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Original Article

Gelatin Potentiates Lead Toxicity Due to Improper Preparation of a Chinese Tea Drug, Choreito. A Study Based on our Previously Published Case Report of Long-Term Choreito Use.

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Abstract

A woman who had used a Chinese tea drug, choreito, for treatment of chronic renal diseases over years, experienced lead poisoning with blood lead concentration over 600 μg/l on admission to the hospital. We found that one of the ingredients in choreito, kasseki, was commonly contaminated by lead (30-50 μg/g of kasseki), but this level of lead contamination in the drug had never caused poisoning previously. Our experiment indicates that another ingredient, gelatin, has lead-extracting ability and an adhesive quality on the walls of teapots. Thus, the possible causes of the toxicity seemed to be: (1) the lead in the kasseki, which was extracted by gelatin that had adhered to the wall of the pot, accumulated in large quantities for a long period of time (the patient used the same pot for more than a year without washing); and (2) a large quantity of the accumulated lead was released into the decocted drug day by day and induced the intoxication. In all, 37.2 mg of lead was extracted by 10 extractions of 4% acetic acid from the patient's pot. Repeated extraction (four times) of lead from the pot which was made by the same manufacturer in the same lot of the patient's pot with acetic acid, only totally 18.5 μg of lead was detected.

Also, it is evident that the intoxication was due to an improper method of decoction, that is, the patient did not prepare the tea according to Japanese pharmsacopoedia. The patient decocted all of the ingredients at the same time.

Key words: lead poisoning; Chinese drug; gelatin; kasseki

1. Introduction

We tried to clarify in this report the mechanism of lead intoxication induced by a Chinese herbal tea, choreito. The case report of lead intoxication (blood lead level; 600 μg/l) induced by choreito was published previously, but we did not describe the induction mechanism of the intoxication [1]. There have been many reports of lead poisoning occurring in domestic environments, for example, lead poisoning due to use of porcelain tableware [2-11] or to Chinese herbal tea [12-21]. In most of these reported cases, the background and the detailed inducing mechanism of the poisoning were not thoroughly clarified, especially in cases of the poisoning caused by Chinese herbal tea. Most of the reports commented only on the lead content in the tea drug as a whole, or that in the prescribed main substances used for the tea. Furthermore, all of the lead intoxication reports were of chancy high levels of lead exposure as shown in Table I. [2, 7, 10-12, 15-19, 21], and there are no discrepancies between the lead level in the drug material and the lead intake. However, in our reported case, the lead level in the drug (microgram order) was exceedingly low and quite different from that in the decocted herbal tea (milligram order). Thus, we had to explain the lead level discrepancy in the drug material and in the extracted drug fluid for our patient and to determine what kind of situation in the decocted drug caused the high blood lead level of the patient. We considered that there was the possibility that the toxicity was not induced by a chance of high lead exposure level and the possible cause of intoxication was due to (1) a large quantity of lead accumulation in the drug decoction teapot; (2) release of the accumulated lead into the decocted extract; and (3) high lead level of the patient's blood after ingestion of a large quantity of lead for a long period of time. The lead content in kasseki and

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the gelatin's lead extracting ability and adhesive quality to the teapot wall were determined to have been the causes of lead poisoning in this patient, because the patient used the pot for more than a year without washing.

2. Materials and Methods

2.1. Drug used by the patient

The ingredients of choreito are (1) kasseki, which is composed mainly of $Al_2SiO_5(OH)_4$ H_2O, Al_2O_3 $2SiO_2$ $2H_2O$, (2) gelatin, (3) alismatis rhizoma, (4) polypolus and (5) hoelen. The main ingredients were identified from the prescribed choreito and applied for the lead analysis. The prescribed drug consisted of 2 packages, one (package no. 1) contained kasseki, and the other (package no. 2) contained gelatin, polypolus, alismatis rhizoma and hoelen. Gelatin was easily separeted from the other ingredients in package no. 2, but the other substances could not be separated. Thus, lead content in kasseki (package no.1), in (1) the gelatin and in (2) the other ingredients as a whole (package no.2) were analysed. We could only perform duplicate analyses of these samples because only a small volume of the sample was available.

2.2. Standard ingredients in the drug

Uchida Wakanyaku Co. Ltd. Tokyo, Japan kindly presented us one sample each of choreito ingredients in separate packages. The lead content was also analyzed in each of the ingredients. We also obtained data on the lead content of different lots of kasseki measured by the Japan Food Analysis Center using the graphite furnace atomic absorption spectrophotometry method.

