Original

DETERMINATION OF LEAD IN PLASMA, WHOLE BLOOD, AND URINE BY ICP-MS AND THE RELATIONSHIPS AMONG THE THREE EXPOSURE INDICES

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(Received: Octover, 23, 2002)

Abstract

The cbjectives of this study were to develop the simple method of datermining lead in plasma (PbP), whole blood (PbB), and urine (PbU) by inductively coupled plasma mass spectrometry (ICP-MS), and to examine the relationships among the three indices of lead exposure. Venous blood treated with heparin and spot urine was collected from 69 male lead workers. For ICP-MS analyses, plasma, whole blood, and urine were diluted 5, 50, and 80-fold, with nitric acid solution, respectively. PbP, PbB, PbU were also detarmined by the method of atomic absorption spectrometry (AAS). PbB and PbU determined by ICP-MS were close to those by AAS, however, PbP levels by AAS were more than 2-times higher than those by ICP-MS. PbP and PbU levels logarithmically increased with elevating PbB. A linear relationship was found between PbP and PbU. PbP, PbB and PbU were simply measured by ICP-MS, and the logarithmic or linear relationships were found among those indices.

(JJOMT, 51 : 50—57, 2003)

—Key words—

Lead, Plasma, ICP-MS

Introduction

In blood, more than 98% of the lead are found in blood cells[1)2)]. Plasma lead (PbP) has an important role in lead metabolism, where it is the active center of the body lead pool, although the concentration is very low levels. PbP is equilibrates with the extra-cellular pool and is directly involved in all the movements of lead among the different biological compartments[3)]. Thus, lead in plasma circulates in the body, affects the body lead burden and causes the toxicity of lead in some soft tissuies, such as bone marrow, kidney brain and so on.

The levels of PbP are sharply elevated with a sudden intake or acute exposure to lead and rapidly diminished by time elapse from it[3)], indicating that PbP is an index for very recnt exposure[4)]. The chelatable lead excreted in urine (MPbU) is considered to be an excellent measue of potentially toxic fraction of the body lead burden[5)], and usually determination in urine collected for 24 hs after calcium disodium ethylenediamine tetraacetic acid (Ca EDTA) administration[6)]. Recently, Sakai et al[7)]. have report that PbP concentration at 2 hs after the start of CaEDTA injection (MPbP) is well correlated with the amount of lead excreted in urine for 24 hs thereafter, and is a useful measure for the chelatable lead.

Thus, PbP as well as MPbU are very impotrant indices of lead as exposure, distribution, and health risk. However, the concentration of lead in plasma or serum is not routinely measured, so far. One of the main reasons is the very low conecentration of PbP, which is difficult to determine by atomic absorption spectrometry (AAS). In the AAS techniques, it needs troublesome procedures of chelation and extraction[1)], which might result in contamination of samples with exogenous lead.

Recently, a highly sensitive instrument, inductively coupled plasma mass spectrometry (ICP-MS), has been introduced for the measurement of lead in plasma or in serum[2)8)~11)]. Lead in whole blood (PbB) and in urine (PbU) can be also determined by ICP-MS more exactly than AAS[12) 9) 12) ~ 14)] .

The aim of the present study is to establish the ICP-MS method for the determination of PbP, PbB, and PbU

in lead workers. We have also investigated the relationships among the three indices of lead exposure, using ICP-MS.

Materials and Methods

The subjects were 69 male lead workers (25-61 years old, mean 43 years). They were employed in a battery smelter and a glass factory. Venous blood (n=69) and spot urine (n=49) were collected during their physical examination. Urine was collected in acid washed polypropylene cups. Venous blood was drawn from the cubital vein into an evacuated and heparinised 5 ml tube (Terumo, Tokyo, Japan). Plasma was separated by centrifugation (10 min, 1,200 g) within 30 min after the sampling and transferred into acid washed polypropylene tubes. The samples of blood, plasma, and urine were stored at −20°C. The analysis was carried out within 3 months after sampling.

