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Metallothionein as a biomarker for mercury in tissues of rat fed orally with cinnabar

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Cinnabar, as one of the most widely used mineral drugs in traditional Chinese medicines, has been proven to have prominent curative effects in clinical use for more than 2000 years. But the safety and toxicity of the drug has been under constant debate in clinic usage. Metallothionein (MT) contains about 30% of cysteine in the molecule, and plays an important detoxification role against heavy metals. In this study, it was used as a biomarker to assess mercurial accumulation in rats fed orally with cinnabar. After feeding rats with cinnabar by gastric gavage at different dosages and at different times, the distribution of heavy metals (including mercury, copper and zinc) and MT was investigated among rat tissues, including liver, kidney, heart, brain, testis and blood. Metals and MT determinations were carried out using inductively coupled plasma mass spectrometry (ICP-MS) and a modified mercury saturation assay technique respectively. The results indicated that mercury was easily accumulated in the tissues of rats exposed to cinnabar, especially in kidney. For example: at a feeding dosage of 5 g kg^{-1} (bw) for 4 weeks, the mercury concentrations in kidney were 13, 8.7, 21.6 and 26 times those in liver, testis, brain and heart respectively; and at 2.5 g kg^{-1} (bw) for 2 weeks, the mercury concentrations in kidney were 21, 2.1, 3 and 21 times those in liver, testis, brain and heart respectively. In addition, mercury in kidney and liver of all cinnabar groups was significantly higher than that of the control group ($P < 0.01$). A high positive correlation observed between MT concentrations and mercury levels in both liver and kidney ($R^2 = 0.9299$, $P < 0.02$ for liver; $R^2 = 0.9923$, $P < 0.0008$ for kidney) indicated that MT could be used as a biomarker for mercury in tissues.

KEYWORDS: metallothionein (MT); cinnabar; mercury; accumulation; rat

INTRODUCTION

Cinnabar, a dark-red powdery drug composed mainly of sulfurated mercury (≥96% HgS), is an important traditional Chinese medicine (TCM) that has proven effects as a sedative and hypnotic, and has been widely used in clinical treatment of antiepilepsy, sore throat, antiarrhythmia and asthma.[1–5] It is abundantly produced in the Guizhou, Hunan, Sichuan, Guangxi and Yunnan provinces of China. Recipes containing cinnabar account for more than 12% of the total listings (i.e. more than 47 compounds containing cinnabar) in the Chinese Pharmacopoeia (1995).[1] Yet, the exact curative mechanism of the mineral drug remains unknown. The special curative effects seem to contradict the high mercury content in a dose of drug containing cinnabar because of the high toxicity of mercury. As a result, the allowable amount of cinnabar for patients regulated in the Pharmacopoeia of China (2000) is 0.1–0.5 g per dose. To investigate the mercuric solubility and

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digestion of cinnabar in the human body, an artificial body fluid including gastric and intestinal digestant was used in our previous study and the results indicated that about 3.39 μg of mercury per gram of cinnabar was soluble in the artificial fluids.[6]

It has been confirmed in literature reports that the toxic phenomena are notable when the amount of mercury accumulated in the human body approaches 100 mg.[7] Taking into account the high level of mercury incepted in human bodies with cinnabar-containing TCM, the detoxification mechanism of mercury in the human body is of great importance and needs further research. In order to investigate the distribution and metabolism of heavy metals in different tissues of rats fed orally with cinnabar, the concentrations of mercury, copper and zinc are measured simultaneously with inductively coupled plasma mass spectrometry (ICP-MS). Because of the affinity between mercury and proteins, the soluble mercury in tissues exists entirely in protein- or polypeptide-bound forms through the thiolate radical (–SH). Among these biomolecules, metallothionein (MT), a low-molecular-weight protein with abundant thiolate radicals, is the one most prone to binding with mercury. Furthermore, MT can be induced in tissues by heavy metals such as mercury, copper and zinc, etc., and this synthesized MT plays an important role in the detoxification of heavy metals such as mercury. In addition, MT also manipulates the homeostasis of essential metals like zinc and copper.[8,9]

The interrelationship between heavy-metal levels and MT concentrations in rat tissues leads to the utilization of MT as a biomarker to assess the mercurial toxicity of cinnabar. There are many reports on the inducement of MT in mammals upon exposure to mercury,[10–15] but little is known about the distribution and metabolic discharge of MT and heavy metals in animal tissues after oral intake of cinnabar. As a biomarker for the accumulation of heavy metals, MT is ideally suitable and is therefore helpful for the study of toxicological effects at a molecular level.