2.3. Lead determination in the individual ingredients

The method of lead content analysis in the sample was as follows. One hundred milligram of one of the choreito ingredients was put in a teflon crucible (Uniseal Vessels, Haifa, Israel) of 10 ml volume. In the case of the drug prescribed for our patient, 100 mg of either (1) gelatin, (2) other ingredients or (3) kasseki was put into the crucible. Then 4 ml of 60% HNO_3 was poured into the crucible, and introduced it into a stainless steal receptacle. The crucible-loaded receptacle was heated at 140℃ for 70 min. An aliquot of the sample digested in the crucible was injected into a graphite furnace atomic absorption spectrophotometer (GFAAS, Shimadzu AA-6500 or AA-6800). All of the samples were assayed by the method of standard addition; 0-25-50 ppb of the standard lead solution was added to the sample.

2.4. Method of assaying lead content in the medium,which was an extract of 4 to 5 choreito ingredient combinations

To examine the lead content difference due to the differences of extraction, each ingredient was combined as shown in Table 3, and lead concentration in each combined material was analysed. One g of each ingredient was weighed and put in a 50 ml volume polyester tube, to which was added 30 ml of water, and the mixture was heated in a thermostated water bath at 75℃ for 60 min. When the contents of the tube were returned to room temperature, the tube was centrifuged at 2, 000 × g. The aliquot of supernatant (5ml) was put in a teflon crucible, 60% HNO_3 was added to the sample, then the same procedure as cited above was performed.

2.5. Testing the lead extracting ability of gelatin and investigating the mechanism of lead extraction and holding ability

Lead extracting and holding ability of gelatin were studied in detail. One g of gelatin was dissolved in 30 ml of water and heated to 75℃ for 60 min, and 5 ml of the aliquot was passed through a G-25 column (1.2 x 12.5 cm) to secure gelatin free from metal contamination. Gelatin contained no detectable lead by GFAA method, though, before the treatment of G-25 column. Five ml of the eluate containing gelatin was added to 10ml of one of the buffers shown in Table 4. The mixture was incubated with 50 μg/ml of lead at 75℃ for 60 min, then 2.5 ml of the gelatin solution was passed through a PD-10 column (Pharmacia Biotech, Wikstroems, Sweden), 3.5 ml of the buffer used for the incubation was added to the column. The initial 2.5 ml of the eluate was discarded and the lead content in this aliquot could never be detectable, thereafter 3.5 ml was eluted from the column with high molecular weight substances, and this was taken for lead and protein assay. The eluting procedure was performed according to the instructions included in the PD-10 column. The assay method of lead content in the eluate was the same as that described above. Protein was assayed by Lowry's method[24].

2.6. Experiments relevant to the pot

We decided to assay by the method recommended by ISO[23] the contents of lead adhered to the wall of the patient's pot with 4% acetic acid as well as two control pots that were never used for decoction of the drug. One pot purchased from the same manufacturer as that of the patient's pot and the other from a different manufacturer.

Table 1 Reports which indicate relationships between the lead intake and blood lead level.

	Pb concentration in the material	estimated Pb intake (mg/day)	total days of Pb intake	patient's age male/female	estimated bllod Pb level (μg/100ml)	paper that cited the data and reference number
lead from pottery	0.6mg/100ml in the drink	3	240	55 (M)	58	Harris and Elsea, 1967, JAMA 202:544 (2)
	1.27mg/L	1.27	280	33 (F)	65	Lob and Berode, 1977, Schw. Med. Wschr. 107:1667 (7)
	50 ~ 600 μg/L	0.8-1.0	216	35 (M)	50	Jouglard et al. 1996, Presse. Med. 25:243 (10)
	120mg/L	120	90	24 (F)	60	Autenrieth et al. 1998 Deut. Med. Wschr. 123:353 (11)
herbal tea drug etc.	0.5mg/pill (30pills/day)	15	120	59 (F)	90	Lightdoote et al. 1977. JAMA, 238:1539 (12)
	26.4mg/g	140	30	33 (M)	70	Mitchell and Heggs, 1990, Hum. Exp. Toxicol. 9:195 (15)
	49.4% (W/W)	7.35-14.7	30	36 (F)	80	Smitherman and Harber, 1991, Am. J. Ind. Med., 20:795 (16)
	79.3mg/tablet 1	429	42	37 (M)	94.3	Dunbabin et al. 1992, Med. J. Aust. 157:835 (17)
	55.9mg/tablet 2					
	301mg/L of tea	141.4	35	45 (M)	71-76	Markonitz et al. 1994, JAMA, 271:932 (18)
	16.7% (w/w)	167	56	48 (M)	74	Brown and Ede, 1995, Brit. J. Hosp. Med. 53:469 (19)
	173mg/kg	43	180	58 (F)	123.3	Phan et al. 1998, Med. J. Aust. 169:644 (21)
				63 (M)	93	