Standard solutions containing a single element (1,000 mg/l) of lead and bismuth were purchased from Wako Pure Chemicals (Osaka, Japan). Ultra pure nitric acid (Tama Pure AA-10) was purchased from Tama Chemicals (Kawasaki, Japan). Distilled pure water (DW) was prepared by filtration of distilled water through a Millipore-Q system (Millipore Japan, Tokyo, Japan).

For preparation of sample of solutions for ICP-MS determination, whole blood (30 μl) was hemolyzed with 270μl of DW, then well mixed with 1.2 ml of nitric acid (0.15 N). Plasma (300 μl) was well mixd with 1.2 ml of nitric acid （0.15 N). Plasma (300 ml) was well mixed with 1.2ml of nitric acid (0.15 N). Thirty microliter of bismuth nitrate (500 μg/l） was added to each sample as an internal standard. In the same way, 30 μl of urite was well mixed with 2.4 ml of the nitric acid and 50 μl of the bismuth solution was added to each sample.

ICP-MS (Model PMS-2,000, Yokogawa Analytical Systems, Tokyo, Japan) was used for the analysis of lead in the sample solutions. The operating conditions were as follows : RF power 1.2 kW, plasma gas 14 l/min, auxiliary gas 1 l/min, carrier gas 0.98 l/min, and sample uptake rate 0.4 ml/min, respectively. The selected isotopes ^{208}Pb and ^{209}Bi were monitored at three points per peak. The dwell time was 10 ms/point for both Pb and Bi. They were scanned 50 times in a determination. One sample was determined 3 times and total analytical time was 9.6 second. The average of five replications was used for calculations. The instrument was calibrated against spiked plasma (+10 and +20 μg/l), whole blood (+500 and +1,000 μg/l) and urine (+100 and +200 μg/l) samples.

In all 69 workers, the lwvels of PbP were determined by the method of ICP-MS. For the comparison, 19 out of 69 lead workers were determined by the method of DeSilva[1], using graphite furnace AAS (Model Z-8,000, Hitachi, Tokyo, Japan). For the comparison, PbB was also measured by both ICP-MS and graphite furnace AAS in 53 out of 69. PbU was determined by ICP-MS in 49 lead workers. In 15 workers of them, PbU was analyzed by the method of Ushio et al[15]. using flame AAS (Model Z-8,000, Hitachi, Tokyo, Japan) for the comparison. For the external quality control of PbB determination, our laboratory was involved in the Quality Control Program conducted by the National Federation of Industrial Health Organizations, with the support of Ministry of Labor, Japan. PbU was corrected for creatinine concentrations. Creatine was determined by the method of Jaffe with the “Creatinine Determination Kit” of Wako Pure Chemicals (Osaka, Japan).

Results

Fig. 1 shows the calibration curves of PbP, PbB and PbU in ICP-MS analyses, indicating ICP-MS has a wide dynamic range in every determination. In the method of ICP-MS, the recovery rate of PbP was 105.9 ± 4.3%, when 10 μg/l of Pb was added to 20 plasma samples (PbP range : 0.34-17.18 μg/l). The mean recovery rate of PbB was 100.0 ± 1.2% when 500 μg/l of Pb was added to 12 blood samples (PbB range : 42~679 μg/l). In the same way, the recovery of PbU was 101.5 ± 2.2% when 200 μg/l of Pb was added to 8 urine samples (PbU range : 7.3~200.8 μg/l, creatinine concentration range : 48.9~193.9 mg/dl).

Table 1 indicates the comparison of mean and range of PbP, PbB and PbU determined by ICP-MS and AAS. Fig. 2 shows the comparison of PbB, PbP, and PbU by the two methods, ICP-MS and AAS. PbB values determined by ICP-MS are well correlated with those by AAS. The PbB values of two methods are almost the same (Table 1).

