Circadian Rhythms of Seven Heavy Metals in Plasma, Erythrocytes and Urine in Men: Observation in Metal Workers

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Abstract: To elucidate circadian rhythms (variation within a day) of 7 toxic or essential metals in plasma and erythrocytes in relation to the rhythms in urine in men, 19 male metal foundry workers were examined; they were exposed to lead (Pb), zinc (Zn) and copper (Cu) occupationally but separated from the exposure during the study. Circadian rhythms were found for plasma concentration of Pb, cadmium (Cd), Zn, Cu and chromium (Cr) in the workers. Circadian rhythms were also found for Pb, inorganic mercury (Hg), Zn and Cr in erythrocytes and for all metals except Zn in urine. Both the plasma and urinary levels of Pb, Cd, Cu and Cr together with urinary excretion of Mn and creatinine tended to decrease during night hours; both the erythrocyte and urinary levels of Hg together with both the plasma and erythrocyte levels of Zn tended to increase during morning hours. The results of profile analysis suggested that the circadian rhythm of Pb in urine was affected more strongly by its plasma and erythrocyte rhythms than by the rhythm of creatinine in urine, i.e. the rhythm of glomerular filtration; the urinary rhythms of Cd, Cr, and Mn were affected more by the creatinine rhythm; and the urinary rhythm of Cu was affected by both its plasma and creatinine rhythms. On the other hand, the urinary rhythm of Hg was assumed to be independent of the creatinine rhythm and be affected by its erythrocyte rhythm. The present study suggested that different biological limit values might be needed for different hours of the day especially for shift workers who are exposed to various heavy metals. Instead, further studies should be conducted to find the adjustment methods by which no circadian rhythms are discerned.

Key words: Circadian rhythms, Essential metals, Heavy metals, Plasma, Erythrocytes, Urine, Creatinine clearance

Introduction

Circadian rhythms in the urinary excretion of toxic or essential metals such as lead (Pb), mercury, cadmium (Cd), zinc (Zn), copper (Cu), chromium (Cr) and manganese (Mn), i.e. variations observed within a day, in human subjects have been elucidated by many workers1-10. On the other hand, the rhythms of these heavy metals in blood (plasma and erythrocytes) have been reported only for Zn and Cu1,6,11-15. In the present study, we analyze the plasma and erythrocyte rhythms of these seven metals in metal workers in relation to their urinary rhythms. The analysis is conducted in smokers and non-smokers, as evidence has been given that smoking affects blood metal concentrations16,17 as well as renal hemodynamics18.

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Subjects and Methods

Subjects

The 19 subjects (8 smokers and 11 non-smokers) were male workers engaged in founding faucets and pipes using gun metal containing Pb, Zn and Cu, and exposed to their fumes. Their age, metal concentrations in whole blood and plasma, years of employment and number of cigarettes smoked are shown in Table 1. Their blood Pb, plasma Cu and Zn concentrations were significantly higher than those in 16 referents (male steel foundry workers at the same factory), i.e. 8–20 (mean 13), 54–97 (mean 72) and 50–100 (mean 73) µg/dl, respectively (p<0.05). The concentrations of mercury (total), Cd and Mn in whole blood were not measured in the referents, but those in the 19 subjects were not substantially different from the reference values reported in the literature, e.g. less than 5 µg/dl26, 2.49 (0.11–6.06) µg/dl26 and 9.0 ± 1.2 µg/l21, respectively, indicating that occupational exposure to these three metals were insignificant among the study subjects. Plasma Cr concentrations in the 19 subjects were higher than reference values reported (0.12 ± 0.05 µg/l – 0.2 ± 0.01 µg/100 g)22, though there was no evidence that they had been exposed to Cr occupationally. No subject had ever suffered from renal disease; neither albuminuria nor glucosuria was found in any subject.

Collection of blood and urine samples

The procedure was explained to all subjects and the study was conducted with their informed consent. All subjects were admitted to a special room for health examination at a medical school, being separated from the work place during the study.

Their urine samples were collected during the following four periods of the day: (1) 7–12 am (morning hours), (2) 0–6 pm (afternoon hours), (3) 6–11 pm (evening hours), and (4) 11 pm–7 am (night hours). Blood samples were collected four times on the same day at 10 am, 3 pm, 10 pm and 5 am (each time represented the morning, afternoon, evening and night hours). They ate every meal at an eating hall of the school hospital at 8 am, 12 am and 6 pm; very little fish and shellfish were eaten to minimize the intake of organic mercury.

