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Urine and Hair Metal Concentrations in Subjects with Long Term Intake of Herbal Medicine

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ABSTRACT. One of the main attractions of treatment with herbal medicine is its apparent lack of side effects compared with the drug therapies used in allopathic medicine. However, evidence from various countries suggest that Asian herbal medicine carry a significant risk of contamination with toxic heavy metals at levels that may seriously threaten health. The aims of this study were to analyze and compare concentrations of heavy metals in urine and hair from 184 patients taking herbal medicines in the form of decoctions and/or pills in comparison to 101 control subjects taking either Western or no medications. Levels of metal concentrations exceeding WHO reference values were observed in a number of hair and urine samples for all subjects. After adjusting for potential confounders, taking decoctions or pills was associated with higher levels of some metals (such as Cu, Pb in urine), as well a higher odds ratio of exceeding the upper limit of reference ranges for Pb, Hg in hair. In contrast, taking decoctions or pills was associated with lower levels of some metals (such as Cu in urine and Cd, Cu, Hg, Pb in hair), suggesting that some herbal medicines may have a chelating effect on heavy metals in the body. Overall, the results obtained in the study show a mixed picture and suggest that heavy metals contamination in herbs is sometimes present, but may also be counteracted by the potential for some herbal medicines to act as chelating agents. Further study must be followed to obtain more concrete evidence.

Keywords: Herbal medicine, Decoction, Metal concentrations, Chelator.

INTRODUCTION

An herb is defined as a crude materials used for its medicinal properties. Herbs may be derived from plants, animals, minerals, and other materials. Herbal medicine may come in variety of forms including capsules, tablets, pills, mixtures, and decoction to name a few. Herbal medicine is one form of complementary and alternative medicine. Alternative medicine, interpreted broadly, may also include acupuncture, moxibustion, yoga, vitamins, chiropractic, massage, and others. Herbal remedies are widely used in many Asian countries, especially China and Korea. One of the main attractions of herbal medicine treatment is its apparent lack of side effects compared with the drug therapies used in allopathic medicine. For this reason, herbal medicine has been used as a main traditional therapy and is becoming increasingly popular as an alternative or complementary therapy in western countries (Ernst, 2005). However, evidences from various countries suggest that the contamination of Asian herbal medicine with toxic heavy metals may constitute a serious health problem (Hasegawa *et al.*, 1997; Cheng *et al.*, 1998 Ernst *et al.*, 2001). Heavy metal contamination of herbal medicine was found to be responsible for several cases of metal poisoning (Ernst *et al.*, 2001; Caldas *et al.*, 2004). According to Californian officials, twenty-four products of the 251 herbal products contained at least 10 ppm lead, 36 contained an average of 14.6 ppm arsenic, and 35 contained an average of 1046 ppm mercury (Ko, 1998). Several possibilities exist to explain the presence of heavy metals in herbal medicine. First, herbal medicine may contain heavy metals when grown in seriously polluted soil (Kabelitz, 1998). Second, the presence of heavy metals might be the result of contamination during the manufacturing process, for example, from grinding weights or leaded containers or other manufacturing utensils (Fong, 2000). Third, heavy met-

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als could be included intentionally for alleged medicinal properties. Ayurvedic textbooks, for example, take note of the toxicity of heavy metals and recommend special physicochemical processes that, according to ancient Indian belief, 'detoxify' such toxic heavy metals (e.g. by heating them until they glow (Prpic-Majicet *et al.*, 1996).

These reports raise concerns about the safety of patients using herbal medicine. In Korea, herbal medicine has been used as a main traditional therapy for many centuries. So far, there has been no report of health problems related to heavy metals contained in herbal medicine in Korea, but few systematic studies have been performed worldwide. In recent, Koh and Woo reported the detection of toxic heavy metals that exceeded Singapore's legal limits in 42 Chinese proprietary herbs (Koh *et al.*, 2000). They collected 2,080 samples of herbal medicine in Singapore and tested them for heavy metal contents. Forty-two different herbs were found to contain metals in amounts exceeding the legal limits. Mercury was found in 28 products, lead in eight, arsenic in six, and copper in one. Considering the fact that 80% of herbs used in Korea depend on herbal products imported from China, potentially important safety issues from Koh and Woo's report should not be neglected in Korea.