2.7. Testing the adhering ability of lead from the choreito to the inner surface of the pot

Adhering ability of lead from the choreito to the inner surface of two control pots were examined. Three g of each choreito ingredient was decocted for lead extraction into 600 ml of water in the pot once a day, and we repeated the experiment 50 times. After every decoction, an aliquot of the extract was taken for lead assay and the pots were washed with water and dried, but not cleansed thoroughly. Five ml of the extract solution added to 60% HNO_3 was treated at 140 ℃ for 70 min in a teflon crucible, as described above, and the lead content was assayed by GFAAS as cited above. When all the decoction experiments were completed, the lead adhering to the wall of pot was extracted by 4% acetic acid, as described above, and the total adhered lead was measured for the 2 pots.

2.8. Statistics and other calculations

All data are presented as mean ± standard deviation. Statistical analysis was performed by two way of analysis of variance with Tukey's test [25] The significance levels were calculated by t- and F-tests [26] The computer used for data analysis was a FM New 7 by Fujitsu Co. Ltd. (Kawasaki, Japan).

3. Results and Discussion

3.1. Lead in the herbal tea drug

We analyzed the lead in the drug used by the patient (Table 2). In both of the samples from the patient and from the pharmaceutical company, the kasseki contained lead. The lead levels of different lots of kasseki as measured by the Japan Food Analysis Center using GFAAS were also described in the footnote. The obtained result in this paper together with the data from the Japan Food Analysis Center , show that kasseki is always contaminated by lead to a certain extent (μg order).

3.2. Lead levels in different combinations of ingredients and addition of gelatin to the solution

According to the results of the preliminary experiment, gelatin seemed to have extracting and holding action on lead. Thus, further experiments were performed. Lead content in the solution differs according to the combination of ingredients used and the procedure of addition of gelatin to the other materials (Table 3). Two ways of analysis of variance (ANOVA) was administered to the data in Table 3, as to the order of ingredient addition for making the drug (A, B and C) and the combination of the materials for making the drug (1, 2, 3 and 4), but the last two treatments (5. K+P+H+A and 6. P+H+A+G) were not included in the ANOVA analysis. Thus, ANOVA was administered for the data consisting of 12 different kinds of experiments and each experiment composed of five different measurements of lead in the sample. The result of ANOVA revealed that both of the factors, the order of ingredient addition and the combination of the materials for the preparation of the drug, were statistically significant factors by F-test [26]. The statistically significant differences were found (1) between the ingredient addition A and that of C, (2) between the ingredient addition B and that of C with Tukey's test [25]. Lead levels were high in Table 3-A, and. lead levels were low in Table 3-C. But when gelatin was added to the drug after the decoction of other ingredients (in Table 3-B), the situation was not different from Table 3-A, because the lead contaminated materials were not discarded by centrifugation or filtration. This suggests that gelatin has a holding action on lead in the solution, if the solution contains a trace of lead. After centrifugation, some of the lead in the solution enters the precipitate, and lead in the supernatant decreases and escapes from the holding action of the gelatin. The comparison of the combined experimental results which were performed with kasseki and gelatin ([A, B and C] x [1, 2, 3 and 4] in Table 3; the mean and standard deviation (x ±

Table 2 Lead content in the ingredients of choreito prescribed for the patient, in standard ingredients of choreito presented by a certain pharmaceutical company and in other kasseki samples from different manufactures.