Although, PbU levels determined by ICP-MS are slightly high in comparison with those by AAS, the correlation coefficient of PbU levels by both the methods is also high (r=0.934). This indicates that PbU measurements by both the methods can be comparable. The mean levels of PbP measured by AAS are more than 2-times higher than those

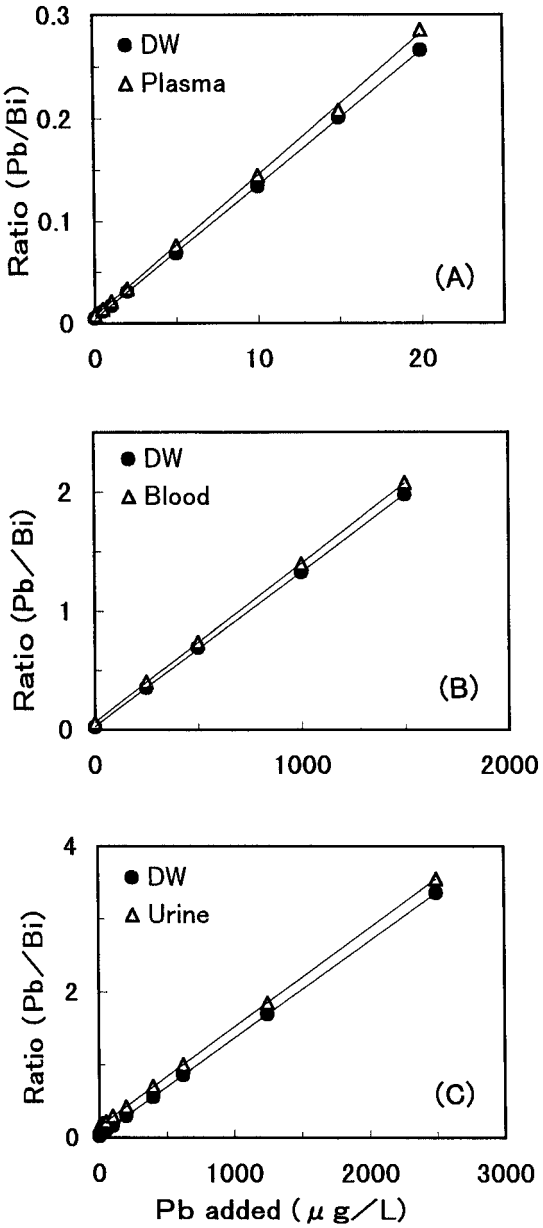

Fig. 1 Calibration curves of PbP (A), PbB (B), and PbU (C). Lead was added to DW or sample.
(A) DW : y=0.013x+0.004 (r=0.999). Plasma : y=0.014x+0.007 (r=0.999).
The mean recovery rate of PbP was 105.8%, when 0.5 to 20 μg/l of lead were added to plasma sample (Original PbP level : 0.57 μg/l).
(B) DW : y=0.001x+0.016 (r=1.00). Blood : y=0.001x+0.061 (r=1.00).
The mean recovery rate of PbB was 100.8%, when 250 to 1,500 μg/l of lead were added to blood sample (Orignal PbB level : 42 μg/l).
(C) DW : y=0.001x+0.025 (r=1.00). Urine : y=0.001x+0.154 (r=1.00).
The mean recovery rate of PbB was 100.0%, when 25 to 2,500 μg/l of lead were added to urine sample (Original PbU levels : 102.9 μg/l, Creatinine level : 100.2 mg/dl).

by ICP-MS (Table 1). Each PbP level measured by AAS was also apparantly higher (max 9 times) than that by ICP-MS. The difference in both the methods is more evident especially in low PbP levels.

Fig. 3 shows the relationships among PbB, PbP and PbU, which were determined by ICP-MS (Table 1, first raw). Fig. 3 (A) shows the relationship between PbB and PbU (n=53). PbP levels logarithmically increase with elevating PbB levels. The correlation coefficient was as high as 0.933. Fig. 3 (B) shows the relationship between PbB and PbU (n=49). PbU levels are also logarithmically increased with elevating PbB levels. The correlation coefficient (r=0.817) was slightly higher than that calculated in a linear correlation mode (r=0.765). although the difference was not significant. Figure 3 (C) shows the relationship between PbU and PbP (n=49). A linear relationship (r=0.657) is found between PbP and PbU. The correlation coefficient was almost the same as that calculated in a