Numerous case reports and case series of heavy metal poisoning associated with the use of herbal medicine have been published. However, there have been few reports of biological markers of heavy metal doses in patients taking herbal medicine. Furthermore, it is possible that differences in method of administration, such as pill vs. decoction, could affect the dose of metals incurred by users. This study aimed to analyze and compare concentrations of heavy metals in urine and hair from patients taking herbal medicine through decoction and pill. In addition, the possibility that herbal medicine may act as a metal chelator was also entertained.

MATERIALS AND METHODS

Study population

For the present study, 308 patients (162 males and 146 females) affected with vitiligo and psoriasis were selected from one Oriental medical clinic located in Seoul, Korea. Treatments were rendered from September to December, 2000 and patients were asked to visit 2 to 4 times a month.

Patients were classified into three groups as decoction, pill, or control, depending on the type of herbal medicine taken. "Decoction" is defined as herbal medicine boiled in water to yield an herbal extract. A "pill" is a mixture of raw herbs taken without boiling. The control group consisted of patients taking neither decoction nor pill. The patients who were only taking decoction were classified as the decoction group. The patients who were taking both pill and decoction were classified as the pill group. The number of patients for each group was 101 for the control group, 184 for the decoction group, and 23 for the pill group (see Fig. 1). The duration for taking decoction ranged from 1 to 124 months (mean = 7.55 months). The duration for taking pills ranged from 1 to 60 months (mean = 1.27 months). Patients were asked to fill out a questionnaire relating to history of jobs, residences, alcohol intake, smoking, etc. A comparison of general characteristics between the subjects with complete data and those with missing data is summarized in Table 1.

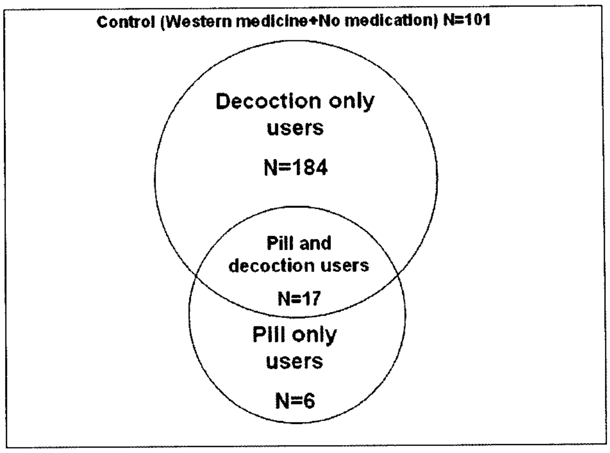

Fig. 1. Study subjects classified by drug use history (N = 308).

Manufacturing procedures for decoction and pill

Decoction. Based on the doctor's prescription, a set of herbs for 15 days worth of treatment is inserted into a bag made of polypropylene. To make an extract, herbs are cooked in an electric herb extractor for 3 hours at 100~120°C. The extraction is then sealed in a retort pouch (material pet, CLP) : 50 cc for children and 100 cc for adults for a single dose. Vacuum packed pouches are either picked up directly or send to the patients.

Pill. According to the prescription, dried herbs were gathered and powdered. The powder is then mixed with rice starch, wheat starch, and/or honey to make a mixture that is then formed by a machine into a pill weighing 1 to 10 g. Pills are normally taken three times a day before or after a meal but depending on the illness, dosage was adjusted to once a day up to five times a day.

Differences of decoction and pill. Decoctions and pills differ not only in shape and methods of intake, but

Table 1. Comparison of general characteristics between the subjects with complete data and those with missing data

