolism and distribution is not known from this study.

The heavy metal ratios by area in rice, the other foods, feces and the urine are indicated in the Table 2-1 through 2-4. The following observation and discussion based on these can be done; (1) The Pb/Cd ratio of the food samples excluding rice showed no difference between the polluted and control areas and showed greater than one. This means the more existence of Pb concentration than Cd in the foods other than rice, even in the Cd-polluted areas. (2) Comparing the other five heavy metal ratios than Pb/Cd in rice to those ratios of the foods except rice, both ratios indicated the similar level. This contaminated by the other heavy metals. (3) The ratios of heavy metals in feces were approximately the same levels as those ratios in rice and the other food samples, but different from those of the urinary metals. This may suggest the different metabolic pathways of those metals in human organs, resulting in the different distribution and excretion between urinary and alimentary tracts.

The Cu/Cd ratio in rice for the polluted area inhabitants was 15.17, that in the foods without rice was 13.66, and that in the feces was 11.55. But that
in the urinary samples was 1.81, about eight times smaller than that obtained from the other three biological samples. Interesting thing is that the Cu/Cd in the urinary samples from the control area residents showed also about eight times higher than that in the rice, foods and fecal samples. Since rice or fecal Cu concentration in the polluted area residents was not statistically different from that of the control area residents, the difference of the Cu/Cd ratio between the urine and the feces may be solely due to the increase of Cd in the urine. The Cu/Cd ratios of rice, the other foods, and the feces were fairly correspondent to the levels reported elsewhere. But the urinary Cu/Cd ratio in the control area inhabitants showed the equivalent level of that in the liver, but not that in the renal cortex. This may suggest that urinary Cu/Cd ratio does not reflect those heavy metal distributions in the renal cortex possibly because of the different affinity of the heavy metals to such organs.

CONCLUSION

The Cd-polluted area is not only contaminated by Cd but also contaminated by Pb and Zn. And the magnitude of contamination by these metals is related to the intensity of the exposure to Cd. Increased Cd possibly suppresses Cu excretion to the bile or the urine, since the positive correlation coefficient between Cd and Cu disappeared in the Cd-polluted area. On the other hand, Cd-Zn relationship was not clear in this study because of the lack of the positive correlation of that in the polluted area.

GENERAL SUMMARY

Three Cd-polluted areas and three control areas in Japan were selected for the present study. A total of 216 men, 50 to 70 years of age, who were clinically healthy (in particular without proteinuria or glucosuria) were subjects of urinary and fecal sampling, with approximately 30 to 40 men from each area. Ten of each dietary sample were collected from the six individual areas.

The analysis of the heavy metals showed the following results; the daily Cd and Cu intake comes chiefly from the rice. Pb and Zn come from the other food sources in the everyday diet. Man's heavy metal intake is highly dependant upon the geochemical characteristics of the area. The polluted area residents take two to three times larger amounts of Cd than the residents of control area, relatively larger amounts of Pb and Zn, and equal amounts of Cu as compared to the residents of control area. The daily intake of the four heavy metals in the control group showed similar values to those earlier reported. The urinary excretion of the four metals, except Zn, in the polluted area residents was also
higher than those of the control area residents. But the difference in the urinary Cd excretion between the polluted and control area inhabitants was smaller than the difference in the fecal Cd excretion between the two areas.

The daily balance study for the heavy metals revealed a negative balance which did not differ significantly by area. This loss of the daily balance may be due to other exposures, such as inhalation by lung, resulting in the increase of heavy metals in the feces.

The residents of the Cd-polluted areas are exposed not only to Cd but also to Pb and Zn. The more the daily Cd amounts are taken, the more another metals are simultaneously taken. The ratios of the four heavy metals in the feces reflected the levels of heavy metal ratios of the dietary sources. The fecal heavy metal ratios, however, differed from those in the urine. This may suggest a different distribution, accumulation and metabolism of individual heavy metals in the body, and hence excretion in the urine at different concentrations.

At the given level of Cd-exposure in the polluted area, definite adverse effect of Cd or the other metals was not obtained. However, possible interrelationship between Cd and Cu was observed, since positive correlation disappeared with the increase of Cd-exposure.

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