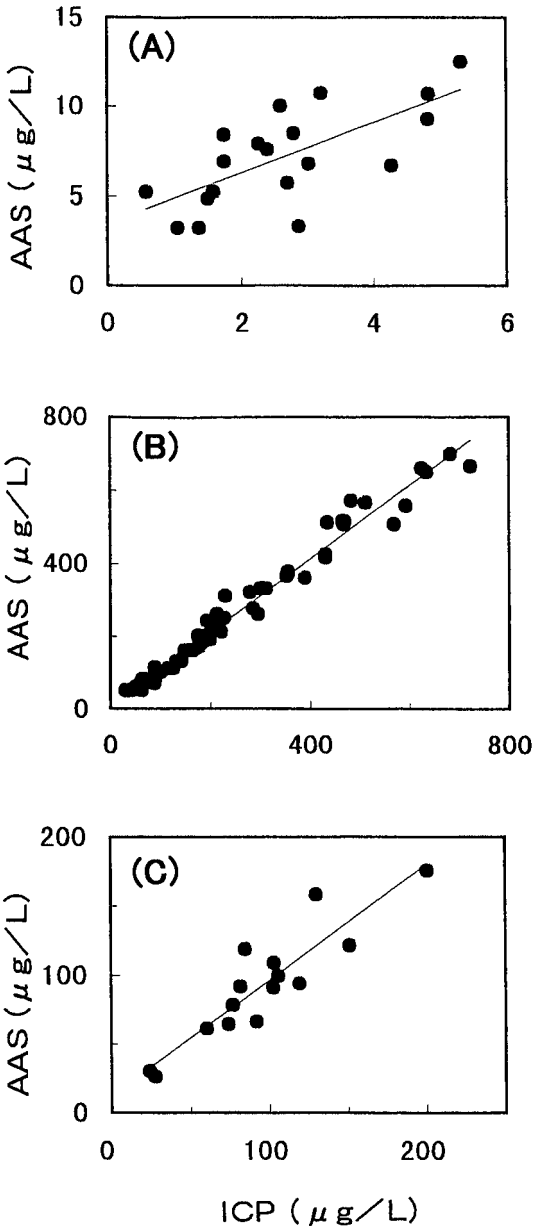

Fig. 2 Comparison of lead determinations by ICP-MS (x) and AAS (y) methods.
(A) PbP : y=1.41x+3.4 (r=0.699, n=19)
(B) PbB : y=1.006x+7.89 (r=0.987, n=53)
(C) PbU : y=0.836x+12.5 (r=0.934, n=15)

logarithmic mode (r=0.656).

Discussion

In the present study, three kinds of indices of lead exposure (PbP, PbB, and PbU) could be simply determined by ICP-MS. For the analysis of plasma, whole blood, and urine, samples were diluted 5, 50 and 80-fold, with nitric acid solution, respectively. Each value of exposure indices was compared by two methods, ICP-MS and AAS (Fig. 2, Table 1). One of the advantages for ICP-MS determination was of a wide range of linearity in the calibration curves (Fig. 1) as compared with AAS, where dynamic range was narrow. Another advantage for ICP-MS determination was of simple procedure and also high sensitivity, which enabled us to determine very low levels of PbP, without complicated and time consuming pretreatment.

Previously we have to determine PbP by graphite furnace AAS after solvent extraction of plasma lead chelat-

Table 1 Comparison of the methods of ICP-MS and AAS for measurement of PbP, PbB, and PbU

Kind of indices	N	Analytical methods	
		ICP-MS	AAS
PbP (μg/l)	19	2.67 ± 1.34 (0.58—5.32)	7.19 ± 2.71 (3.2—12.5)
	69	*1.87 ± 2.26 (0.25—17.18)*	
PbB (μg/l)	53	270 ± 183 (32—724)	276.4 ± 186 (50—698)
PbU (μg/l)	15	95.7 ± 44.9 (24.2—200.8)	92.5 ± 41.4 (26.1—175.9)
	49	*47.4 ± 42.6 (7.3—200.8)*	

Mean ± SD (range)