Ingredients in the tea prescribed for the patient	Pb (μg/g)	Standard ingredient	Pb (μg/g) Mean ± SD	n
Kasseki	41.5 (duplicate)	kasseki	9.57 ± 4.65	5
Gelatin	0.46 (duplicate)	Gelatin	N.D.	5
Others	<1 (duplicate)	Polypolus	1.12 ± 0.36	5
		Alismatis rhizoma	0.14 ± 0.15	5
		Hoelen	0.03 ± 0.08	5

The prescribed drug consisted of 2 packages; one (package no. 1) contained kasseki and the other (package no. 2) contained gelatin, polypolus, alismatis rhizoma and hoelen. Only gelatin could be separated from the other ingredients in package no. 2. Thus, gelatin was taken from package no. 2, and the lead content was meansured in the gelatin and in the other ingredients as a whole for package no. 2. The following data: 32.0, 38.1, 57.7, 27.7 and 37.3 for the kasseki of the other lots were from the Japan Food Analysis Center.

Table 3 The lead content in different combination of ingredients with different treatments in the extraction process, suggesting the lead extracting action of gelatin.

No.	Combination of materials	Treatment		
		A	B	C
1	K+P+H+A+G	0.59 ± 0.11 (μg)	0.45 ± 0.12 (μg)	0.21 ± 0.05 (μg)
2	K+H+A+G	0.73 ± 0.07	0.59 ± 0.16	0.23 ± 0.10
3	K+P+H+G	0.47 ± 0.03	0.49 ± 0.08	0.28 ± 0.02
4	K+P+A+G	0.39 ± 0.07	0.39 ± 0.07	0.17 ± 0.04
5	K+P+H+A	0.19 ± 0.03	-	-
6	P+H+A+G	0.09 ± 0.09	-	-

The total extracted lead value is shown in the table. The combinations of ingredients were diffrtrnt. Abbreviations are: K, kasseki; P, polypolus; H, hoelen; A, alismatis rhizoma and G, gelatin. Treatment A, 1g of gelatin was added at the same time as the other ingredients (1g each of the material was used) to the incubation medium and it was decocted for 1h; Treatment B, gelatin was added to the solution after the decoction of other materials; Treatment C, gelatin was added to the supernatant solution after the decoction of other materials and centrifugation (mean ± SD; n=5). For statistical analysis concerning this table, see text

sd) was 0.41 ± 0.18, n=60) with the experiments performed without kasseki, or gelatin (the lower lines 5 and 6 in Table 3., x ± sd was 0.14 ± 0.077, n=10). There was a statistically significant difference between them by t-test ($p<0.001$). This also suggests that gelatin has an extracting and holding action on lead and the lead held by the gelatin comes from kasseki.

3.3. Experiments investigating the lead holding action of gelatin

The lead holding action was tested by PD-10 column treatment with different pH of incubating solutions. Table 4 shows the lead holding ability of gelatin in incubation solutions with different pHs. No statistically significant differences were found among the pHs so far tested. Thus, the lead holding ability of gelatin does not change according to the light acidic and neutral pHs tested. The incubation medium (15 ml) contained 50 μg/ml of lead, and the lead level detected after PD-10 column treatment was calculated in terms of a starting incubation buffer of 15 ml equivalent, ca. 30 μg/ml (60% lead), which was held by some component of the gelatin, which was a mixture of various substances. The amount of lead held by the gelatin was dependent on a gelatin concentration using 25mM acetate buffer, pH 5.0 (Fig. 1).

3.4. The teapot used by the patient left a record of lead contamination by the drug

Control teapots contained only trace amounts of lead and did not seem to be able to cause the intoxication, as shown in Table 5. The most important point is whether the lead extracted from the ingredients and the lead that came off the wall of the teapot during decoction can induce intoxication or not. First, we twice decocted all of the ingredients in the pot ordinarily used by the patient for more than a year, and the lead content in the extract was assayed each time after the decoction. We were surprised to find that the total lead contents assayed were 7.68 and 2.04 mg at the first and second decoctions, respectively. Thus, we performed extraction experiment by the ISO [23] using 4% acetic acid to determine whether or not a large amount of lead was extracted from the patient's teapot. The extraction of lead was repeated 10 times. The results show in Table 5 and indicated that a considerable amount of lead, 37.2 mg in total, was extracted from the patient's pot. This extraction experiment was also performed using 2 control tops. Four times of extraction were performed concerning the two pots, and the lead level attained zero level at 3 and 4 time. This result and the detection of high lead levels at the first and second detections as mentioned above indicated the accumulation of lead on the teapot wall, because of gelatin's lead extracting ability and its adhesive quality on the wall. Moreover, it is possible that the lead adhered to the wall of the teapot every day by decoction. These results suggested that the accumulated lead on the teapot wall falling off day by day could have induced intoxication.