The purpose of this paper was to determine the total MT concentration and heavy metals distribution in rat tissues (including liver, kidney, heart, brain, testis and blood) after mercury exposure. The rats were exposed to different mercurial agents, including cinnabar and $HgCl_2$, by gastric gavage at different dosages and different times. The heavy metals, including mercury, zinc and copper, were measured in rat tissues by ICP-MS, and the MT contents were determined by a modified mercury saturation assay. The relationships between metals and MT content in tissues are discussed.

MATERIALS AND METHODS

Apparatus

The ICP-MS instrument used was an HP4500 (Agilent, USA). The sample introduction system used included a glass double-path spray chamber fitted with a concentric nebulizer. Nickel sampler and skimmer cones were used for all experiments. The microwave-assisted digestion oven used to digest all of the samples was an MK-III fitted with an optical-fiber pressure-controlling system (manufactured by the Institute of Xinco Microwave Technology, Shanghai, China).

Reagents

The official cinnabar (⩾98% HgS) used in this experiment was purchased from Jimei Hospital, Xiamen, China.

MT-2 standard (extracted from rabbit liver) was purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). The purity limit for the standard of MT-2 was not specially defined from batch to batch; the lot number of MT-2 used in this assay was 052K7002. The product contained 6.49% cadmium, 1.95% zinc and 0.17% copper.

The standard reference material of human hair (GBW07601) was purchased from the Institute of Physical and Chemical Survey, Ministry of Geology and Minerals, China. The stock standard solution containing 100 μg ml^{-1} of multi-elements (including mercury, zinc, copper and cadmium) in 2% nitric acid was stored at 4 °C in a plastic bottle. Working standard solutions for ICP-MS were prepared daily from the stock solution by diluting it with 2% nitric acid. Thallium, used as an internal standard, was diluted from a 1000 μg ml^{-1} stock solution (GSB G62070–90, National Analytical Center of Steel Material, China).

For MT determination, a 20 μg of mercury per milliliter mercuric working solution was prepared as followed. 1.353 g $HgCl_2$ (Peking Reagent Factory, China) was dissolved in 10% (w/v) trichloroacetic acid (TCA; China) in a 100 ml volumetric flask as stock solution. 100 μl of this stock solution was then transferred to a 50 ml volumetric flask and brought to the volume with 10% TCA. A 50% (v/v) chicken egg-white solution was prepared by mixing the same volume of chicken egg white and 0.9% (w/v) saline, prepared from aqueous NaCl.

The analytical reagent 65% HNO_3 (Peking Reagent Factory, China) was of high purity grade. All other chemicals used, including mercuric chloride (China), were A.R. grade. Milli-Q water (18.2 MΩcm^{-1}; Millipore) was used throughout.

Animal treatment

The 60 male Wistar rats, weighing between 180 and 240 g, used in this experiment (SC4K11-00-0006) were purchased from the Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College, China. Rats were maintained in air-conditioned metabolic boxes in standard conditions (18–24 °C) on a 12 h/12 h light/darkness cycle. They were randomly divided into six groups, with 10 rats in each group. One group was used as the control group and fed only with distilled water. A second group was fed with $HgCl_2$ for 2 weeks and is termed the $HgCl_2$ group. The remaining four groups were all fed cinnabar with different feeding dosages and feeding times and were termed cinnabar groups I, II, III or IV. The rats of each group were also fed daily with gastric gavage as described in Table 1.