Variable	No. of subjects (N = 308) 3	Mean(SD)or %	No. of subjects (N = 48)^	Mean(SD)or %	P-value
Age (years)	308	30.82(14.37)	48	25.83(13.35)	0.02
Sex					
Male	162	52.6%	21	43.8%	0.25
Female	146	47.4%	27	56.3%	
Marriage					
Yes	155	50.3%	23	47.9%	0.82
No	153	49.7%	25	52.1%	
Food preference					
Meat	29	9.4%	6	12.5%	0.41
Vegetable	39	12.7%	4	8.3%	
Mixed	224	72.7%	33	68.8%	
Others	16	5.2%	5	10.4%	
Alcohol					0.94
Yes	114	37.0%	18	37.5%	
No	194	63.0%	30	62.5%	
Smoke					
Yes	73	23.7%	6	12.5%	0.08
No	235	76.3%	42	87.5%	
Residential Area					
Big city	156	50.7%	21	43.7%	0.55
Rural	104	33.8%	15	31.3%	
Industrial City	20	6.5%	6	12.5%	
Etc	28	9.1%	6	12.5%	
Job					
White collar	45	14.6%	3	6.3%	0.077
Blue collar	104	33.8%	12	25.0%	
Unemployed	159	51.6%	33	68.8%	
Body mass index	308	17.5(9.16)	48	13.44(10.78)	0.006
Medication History					
Decoction only	184	59.8%	2	4.1%	
Pill only	6	1.9%	2	4.1%	
Decoction and pill	17	5.5%	13	27.1%	0.000
Control	101	32.8%	31	64.6%	

[a]subjects with complete data. ^ subjects with missing data.

also in components, action mechanism, contamination, and others. Decoctions only yield components of the herbs during the boiling process and most of the improper minerals salts are either eliminated or not eluted in the process. The metal contents of the herbs, proteins, saponin, flaconoid, coumarin, and others form compounds and these compounds are normally eliminated and precipitated during the extraction process. Pills are basically herbs in a different shape and contains every component of the herbs including heavy metals which are readily exposed in the body.

Sample collection

Urine. A total of 374 spot urine and hair samples were collected. Immediately after the collection, samples were stored in a salt-ice mixture and kept at -20°C in the laboratory. Prior to analysis, the urine samples were centrifuged at 3000 rev./min for 10 min and filtered through a 0.45 μm membrane filter (Chromatodisc, 25A) to remove the suspended particulate. The filtered urine samples were diluted properly with water before analysis.

Hair. Regarding hair, the removal of exogenous contamination is mandatory prior to analysis. Samples were repeatedly washed under stirring with a mixture of ethyl ether andacetone (3 + 1, v/v) (Merck, Germany). After drying, 5% EDTA solution (Merck, Germany) was added and stirred for 1 h. Samples were then added with 2 ml of HNO_3 and digested overnight. One milliliter of H_2O_2 was then added. The MW digestion settings were as follows: (i) 3 min at 250 W followed by 6 min at 0 W; (ii) 5 min at 250 W and 5 min at 0 W; (iii) 5 min at 450 W; and (iv) 5 min at 500 W. The digestion solutions were diluted up to 20 ml and stored at 2°C.

Sample pretreatment and metal analysis

Sample pretreatment and analytical methods followed US EPA procedures Goulle's and Mortada's study (US

EPA, 1981; Mortada *et al.*, 2002; Goulle *et al.*, 2005).

Cd, Cu, Pb. Samples were treated using 65 ml of cleaning solution (186 ml of ethyl alcohol, 93 ml of acetone and 371 ml of n-hexane to 1 l volumetric flask) for 12 hours. Each aliquot (0.1~0.2 g) from well homogenized samples was moved to a digestion vessel. The aliquots were mixed with 10 ml of 1 : 1 HNO_3, and then covered with a watch glass. Treated samples were heated to 95 ± 5 and refluxed for 10 to 15 minutes without boiling. After adding 5 ml of concentrated HNO_3, the samples were allowed to be cool and were refluxed for 30 minutes. This step was repeated until no brown fume was given off by the sample, showing the complete reaction with HNO_3. Using a ribbed watch glass, the solution was evaporated to approximately 5 ml at 95 ± 5°C for two hours. The samples were cooled again and 3 ml of 30% H_2O_2 was added. In order for the peroxide reaction the vessels were covered with a watch glass and placed on the heat source for warming until effervescence subsides. Then peroxide solution was added in 1 ml aliquots. The aliquots were warmed until the effervescence was minimal. The aliquots were covered with a ribbed watch glass. The acid-peroxide digestate of the aliquot was heated until the volume was reduced to approximately 5 ml. The solution was covered over the bottom of the vessel at all times. After cooling, it was diluted to 50 ml with water. The particulates in digestates were then removed by filtration. The filtered samples were analyzed by ICP-MS. The ICP-MS analysis for seven elements (Cu, Cd, and Pb) was carried out using ICP-MS spectrometer Varian Ultramass 700 (USA, 1998) with cross flow nebulizer. The operating conditions are given in Table 2.