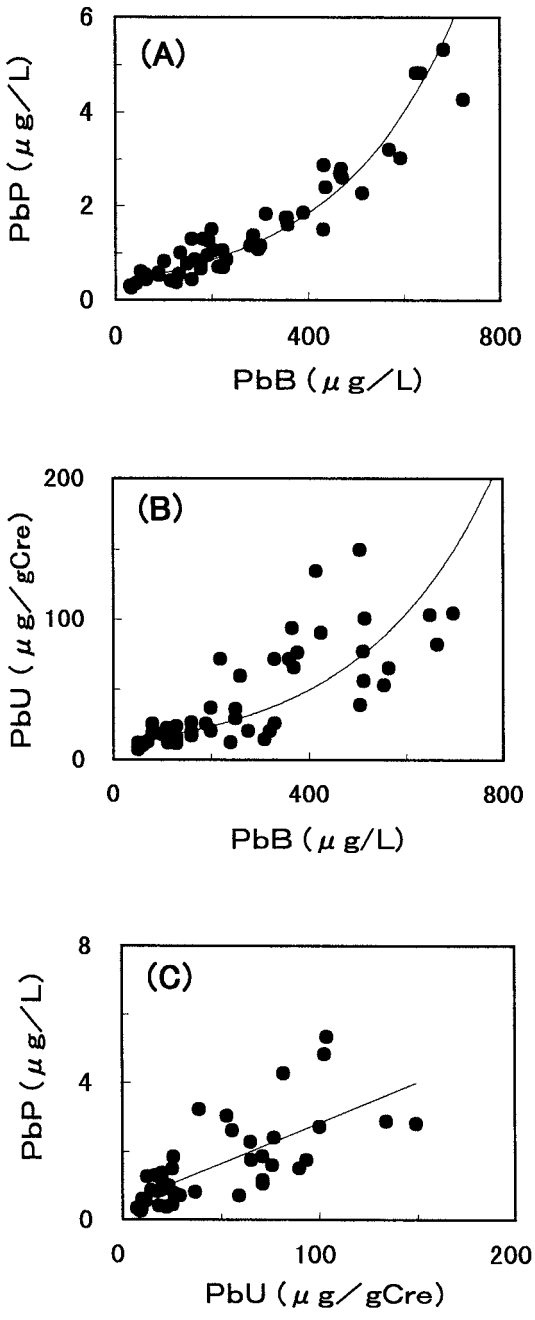

Fig. 3. Relationships among three exposure indices determined by ICP-MS.
(A) PbP (y) vs. PbB (x) : y=0.392e (r=0.933, n=53).
(B) PbU (y) vs. PbB (x) : y=11.78e 0.0035x (r=0.817, n=49).
(C) PbP (y) vs. PbU (x) : y=0.219x+0.481 (r=0.657, n=49).

ed with ammounium pyrrolidinedithiocarbamate (APDC)[4]. The procedure included so many steps and chemicals which mighe cause the contamination of samples with exogenous lead. Acutually PbP levels measured by AAS were much higher than those by ICP-MS. It might be attributable to the contamination of lead during the chelating and extraction procedure befor introducing the sample into AAS. The differences in PbP levels between two methods were especially large at low PbP levels, where the lead contamination might be more obviously observed.

On the contrary, the PbB levels by ICP-MS were almost the same as those by AAS (Fig. 2). The fact indicated that both the methods were useful for the routine analysis. The values of PbU by ICP-MS were also identical to those by AAS. However, it should be further confirmed the exact relationship between PbU levels by two analytical methods in a larger numbers of samples. Thus, ICP-MS method will be used widely in future because the analytical procedure is simpler than by AAS.

Using ICP-MS, Schütz et al[2]. reported the ratio of PbP/PbB increased with increasing PbB, in other words, there was a logarithmic relationship between PbB and PbP. Our result (Fig. 3) also showed that PbP levels logarithmically increased with elevating PbB. Based on the correlation equation in the present study, the PbP level corresponding to PbB levels of 400 μg/l was calculated to be about 1.9 μg/l. The PbP level corresponding to PbB of 400 μg/l was close to that in the report by Schütz et al[2]. from whose data it was calcuated to be about 2.2 μg/l.