Table 1. Grouping method of rats fed with cinnabar or $HgCl_2$ by gastric gavage

Group name	No. of rats	Substance fed	Dosage of intake (g kg^{-1} (bw))[a]	Volume of intake (ml/100 g (bw))[b]	Time (weeks)
Control group	10	Distilled water		1	4
$HgCl_2$ group	10	$HgCl_2$	0.010	1	2
Cinnabar group I	10	Cinnabar	2.5	1	2
Cinnabar group II	10	Cinnabar	5.0	1	2
Cinnabar group III	10	Cinnabar	2.5	1	4
Cinnabar group IV	10	Cinnabar	5.0	1	4

[a] Amount (g) of substance intake per kilogram of body weight each week.
[b] Intake volume (ml) per 100 g of body weight each day.

At the end of the continuous feeding period, whole blood samples were taken from the rats by extraction from the ventral aorta. This whole blood was then separated into plasma and erythrocytes fractions. Then rats were then sacrificed and the organs dissected into parts of liver, kidney, brain, heart and testis for experiments. The dissected tissues were washed with physiological saline solution, cleaned, dried on filter paper, weighed and stored at −80 °C.

Analytical procedures

Heavy-metal measurement

For metal quantification, the frozen samples were lyophilized directly for 24 h to obtain the dry weights. The dried tissues were ground in a mortar. About 0.3 g samples were weighed and digested with 5 ml of 65% HNO_3 in a microwave oven. After predigesting overnight, the sample was digested for 5 min and then for another 2 min with 1 ml H_2O_2 in a microwave-assisted oven. Then, the digest was diluted with 4% (v/v) HNO_3 to a final volume of 25 ml and stored at 4 °C for analysis by ICP-MS. Procedural blanks were run for background subtraction. 10 ng ml^{-1} Tl solution was used as an internal standard to correct for the signal fluctuation during long-term measurement.

Because of a significant 'memory effects' in ICP sample introduction systems, a long washing time with nitric acid (1–5%, v/v) was needed to eliminate residual mercury absorbed onto the spray chamber walls or retained as mercury vapor in the dead volumes of the spray chamber. In addition, samples with a high level of mercury should be diluted with 3% HNO_3 before ICP-MS analysis.

Quality assurance for metal analysis was achieved by the use of standard reference material, such as human hair (GBW07601).

MT measurement

The MT concentrations in rat tissues were measured by a modified mercurial saturation assay proposed by Klaverkamp *et al.*[16] Instead of adding ^{203}Hg as a tracer for measuring the MT-bound mercury, the amount of mercury bound to MT was accurately measured by ICP-MS.

For MT measurement, the frozen tissues were thawed and disaggregated. About 2 g of sample was then homogenized with 6 ml of 0.9% (w/v) ice-cold deoxygenated NaCl (pH 7.0), containing 250 mmol l^{-1} glucose, in an glass homogenizer. This procedure was conducted in an argon atmosphere. The homogenates were centrifuged at 6000 rpm for 30 min (4 °C). The supernatants were heat-treated at 85 °C for 10 min in 2.0 ml polypropylene microcentrifuge tubes to precipitate non-MT proteins. A needle was used to puncture a hole on the cover of the tube to prevent the cover from swelling. The heat-treated homogenates were cooled in iced water for 5 min and centrifuged for 20 min at 15 000 rpm (4 °C). The supernatants were stored at −40 °C for MT determination.

100 µl of the supernatant solution was transferred into 2.0 ml polypropylene microcentrifuge tubes (for liver and kidney of the $HgCl_2$ group, 50 µl volumes of supernatants were transferred), and the volume was made up to 400 µl with saline for each tube. 400 µl of $HgCl_2$ working solution ([Hg] = 20 µg ml^{-1}) was added to each of these tubes and mixed sporadically by floating the tubes in an ultrasonic groove. After 20 min of incubation, 400 µl of 50% (v/v) chicken egg-white solution was added to each assay tube and mixed again ultrasonically for 10 min to remove excess (non-MT-bound) mercury. After the mixture was heat-treated at 80 °C for 10 min and then cooled in iced water for 5 min, it was centrifuged at 15 000 rpm for 15 min at room temperature.