Pb. Samples were treated by using 65 ml of cleaning solution (186 ml of ethyl alcohol, 93 ml of acetone and 371 ml of n-hexane to 1 l volumetric flask) for 12 hours. Each aliquot (0.1 0.2 g) from well homogenized samples was placed in the bottom of a BOD bottle. The aliquots (0, 1, 3, 5 ml) of mercury working standard containing 0~5 ug/l of mercury were transfer to a series of BOD bottles. The reagent water (5 ml) and concentrated sulfuric acid (5 ml) were added to the aliquots of standard and sample. Also 2.5 ml of concentrated Nitric acid was added to them and then they were heated for two minutes at 95 ± 3°C. After cooling, samples and standard were added by 15 ml of 5% potassium permanganate solution and mixed. They were heated for 30 minutes at 95 ± 3°C. After cooling, they were mixed with sodium chloride-hydroxylamine hydrochloride to reduce the excess permanganate. The standard and sample were diluted to 100 ml with de-ionized water. The particulates in digestates were removed by filtration. The filtered samples and standards were analyze by mercury analyzer (USA, Cetac, M-6000A).

Table 2. ICP-MS operating conditions

Nebulizer	Babington
Spray chamber	Scott, 2°C
rf power	1300 W
Sampling depth	6.4 mm
Plasma gas flow rate	15.0 l min^{-1}
Auxillary gas flow rate	1.0 l min^{-1}
Nebulizer gas flow rate	1.05 l min^{-1}
Sampler	0.5 mm, Ni
Skimmer	0.5 mm, Ni
Masses measured	^{65}Cu, ^{111}Cd, ^{207}Pb
Integration time	0.3 s/channel (three channels per mass)
Repetitions	5

Reagents

All reagents were of analytical reagent grade. High purity de-ionized water (Milli-Q system, Millipore, USA) was used throughout. Analytical reagent nitric acid (Merck, 70%) was used after additional purification by sub-boiling distillation in quartz still. Plastic bottles and glassware were cleaned by soaking in 20% (v/v) HNO_3 for 24 h. This material was then rinsed three times with de-ionized water.

Statistics

Statistical analyses were performed separately by treatment status including no treatment, and treatment groups were further stratified by treatment method: pill only, decoction only, and combination of pill and decoction. We calculated geometric means for Cd, Cu, Hg, and Pb in urine and hair samples of the test subjects. We performed ANOVA to compare the geometric means between the experimental groups and the control group. We also calculated percents exceeding the upper limit of the reference value ranges for each metal. We adjusted for potential confounders such as age, sex, smoking, drinking, job, and residence in the ANOVA.

Multiple regression values (β(SE))for duration of decoction and pill use were calculated after adjusting for potential confounders. Dependent variables were the natural logarithm of each metal level plus one, for example, log (Al + 1). Odds Ratios were taken for exceeding reference values in urine and hair, adjusting for potential confounders. Odds ratios of the control and experiment groups were compared after adjusting for potential confounders.

For statistical analysis, Stata (2001) statistical package was used for mean ± standard deviation (SD), geometric mean, ANOVA, multiple regression, and odds ratios.

Table 3. Geometric mean and SD of heavy metals in urine and hair in Korean herbal users (N = 308)

Variable	Mean (SD)	% exceeding upper of limit reference range	Reference* Value (WHO)
Urine (ug/l)			
Cd	0.87 (0.57)	0.97	1~5.00
Cu	3.88 (1.49)	3.57	30~60.00
Hg	0.49 (0.50)	0.32	5~20.00
Pb	1.80 (0.94)	6.17	10~20.00
Hair (ug/l)			
Cd	0.39 (0.41)	11.69	0.25~1.00
Cu	4.62 (0.79)	26.95	15~25.00
Hg	0.75 (0.74)	20.78	0.5~2.00
Pb	0.97 (0.74)	12.66	2~20.00

*WHO (1996), Trace elements in Human Nutrition and Health. World Health Organization, Genova. 258-9.