Analytical methods

The concentration of Pb in whole blood, erythrocytes and urine were measured by the atomic absorption spectrophotometry (AAS) (Hitachi Polarized Zeeman Atomic Absorption Spectrophotometer 180-80) after wet ashing, chelation by sodium diethylthiocarbamate (DDTC) and extraction to water-saturated methyl-isobutylketone (MIBK); the plasma Pb concentration was measured by the method of Desilva21. The concentrations of inorganic mercury (Hg) in plasma, erythrocytes and urine were determined by the method of Magos (Sugiyama-gen Mercury Vapor Meter MV-253R)24, 25. The concentration of Cd and Cu in plasma and erythrocytes were measured by the flameless AAS after deproteinization by trichloroacetic acid (TCA); urinary Cd concentration by the method Subramanian et al.26. The concentrations of Zn in urine and plasma were determined by AAS after deproteinization by TCA; the erythrocyte Zn concentration by AAS after wet ashing. The concentrations of Cu, Cr and Mn in urine were analyzed by AAS after wet ashing, chelation by DDTC and extraction to MIBK; the plasma and erythrocyte concentrations of Cr and Mn by the flameless AAS using the standard addition technique after deproteinization by TCA. The concentration of creatinine in urine was measured by Jaffe’s reaction.

The detection limits for metals in plasma, erythrocytes, whole blood and urine were 0.05, 0.2, 0.2, 0.2 µg/dl for Pb in plasma, erythrocytes, whole blood and urine, respectively; 0.05 µg/dl for Hg in plasma, erythrocytes and urine; 0.01 µg/dl for Cd in plasma and erythrocytes, and 0.05 µg/dl for Cd in urine; 2 µg/dl for Zn in plasma, erythrocytes and urine; 2.5 µg/dl for Cu in plasma and erythrocytes, and 0.2 µg/dl for Cu in urine; 0.2 µg/dl for Cr in plasma and erythrocytes, and 0.1 µg/dl for Cr in urine; 0.1 µg/dl for Mn in plasma, erythrocytes and urine; and 0.5 mg/dl for creatinine in urine.

The reproductibility of analysis for Pb, Zn and Cu in plasma, erythrocytes, whole blood and urine, and creatinine in urine has been reported previously16, 27, 28. The coefficient of variation for determinations of other metals were 6.5, 5.1

Table 1. Age, concentrations of lead, total mercury, cadmium and manganese in whole blood (BPb, BHg, BCd, BMn) and zinc, copper and chromium in plasma (PZn, PCu, PCr), years of employment and number of cigarettes smoked in 8 smokers and 11 non-smokers (mean with ranges in parentheses)

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Smokers</th>
<th>Non-smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPb (µg/dl)</td>
<td>34 (25–40)</td>
<td>41 (22–59)</td>
</tr>
<tr>
<td>BHg (µg/dl)</td>
<td>3.5 (2.5–4.4)</td>
<td>4.8 (2.3–8.5)</td>
</tr>
<tr>
<td>BCd (µg/dl)</td>
<td>0.27 (0.14–0.46)</td>
<td>0.37 (0.10–0.57)</td>
</tr>
<tr>
<td>BMn (µg/l)</td>
<td>2.35 (1.1–4.10)</td>
<td>2.37 (1.20–3.37)</td>
</tr>
<tr>
<td>PZn (µg/dl)</td>
<td>9.5 (75–110)</td>
<td>84 (67–95)</td>
</tr>
<tr>
<td>PCu (µg/dl)</td>
<td>93 (64–134)</td>
<td>95 (76–122)</td>
</tr>
<tr>
<td>PCr (µg/dl)</td>
<td>4.5 (3.5–6.3)</td>
<td>5.0 (3.9–6.2)</td>
</tr>
<tr>
<td>Employment (years)</td>
<td>8 (2–17)</td>
<td>11 (3–16)</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>0 (0–15)</td>
<td>0</td>
</tr>
</tbody>
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and 7.7% for Hg in plasma and erythrocytes and urine, respectively; 3.4% for Cd in plasma and erythrocytes, and 4.8% for Cd in urine; 4.1% for Cr in plasma and erythrocytes, and 5.8% for Cr in urine; and 6.4% for Mn in plasma and erythrocytes, and 6.8% for Mn in urine.