Then, we calculated the generalizable tendency of lead absorption using the human lead exposure data reported by the authors cited in Table 1. In the first place, we picked up the data of the patients who drank the drug, or the beverage more than 100 days and the lead intake per day was less than 20 mg from Table 1, because the patient used the same pot for decoction without thorough washing for about a year and the lead intake per day seemed to be less than 20 mg/day. Then,we calculated the equation using the data concerning the lead intake per day (x_1; mg/day) and how many days they took the lead (x_2; days). The derived equation is;

$y=4.32\ x_1+0.205\ x_2$ (1). The square value of the multiple correlation coefficientt is $R^2=0.9715$ and the critical level of statistical significance tested by F- test [26] (n= 4, the predictors, k = 2) was $p< 0.05$. Then,we estimated how many days and how much lead per day were needed to raise the blood lead level to 60 μg/100ml. The calculation was performed by inserting the blood lead level of the patient (y=60 μg/100ml) and possible days of dosing the lead polluted drug (x_2=120-280 days; because the data concerning total days of lead uptake more than 100 days are 120-280 days in Table 1.) and calculated x_1. The obtained x_1 for the patient is 0.6-8.2mg/day, as shown in Table 6. If such quantities of lead was ingested by the patient for 120-280 days and the extrapolation into the equation is allowable for the data of the patient, there is a possibility to reach the blood lead level to 60 μg/100ml. During the long days of using

Table 4 The lead holding ability of gelatin at different incubation pHs.

pH	Buffer used	Pb detected (μg/ml)	Gelatin detected in the medium as protein (mg/ml)
4.6	acetate	28.3 ± 3.99	3.59 ± 1.42
5.7	phosphate	32.2 ± 1.54	4.30 ± 0.73
6.8	phosphate	32.6 ± 2.53	3.64 ± 1.06

The incubation medium (15ml) contained 50 μg/ml of lead, and the detected lead level after PD-10 column treatment was calculated in terms of a starting incubation buffer of 15ml equivalent, ca. 30 μg/ml (60% lead), which was held by some component of gelatin. Protein in the medium is also shown in terms of a starting incubation buffer of 15ml equivalent (mg/ml)(mean ± SD; n=5). There was no statistically significant difference among these data.

Table 5 Lead (μg) adhered to the teapot wall with gelatin was washed out by ten rinses using 4% acetic acid. [n. d.]: not detectable; [−]: don't determine.

No.	Pot used by the patient	a pot from the same manufacturer as that used by the patient	a pot from another manufacturer
1	14261	9.5	7.0
2	6512	9.0	19.4
3	5696	n. d.	n. d.
4	2791	n. d.	n. d.
5	2184	-	-
6	1876	-	-
7	951	-	-
8	1186	-	-
9	1003	-	-
10	793	-	-

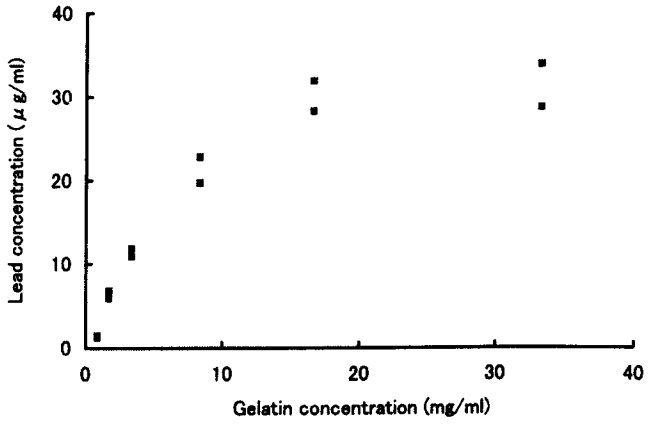

Figure 1 The amount of lead held by gelatin was dependent on the gelatin concentration using a 25mM acetate buffer of pH 5.0.

Table 6 How much lead was necessary over 120 to 280 days to attain a blood lead level of 60 μg/100ml for a patient of 33 years of age. The data were calcuated using equation (1).

the lead dose days	the necessary amount of lead per day (mg/day)
120	8.19
150	6.77
200	4.40
250	2.03
280	0.60

the pot, it is possible the high lead pollution in the drug. Table 5 suggests the possibility.