RESULTS

Table 3 shows geometric mean and SD of heavy metal concentrations in urine and hair. Also WHO reference values (trace elements in Human Nutrition and Health) for heavy metals analyzed are listed and compared to those of samples. In urine, there were 0.87 ± 0.57, 3.88 ± 1.49, 0.49 ± 0.50, 1.80 ± 0.94 μg/l urine for Cd, Cu, Hg, and Pb, respectively. Compared to the range of WHO reference value for each metal, there was no sample for Cd and Hg exceeding the upper limit. However, 3.57% and 6.17% of total samples for Cu, and Pb exceeded the upper limit for WHO reference value. In hair, there were 0.39 ± 0.41, 4.62 ± 0.79, 0.75 ± 0.74, 0.97 ± 0.74 μg/g hair for Cd, Cu, Hg and Pb, respectively. In addition, 11.69%, 26.95%, 20.79% and 12.66% of the total samples showed concentrations exceeding the upper limit of WHO reference value for Cd, Cu, Hg, and Pb, respectively.

Table 4. Geometric mean and SD of heavy metal concentrations in urine and hair in Korean herbal users adjusted for potential confounders (N = 308)

Variables	Decoction N = 184 Mean (SD)	Pill N = 23 Mean (SD)	Control N = 101 Mean (SD)
Urine (ug/l)			
Cd	0.52 (0.43)	0.44 (0.45)	0.51 (0.41)
Cu	4.62 (0.63)*	0.48 (0.74)	2.92 (0.53)
Hg	0.56 (0.41)	0.65 (0.43)*	0.54 (0.40)
Pb	1.60 (0.52)*	1.58 (0.58)*	0.87 (0.47)
Hair (ug/g)			
Cd	0.40 (0.38)	0.39 (0.39)	0.40 (0.38)
Cu	3.06 (0.51)	2.53 (0.56)	3.53 (0.46)
Hg	1.20 (0.50)	1.30 (0.73)	1.35 (0.45)
Pb	1.21 (0.49)	2.23 (0.53)*	1.36 (0.45)

[a]Adjusted for age, sex, smoking, drinking, blue collar job, residence in big city.
*$p < 0.05$.

Table 4 shows geometric means and SD of metal concentrations after adjusting potentially confounding factors such as age, sex, smoking, drinking, job, and residence. In urine, the level of Cd was not significantly different among all three groups. In the case of Cu, the decoction group showed 1.6 times higher level than that in the control group (a significant difference at $p < 0.05$). In contrast, the pill group was 0.48 ± 0.74 μg/l compared to the level of control group, 2.92 ± 0.53 μg/l, showing about 6 times higher level. For Hg, each level was 0.56 ± 0.41 μg/l, 0.65 ± 0.43 μg/l, 0.54 ± 0.40 μg/l, respectively for the decoction, the pill, and the control group. The Hg level of pill group showed a significant difference ($p < 0.05$) compared to other groups. The level of Pb in the decoction and pill group were 1.60 ± 0.52 μg/l, 1.58 ± 0.58 μg/l, respectively and significantly higher than the level of control group, 0.87 ± 0.47 μg/l

Table 5. Regression coefficients (SE) for duration of decoction and pill use, respectively, from multiple regression of heavy metals in urine and hair in Korean herbal users (N = 308[#])

Dependent Variable^	Duration of Decoction Use (month) Beta (SE)	Duration of Pill Use (month) Beta (SE)
Urine (ug/l)		
Cd	0.0 (0.002)	-0.006 (0.032)*
Cu	0.008 (0.05)	-0.012 (0.011)
Hg	-0.001 (0.001)	0.016 (0.002)*
Pb	0.013 (0.003)*	0.011 (0.007)
Hair (ug/l)		
Cd	0.0 (0.0)	0.0 (0.0)
Cu	0.0 (0.003)	0.002 (0.006)
Hg	-0.002 (0.003)	-0.001 (0.006)
Pb	-0.002 (0.003)	-0.002 (0.006)

*$p < 0.05$.
^Dependent variables are natural logarithm of each metal level plus one. For example, log (Al + 1).
[#]Adjusted for age, sex, smoking, drinking, blue collar job, residence in a big city.