Statistical analysis

Differences in the plasma, erythrocyte and urinary levels of heavy metals and urinary creatinine excretion among four periods of the day were tested by the paired sample t test. Parallelisms of the circadian rhythms of the plasma, erythrocyte and urinary levels of these substances in smokers to their rhythms in non-smokers were analyzed by the profile analysis (F test)29). Similarly, parallelisms of the circadian rhythms in the urinary excretion of heavy metals to their plasma and erythrocyte rhythms and to the urinary rhythms of creatinine were analyzed by the profile analysis. The profile analysis is a test to examine if the population mean profiles on several measurements are similar between the two groups of samples;29 we used this test in our previous study to compare the circadian rhythms of metals and organic substances with those of urinary flow and creatinine excretion10.

Results

Circadian rhythms were found for five metals (Pb, Cd, Zn, Cu and Cr) in plasma; and for four metals (Pb, Hg, Zn and Cr) in erythrocytes (Figs. 1–4). Circadian rhythms were also found for all heavy metals except Zn and creatinine in their time excretion into urine (Figs. 1–4).

The plasma rhythms of Cd, Zn, Cu and Cr were more distinct than their urinary and erythrocyte rhythms (Figs. 2–4). On the other hand, the urinary rhythms of Pb and Hg were more remarkable than their plasma and erythrocyte rhythms; the circadian rhythm of Mn was only found in urine (Figs. 1, 2 and 4). The erythrocyte rhythm of Hg sharply contrasted with absence of its plasma rhythm (Fig. 2).

![Fig. 1. Circadian rhythms of lead in urine, plasma and erythrocytes (UPb, PPb and EPb) and creatinine in urine (UCn) in 8 smokers (صوم) and in 11 non-smokers (نشمل). UPb, PPb, EPb and UCn during each time period of the day are expressed as mean ± standard deviation. * and ** indicate significant differences between two time periods of the day at the levels of p<0.05 and 0.01, respectively (paired sample t test). No significant circadian rhythm was found for lead in whole blood in smokers and non-smokers, respectively (p>0.05).](image-url)
Both the plasma and urinary levels of Pb, Cd, Cu and Cr tended to decrease during night hours (urinary excretion of Mn and creatinine also decreased during night hours); the plasma (and erythrocyte) level of Cr also decreased during evening hours despite the fact that the urinary excretion of the metal increased (Figs. 1–4). Both the erythrocyte and urinary levels of Hg tended to increases during morning hours; both the plasma and erythrocyte levels of Zn tended to increase during morning hours (Figs. 2 and 3).

The circadian rhythms of all heavy metals in urine, plasma and erythrocytes in smokers were parallel to those in smokers (p>0.05), i.e. there was no significant differences in the patterns of changes between the smokers and non-smokers, except for the rhythm of Hg in erythrocytes (F=5.356, p<0.05). In smokers and non-smokers combined, the circadian rhythms of Pb, Hg and Cu in urine were parallel to their plasma and erythrocyte rhythms; the rhythms of Cd, Cu, Cr and Mn in urine were parallel to the circadian rhythm of creatinine in urine (Table 2).

When creatinine-adjusted urinary concentration (excreted amount per creatinine in urine) was used, patterns of the urinary rhythms were as follows: The excretion were not significantly different between the four periods for Cu, Cr and Mn in either smokers or non-smokers (p>0.05). The excretion showed significant differences (p<0.05) only between the morning and night hours and between the afternoon and night hours in non-smokers for Pb, and only between the morning and evening hours in smokers and non-smokers and between evening and night hours in non-smokers for Hg. The Cd excretion was significantly different between the afternoon and evening hours in smokers (p<0.05). The difference became significant between the morning and evening hours in smokers and between the evening and night hours in non-smokers for Zn (p<0.05).