3.5. The experiment using the same kind of pot as the patient used

We decocted ordinary ingredients 50 times with control pots(Fig. 2). After 50 decoctions, the lead contents were 9.0 and 19.4 μg in the two control teapots, respectively. Observing in detail the lead level values of the two teapots in Fig. 2, the lead level gradually increased. We only repeated 50 decoctions, and the total lead content after the treatment of 4% acetic acid was in the microgram range, and we could not determine the time when the milligram order of lead might come off of the teapot wall. However, the experiment showed us the possibility of a higher level of lead in the extract after hundreds of decoctions.

3.6. The reason why the drug that the patient used was contaminated by lead

The patient decocted the drug with incorrect method. The doctor did not inform the patient as to the correct method of decoction in detail, or perhaps the doctor himself did not know the right process of decoction, since the doctor wrapped the kasseki in one envelope and the other ingredients in another one. The correct method for preparation of choreito is written in a guide book for preparation of Chinese drugs[22]: add 3 g each of kasseki, alismatis rhizoma, polypolus or hoelen to 500 ml of water, decoct them, turn off the heat when the volume is reduced to about one half, filter and discard the precipitate, add gelatin to the filtered solution, and heat for 5 min, until the gelatin is dissolved in the solution. Commercially available powdered choreito is prepared by drying the decocted solution according to the correct method of preparation in a frozen state. Our experiments revealed that lead levels were low when gelatin was added to the supernatant solution after the centrifugation of other decocted ingredients. After centrifugation, some of the lead in the solution enters the precipitate, and lead in the supernatant decreases and escapes from the holding by the gelatin. When this process is omitted, the lead contamination in the drug prepared by the decoction of all the ingredients at the same time seems to be greater than in the drug prepared by the correct decoction method. We must prepare the drug by the correct decoction method.

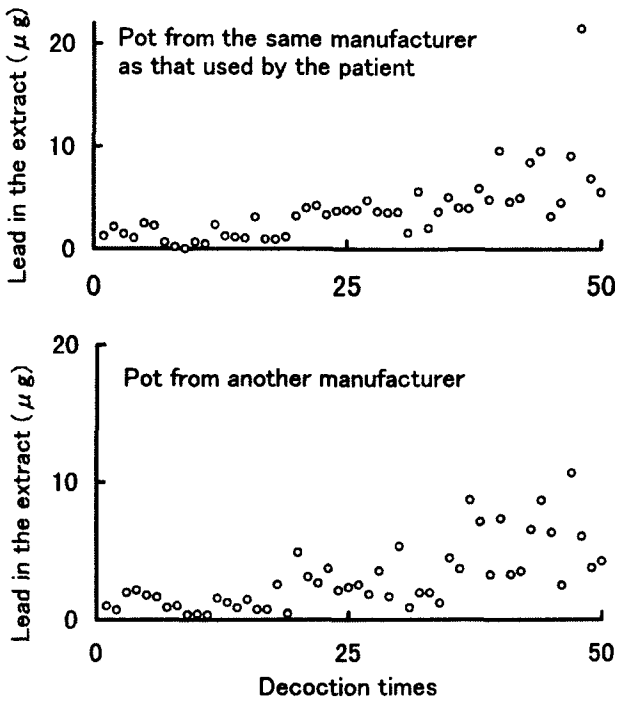

Figure 2 The lead levels in the extract after decoction when all of the ingredients were added at the same time.
The results were obtained using 2 different pots; a pot made by the same manufacturer as that of the patient's pot and a pot from another manufacturer. Fifty decoctions were administered and the lead level was measured after every decoction.

4. Conclusion

The patient had bought a new teapot at the end of February 1994, and then she used it for about a year. She had not cleansed the teapot thoroughly for a long time, so evidence of the contamination remained on the internal surface of the teapot. Kasseki is invariably contaminated by lead. Gelatin extracts the lead from the kasseki, causes accumulation of it to the wall of the pot and holds it. Moreover, the adhesion of lead to the wall of the teapot every day by decoction is possible. Thus, the combined materials adhered to the wall of the pot, the concentration of lead on the walls of the teapot gradually increased, and at the same time, the concentration of lead into the extract also gradually increased. The lead that had come off eventually must have induced the intoxication. Also, it is evident that the intoxication was due to an incorrect method of decoction, that is, decocting all of the ingredients at the same time.

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