($p < 0.05$). In hair, the levels of Cd, Cu, and Hg in both the decoction and pill groups showed no statistic significance compared to level in the control group. However, the level of Hg in the pill group was 0.87 ± 0.47 μg/l and significantly ($p < 0.05$) higher than those in the decoction and pill group, 1.21 ± 0.49 μg/l and 1.36 ± 0.47 μg/l, respectively.

Table 5 presents regression coefficients and standard error (SE) for duration of decoction and pill use from multiple regressions of metals in urine and hair. The regression coefficients indicate the levels of metals increased or decreased in urine and hair after decoction and pill use for one month, compared to those of the control group. Regression coefficient (SE) for duration of decoction was -0.001~0.0013 in urine and -0.002~0.0 in hair, also pill use was -0.012-0.016 in urine and -0.002-0.002 in hair. In urine, the regression coefficient of Cd was 0.00 ± 0.002 μg/l for decoction use which is not statistically significant from the control group. However, in the case of pill use, the value was 0.006 ± 0.032 μg/l and significant ($p < 0.05$) from that of the control group. This can be expressed that the Cd level in urine is expected to be increased by 0.006 ± 0.032 μg/l after pill use for one month. The regression coefficients for Cu in both the duration of decoction and pill use showed no significant difference ($p > 0.05$) from that of the control group. For Hg, the regression coefficients of the duration of decoction use and pill use were 0.001 ± 0.001 μg/l and 0.016 ± 0.002 μg/l for the duration of pill use, respectively. The regression coefficient of pill use is significantly different from that of the control, indicating about 0.016 ± 0.002 μg/l in urine increased by pill use for one month. The regression coefficient was 0.013 ± 0.003 μg/l between Pb and duration in decoction use and was 0.011 ± 0.007 μg/l between Pb and duration in decoction use. The regression coefficient of decoction use is significantly different ($p < 0.05$) from that of the control group, indicating about 0.016 ± 0.002 μg/l increased by pill use for one month. In hair, the regression coefficients for both duration of decoction use and pill in all kinds of metals were not significant for that of the control group.

Table 6 presents odds ratio and standard error from case-control study, using the samples which exceed the upper limit of WHO reference values. In urine, only the pill group showed a significant odds ratio higher than that of the control group. Its odds ratio was 5.94 ± 6.85 indicating that pill use causes about 6 times higher possibility of the Hg level compared to that of the control group in urine. However, other values were insignificant as odds ratios of decoction were 0.90~2.31 in urine and 0.49~0.76 in hair, pill use was also 1.52~5.94 in urine and 0.41~1.13 in hair among those exceeded WHO reference values.

Table 6. Odds ratios and SE among those have exceeded WHO reference values in urine and hair in Korean herbal users adjusted for potential confounders^

Variable^	Decoction OR (SE)	Pill OR (SE)
Urine (ug/l)		
Cd	1.15 (1.47)	NC^^
Cu	0.90 (0.60)	1.52 (1.82)
Hg	NC	NC
Pb	2.31 (2.44)	5.94 (6.85)*
Hair (ug/g)		
Cd	0.53 (0.20)	0.53 (0.43)
Cu	0.51 (0.14)*	0.41 (0.23)
Hg	0.76 (0.24)	1.02 (0.66)
Pb	0.49 (0.18)*	1.13 (0.72)

Note: odds ratios and SE in Table 5 was calculated using only samples exceeding upper of WHO limit reference values, respectively.
^Adjusted for age, sex, smoking, drinking, blue collar job, residence in big city.
^^ NC (Not calculated), there were no calculated odds ratios due to exceed WHO reference values in 3/308 Cd samples and 1/308 Hg samples only.
*$p < 0.05$.

Table 7. The ratios of odds ratios for the metal concentrations in urine and hair

Metals	Odds ratio of Urine/Odds ratio of Pill	
	Decoction	Pill
Cd	2.17	NC
Cu	1.76	3.71
Hg	NC	NC
Pb	4.71	5.4

^^NC (Not calculated), there were no calculated odds ratios for Hg and Cd in Table 7.

The ratios of odds ratios for the metal concentrations in urine and hair are presented in Table 7. These ratios were based on the comparisons of mean odds ratio for metal concentration in urine and hair. The ratios were calculated by dividing the odds ratio of urine by the odds ratio of hair for each metal. In a case of decoction, the ratios were 2.17 for Cd, 1.76 for Cu, 4.71 for Pb. The ratios for pill were 3.71 for Cu and 5.4 for Pb.