PbP is considered to be “biologically active lead” and it is in the equilibrium with the extra-cellular pool of every tissue or organ[3]. The concentration of PbP and serum lead (PbS) has been shown to be very low, usually less than 1% of that PbB, but those percentage are increased with rising lead level[16]. Moreover, Bergdahl et al[17] , who were in the same study group of Schütz, further studied the relationship among PbP, PbB, and bone lead, and reported again positive correlation not only between the ratio of PbP/PbB and bone lead, but also between that and PbB. By the correlation equation[10], the PbP level corresponding to PbB level of 400 μg/l is calculated to be about 6.7 μg/l. The value is 2~3 times higher than those by the data in the present study (1.9 μg/l) and by Schütz et al (2.2 μg/l)[2]. Thus, the data in the present study supported the Schütz, although the bone lead was not determined in the present study.

Since 90% or more of lead in an adults is found in bone and the biological half time of lead in bone is very long (more than several years), it has been considered that bone lead is an important index for internal exposure to lead, but not for external lead exposure[10]. PbP is an index for very recent external exposure[4], might be more directly related to PbU rather than PbB. To examine the relationship between external and internal exposure, it is necessary to examine the relationship among PbP, PbB, and also PbU other than bone lead. In the present study, we demonstrated the relationships among the three exposure indices which could be determined by ICP-MS.

Hirata et al.[18] reported the relationship among PbP, PbB, and PbU using AAS. They found a linear relationship between PbP and PbU and the present data agreed with them. The PbP level corresponding to 100 μg/l of PbU was reportedly be 3.8 μg/l in their workers, although the level was 2.7 μg/g Cre in our present date. They also reported a liner correlation between PbB and PbP or PbU. In the present study, however, a logarithmic relationship was found between PbB and PbP or PbU. Logarithmic relationship between PbB amd PbP are also reported by Schütz et al.[2] and Bergdahl et al.[16]. Furthermore, the PbP level (4.5 μg/l) corresponding to the PbB level of 400 μg/l in the study of Hirata et al.[18] is 2-times higher than those of Schütz et al. (2.2 μg/l)[2], Bergdahl et al (2.1 μg/l)[16] and ours (1.9 μg/l). In the same way, the PbU level (99 μg/l) at the PbB levels of 400 μg/l in their study[18] was also 2-times higher than those in ours (48 μg/gCre, Fig. 3). These disagreements might be due to the differences in the method of determination (AAS) and in the levels of lead exposure (PbB : 308-1,020 μg/l) in their study.

The relationships among three exposure indices were examined in the present study, using ICP-MS, where the sample was simply diluted. Thus the ICP-MS method was indicated to be useful for the determination of three exposure indices and therefore for the examination of the body lead burden.

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（原稿受付 平成14. 10. 23）

別刷請求先 〒143-0013 東京都大田区大森南4―13―21
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ICP-MSによる血漿，血液，および尿中鉛の測定と暴露指標としてのこれら3指標の関連

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—キーワード—

鉛，血漿，誘導結合プラズマ質量分析計（ICP-MS）

要旨：誘導結合プラズマ質量分析計（ICP-MS）を用いた血漿，血液および尿中鉛の簡便・高感度な測定法を開発し，鉛の暴露指標としてのこれら3指標の関連について検討した．測定検体は男子鉛作業者（69名）の検診時に採取されたヘパリン血およびスポット尿である．ICP-MSによる測定では硝酸溶液で検体を希釈するが，血漿，血液，尿の希釈倍率はそれぞれ5倍，50倍，80倍である．血漿鉛，血液鉛，尿中鉛については従来の原子吸光光度方（AAS）による測定も行い比較した．血液鉛と尿鉛ではICP-MSによる値はAASによる値に近似していたが，AASによる血漿鉛濃度はICP-MSによる値の2倍以上となった．血液鉛の上昇につれて血漿鉛，尿中鉛とも指数的に増加した．血漿鉛と尿中鉛濃度との間には直線的な関係がみられた．