Creatinine clearance during the morning, afternoon, evening and night hours (mean with range in parenthesis) in 19 workers was 48.3 (26.7–79.3), 53.1 (31.7–103.6), 58.1 (32.7–93.2) and 48.4 (22.6–94.1) dl/hr; it was significantly lower in the night and morning than in the evening (p<0.05).
Fig. 3. Circadian rhythms of zinc in urine, plasma and erythrocytes (UZn, PZn and EZn) and copper in urine and plasma (UCu and PCu) in 8 smokers and 11 non-smokers.
Signs same as in Figs. 1 and 2.

Fig. 4. Circadian rhythms of chromium in urine, plasma and erythrocytes (UCr, PCr and ECr) and manganese in urine (UMn) in 8 smokers and 11 non-smokers.
Signs same as in Figs. 1 and 2.
was significantly higher during morning hours than during each of other three time periods (p<0.05). The increase in urinary Zn excretion during morning hours has been found by Bhattacharya9) and by us8, 9).

Pb, Cd and Cu in plasma decreased during the night hours, resembling their urinary rhythms; the rhythm of Hg in erythrocytes was similar to its urinary rhythm, showing the lowest level in the afternoon (Figs. 1–3). The plasma rhythms of Cd and Cu were more distinct than their urinary rhythms. It is thus suggested that the circadian rhythms of Pb, Cd and Cu in urine are affected by their plasma rhythms; the rhythm of Hg in urine reflects its erythrocyte rhythm.

It has been reported that glomerular filtration (inulin or creatinine clearance) is low at night30). In the subjects examined here, creatinine clearance was decreased during the night hours. Thus, reduction in glomerular filtration may account for the decrease in the urinary excretion of creatinine during night hours (Fig. 1). The time excretion of all heavy metals into urine except Zn also tended to decrease during night hours (Figs. 1–4). Therefore, the reduction in glomerular filtration rate (GFR) is assumed to be also responsible for the decrease in the urinary excretion of these heavy metals during night hours.

The results of profile analysis (Table 2) further simplified the data collected in the present study. It is indicated that the circadian rhythm of Pb in urine was affected more strongly by its plasma and erythrocyte rhythms than by the GFR rhythm; the urinary rhythm of Hg was affected by its erythrocyte rhythm. Conversely, it appears that the urinary rhythms of Cd, Cr and Mn were more distinct than their urinary rhythms. The urinary rhythm of Cu was affected both by its plasma rhythm and by the GFR rhythm.

The results of our previous study have shown that glomerular filtration plays an essential role in the urinary excretion of all heavy metals examined in the present study except Hg; Hg undergoes little filtration by the glomeruli31). Recently, we have also found that glomerular filtration may be the major factor determining renal excretory mechanisms of Pb, Cd, Cu and Cr in man32). These data underlie the results by the present study. However, as it was also observed in our previous study31) that Pb is reabsorbed and Cr, Cu and Hg are secreted by uniriniferous tubules, effects of tubular reabsorption and secretion on the urinary circadian rhythms should be further examined. In the present study, the creatinine-adjusted concentration of Zn in urine showed more distinct rhythm than its time excretion did whereas creatinine-adjusted concentrations of other metals were less distinct. This may be due to influence of the rhythm of creatinine excretion, which is greatly affected by urinary flow32).
No significant differences between smokers and non-smokers were found in the circadian rhythms of all heavy metals in plasma, erythrocytes and urine except Hg in erythrocytes. Therefore, long-smoking hours in the daytime might not significantly affect the circadian rhythms of most heavy metals in plasma, erythrocytes and urine at the level of tobacco consumption in the subjects of the present study. Further studies are needed to examine the effects of heavier smoking.

The present study thus revealed that the plasma and erythrocyte levels of many heavy metals were significantly different among the morning, afternoon, evening and night hours of the day, in light of results from previous studies which revealed the alterations of their urinary levels. To further confirm the findings obtained here, studies on larger number of subjects over successive days would be necessary. This observation must be accounted for in various aspects of occupational and environmental health practice. For example, different biological limit values might be needed for different hours of the day especially for shift workers who are exposed to various heavy metals. Instead, further studies should be conducted to find the adjustment methods by which no circadian rhythms are discerned. Concerning adjustment methods for urinary excretion, we are preparing another paper on this subject, in which various methods such as adjustment to urinary volume, specific gravity and creatinine are examined. The first method of adjustment would be promising, as we have reported that only the adjustment to urinary volume could eliminate the effects of urinary flow on 24-hour urinary excretion of various heavy metals.

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References