Blanks were obtained by adding 400 µl of 0.9% NaCl was to a vial with no tissue sample. Afterward, the same operating procedure as the sample assay was followed.

From each tube, a 400 µl volume of the respective supernatant was transferred into a 25 ml volumetric flask and diluted with 4% HNO_3 (v/v). The mercury concentration in each tube was determined by ICP-MS.

After comparing data of mercury binding, MT concentrations were calculated by using an MT-2 standard calibration curve constructed from standards with MT-2 concentrations ranging from 5 to 150 µg ml^{-1}.

Statistical analysis

All the results, including metal concentrations and MT concentrations, were reported as the mean plus/minus the

standard deviation ($\overline{X} \pm$ SD). One-way analysis of variance (ANOVA), with software from Microcal Origin, was used for the changes among the different groups with a preset probability level of $P < 0.05$ or $P < 0.01$. Linear regression was carried out to estimate the correlation between MT and metal concentrations.

RESULTS AND DISCUSSION

Heavy metals determined with ICP-MS

When measuring the concentration and distribution of heavy metals in each tissue of rat after oral intake of cinnabar, the digested sample solutions were determined in the same conditions as that of standard reference materials. The results were shown in Table 2.

The mercury distribution results in each rat tissue presented in Table 2 indicate that the mercury in kidney was substantially higher than that of other tissues for all the cinnabar groups and the control group. For example, for cinnabar group IV the nephritic mercury concentrations were respectively 13, 8.7, 21.6 and 26 times those of the liver, testis, brain and heart; and cinnabar group I the nephritic mercury concentrations were respectively 21, 2.1, 3 and 21 times those of the liver, testis, brain and heart. In addition, mercury in kidney and liver for all cinnabar groups was significantly higher than that of the control ($P < 0.01$). The mercury contents in rat kidney and liver increased with longer feeding time and the larger dosages of cinnabar, but no cinnabar-induced allergy was observed during the rat breeding experiment.

Another interesting result revealed from the data in Table 2 is that the copper and zinc levels increase in kidney, liver and testis compared with that in the control group, and they also increase with increasing levels of mercury contained in the tissues. For example, the amount of nephritic copper and zinc in the $HgCl_2$ group was respectively 3.05 and 1.41 times higher than that of the control group; and the levels of hepatic copper and zinc in the $HgCl_2$ group were respectively nearly 1.2 and 1.5 times higher than that of the control group.

The mercury contents in blood erythrocyte and plasma fractions were negligible for all cinnabar groups, but a high mercury content was found even in the two blood phases (about 0.7 $\mu g\ g^{-1}$ in both serum and plasma) in the $HgCl_2$ group.

It is well known that cinnabar is toxic due to its mercury content. However, the toxicity of this mineral drug is not prominent when it is consumed orally, because only a very small amount of mercury is absorbed in the digestive system. For instance, only about 5% of inorganic mercury was assimilated in the intestine, whereas most of the mercury was excreted out of body with dejecta as HgS.[7] The distribution of mercury in tissues correlated closely with the amount of blood flowing through them, but the mercury initially distributed in the tissues was then transferred out and finally accumulated in kidney several hours later.[17] The mercury bound with proteins in kidney was slowly excreted with a half-life of about 64 days. This is the reason why the mercury in kidney is much higher than that of other tissues, as shown in Table 2. The uric mercury, which existed as mercury bound with different molecules, was one of the most important routes to excrete mercury.[18]

The solubility product constant K_{sp} of HgS, the main component of cinnabar, is 10^{-52} in water.[19] Similarly, HgS is almost insoluble in gastrointestinal fluid, which has been verified in previous studies using artificial digesting fluids.[6] Given these pieces of information, why then does the cinnabar present a serious toxicity problem for the human body, especially at a large dosage? The main reason is that the dissociated mercury ions in cinnabar derive mostly from the concomitant $HgCl_2$ in the material. The dissociated mercury in cinnabar used in this experiment is 57.8 $\mu g\ g^{-1}$, which falls in the range commonly observed for ground commercial cinnabar.[2] The dissociated mercury from $HgCl_2$ is readily assimilated in each rat tissue (as seen in Table 2), and especially accumulates in kidney, which is in accord with previous reports.[20,21]