DISCUSSION

Evidence from various countries suggest that toxic heavy metals in Asian herbal medicine may constitute a serious health problem (Ko, 1999; Ernst, 2000; Melchart *et al.*, 2001). However, the majority of the data is anecdotal and insufficient to define prevalent figures. Our study was carried out using the samples from people taking herbal medicine for a long-term, ranging from 1 to 124 months. This fact would reflect a relative figure of risk by heavy metals in herbal medicine.

In this study, metal concentrations in urine and hair of study subjects showed higher than WHO reference concentrations, especially in urine (0.32 6.17%) and in hair (11.69 26.95%). The percentage indicates more samples exceeding WHO reference values were observed in hair than in urine. It is not easy to estimate the potential risk of these results obtained from the comparison with WHO reference value, since the reference values were obtained in different fashion. Thus in other to retrieve toxicological information of metal in herbal medications, our analytical approaches for this study has been diversely carried out.

After adjusting confounding factors, all levels of the decoction and pill groups for metals were compared to those of the control group not taking any herbal medications. In urine, the level of Cu from the decoction group was significantly higher than that of the control group. However, their levels showed no significance in hair. In the case of pill group, only Pb levels in both urine and hair were significantly increased. It seems decoction stimulates the metals to be accumulated in other tissues rather than in hair. However, the aspect of metal accumulation would be dependent greatly on the method of herbal intake. Special attention in this study was identified on the level of Cu from the pill group in urine. All of metal levels showed a significant increase or no difference compared to those of the control group except the level Cu. The level Cu in urine from pill group was significantly decreased by about five times compared to that of control. It is assumed that some of components in herbal medicine show chelating effects on Cu. This assumption can be supported, in part, by an analysis of regression coefficients for duration and pill use from multiple regressions. In this analysis, herbs taken by decoction for one month showed increased the levels for Cu and Pb, but decreased the level of Hg in urine. In addition, it was identified that the levels of Cd and Cu in urine and Hg and Pb in hair were decreased by the pill use for one month even if the levels of other metals were increased compared to the level of the control group. Thus it is believed that herbal medicine caused not only the accumulation of metals but the possible decrease of metals in human body. The decreased levels of metals would be due to the role of herbal components for chelating metals. The assumption for the role of herbal medicine as a chelator may be supported by the comparison of odds ratio from case-control study, using the samples those exceed the upper limit of WHO reference values. The level of Pb in the pill group showed a significantly high odds ratio, about 5 times compared to that of the control group. However, there is a trend of low odds ratios by decoction and pill use. In addition, the odds ratio for Cu and Pb were significantly lower than 1, indicating the possible role of herbs decreasing metal levels in hair. Thus there is possibility that herbal medicine acts as a chelator which decrease the level of body burden for heavy metals. This suggestion can be also supported by the ratios of odds ratios for the metal concentrations in urine and hair. In a case of decoction, the ratios of each metal for odds ratios of urine/odds ratios of hair were over 1, indicating higher levels of metals excreted in urine than those accumulated in body. Furthermore, the same trend was identified in a case of pill. It is assumed that some compounds of herbal medicine bind or chelate metals, resulting in accelerating the excretion of metals outside the body. In general, heavy metals to be excreted should be captured or bound to chelating agents. There are a number of natural (non-synthetic) chelators such as herbs, amino acids, and other nutritional supplements (Georgiou, 2005). One of many herbal component, flavonoid and derivatives, dislodges heavy metal molecules from cell receptors and captures them so that they may be excreted from the body (Lim *et al.*, 2001; Pierre *et al.*, 2003).

In summary, the levels of metals exceeding WHO reference values were observed in hair and urine samples. In addition, some of metals showed the possibility of accumulation in body and the high odds ratio after adjusting confounding factors. However, some of metals in samples from people taking herbal medicine showed the decreased leveland the low odds ratio compared to those of the control group. This may explain the possible role of herbal medicine as a chelator for heavy metals in body. Nonetheless, despite small number of experiment samples, the results obtained in the study suggest that heavy metal contamination in the herbs don't necessarily cause metal poisoning because of the possible role of herbal medicine as a chelator. Further study must be followed to obtain more concrete evidence.

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