Contrary to the above, the mercury contents in other tissues, including brain, heart and serum, were not significantly different among the different cinnabar groups ($P < 0.05$). The mercury contents in the erythrocyte and plasma fractions were negligible for all cinnabar groups, but they were significantly higher in the $HgCl_2$ group and almost the same in the two blood phases (about 0.7 $\mu g\ g^{-1}$ in both erythrocyte and plasma fractions). The results might be interpreted as that the dissociated mercury assimilated by the rats was initially transferred into the blood, and then rapidly transferred to the other tissues of the body. Before distributing into the other tissues, the soluble mercury was partially bound with hemoglobin in the erythrocytes and the rest was bound with other proteins in the blood plasma.[22]

MT concentrations in rat tissues

In studying the concentration and distribution of MT among the different groups, the MT concentration in the supernatant of each tissue was determined under the same conditions as that of the calibration curve. The analytical results are shown in Table 3.

The MT contents in each organ of the different rat groups indicated that much more MT was synthesized in liver and kidney, followed in testis for every rat groups. In the liver and kidney of the cinnabar group IV and $HgCl_2$ group, large amounts of MT were induced in the two organs with high contents of mercury. In order to validate the accuracy of the measurement, recovery studies were carried out, using different concentrations of MT-2 standard solutions in the range of 10 to 200 $\mu g\ ml^{-1}$. The results, shown in Table 3, indicate an essentially quantitative recovery, with values ranging from 89.5 to 112%.

The high affinity between mercury and thiolate (–SH) is the main reason why mercury is a strong poison to human

Table 2. Heavy-metal contents ($\overline{X} \pm$ SD) in tissues of each rat group

		Control	Cinnabar I	Cinnabar II	Cinnabar III	Cinnabar IV	$HgCl_2$
Liver (μg g^{-1} wet weight)	Cu	6.84 ± 0.14	6.94 ± 0.07	7.02 ± 0.13	7.33 ± 0.07^a	7.49 ± 0.005^a	7.91 ± 0.05^a
	Zn	51.2 ± 0.8	50.1 ± 0.6	53.0 ± 0.5	55.6 ± 1.1^a	52.5 ± 0.2^b	75.7 ± 0.8^a
	Hg	$0.001\,31 \pm 0.000\,42$	0.0123 ± 0.0023^a	0.0340 ± 0.0157^a	0.0290 ± 0.0052^a	0.104 ± 0.016	10.4 ± 0.3^a
Kidney (μg g^{-1} wet weight)	Cu	8.49 ± 0.17	15.4 ± 3.8^b	12.4 ± 0.1	24.6 ± 0.2^a	23.8 ± 0.4	25.9 ± 0.2^a
	Zn	28.0 ± 0.2	28.2 ± 0.4	32.0 ± 0.3	32.1 ± 0.1^a	32.9 ± 2.7	39.6 ± 0.5^a
	Hg	0.100 ± 0.015	0.211 ± 0.003^a	0.334 ± 0.013^a	1.22 ± 0.11^a	1.30 ± 0.00	56.7 ± 0.5^a
Testis (μg g^{-1} wet weight)	Cu	3.15 ± 0.05	2.98 ± 0.05^b	3.03 ± 004^b	3.21 ± 0.01	3.05 ± 0.03	3.54 ± 0.02^a
	Zn	42.0 ± 0.7	42.7 ± 0.5	41.9 ± 0.3	45.8 ± 0.7^a	41.8 ± 0.2	47.6 ± 0.6^a
	Hg	0.118 ± 0.007	0.100 ± 0.002	0.104 ± 0.008	0.0926 ± 0.0417	0.154 ± 0.068	1.04 ± 0.06^a
Brain (μg g^{-1} wet weight)	Cu	3.85 ± 0.07	4.00 ± 0.02^b	3.80 ± 0.00	3.96 ± 0.07	4.55 ± 1.42	3.96 ± 0.01
	Zn	20.9 ± 0.4	19.9 ± 0.2^b	20.5 ± 0.1	20.8 ± 0.4	19.4 ± 0.4^b	20.8 ± 0.2
	Hg	0.0450 ± 0.0043	0.0697 ± 0.0155	0.0661 ± 0.0149	0.0736 ± 0.0018^a	0.0658 ± 0.0278	0.312 ± 0.012^a
Heart (μg g^{-1} wet weight)	Cu	8.85 ± 0.14	9.35 ± 0.05	8.78 ± 0.16	9.10 ± 0.23	9.90 ± 0.11	10.3 ± 1.0
	Zn	32.3 ± 0.2	32.1 ± 0.4	31.6 ± 0.4	31.6 ± 1.2	33.2 ± 1.0	35.8 ± 3.6
	Hg	0.0562 ± 0.0118	0.0631 ± 0.0149	0.0729 ± 0.0210	0.0553 ± 0.0187	0.0489 ± 0.0266	0.714 ± 0.077^a
Erythrocytes (μg ml^{-1})	Cu	3.02 ± 0.63	1.74 ± 0.18^b	2.08 ± 0.63	1.71 ± 0.42^b	1.97 ± 0.15^b	2.36 ± 0.18
	Zn	2.53 ± 0.58	1.99 ± 0.22	2.70 ± 0.90	1.88 ± 0.52	2.02 ± 0.17	1.81 ± 0.16
	Hg	$0.007\,82 \pm 0.001\,39$	$0.004\,70 \pm 0.001\,12^b$	$0.002\,53 \pm 0.001\,44^b$	$0.000\,770 \pm 0.000\,450^a$	$0.000\,795 \pm 0.000\,256^a$	1.16 ± 0.09^a
Blood plasma (μg g^{-1} wet weight)	Cu	0.777 ± 0.059	0.717 ± 0.007	0.666 ± 0.018	0.757 ± 0.035	0.847 ± 0.016	0.581 ± 0.009^a
	Zn	8.56 ± 0.44	9.07 ± 0.07	7.95 ± 0.38	9.43 ± 0.70	10.6 ± 0.2^a	8.42 ± 0.37
	Hg	0.0115 ± 0.0311	0.0252 ± 0.0266	0.0126 ± 0.0051	0.0351 ± 0.0316^a	0.0204 ± 0.0018	0.679 ± 0.013^a

[a] ANOVA used for comparing the differences at $P < 0.05$ versus control group.
[b] ANOVA used for comparing the differences at $P < 0.05$ versus control group.

Table 3. MT concentration of each organ in different groups

	Control	Cinnabar I	Cinnabar II	Cinnabar III	Cinnabar IV	$HgCl_2$
Liver (μg g^{-1})	152.6 ± 11.1	163.2 ± 15.0	179.4 ± 27.0	148.2 ± 17.8	226.1 ± 48.9	282.6 ± 37.7
Kidney (μg g^{-1})	120.7 ± 57.1	123.2 ± 14.1	133.65 ± 73.1	203.9 ± 47.7	218.2 ± 49.0	190.3 ± 27.1
Testis (μg g^{-1})	104.8 ± 29.8	132.7 ± 14.4	162.1 ± 22.2	175.2 ± 41.2	167.9 ± 57.2	146.1 ± 40.3
Brain (μg g^{-1})	29.1 ± 24.0	66.6 ± 17.6	32.6 ± 21.3	39.4 ± 15.0	52.6 ± 19.6	40.9 ± 12.5
Heart (μg g^{-1})	14.6 ± 17.0	35.5 ± 6.1	24.9 ± 3.8	30.7 ± 6.7	83.0 ± 21.1	20.4 ± 2.2
MT-2 standard (μg ml^{-1})		10.0		50.0	100	200
Measured value (μg ml^{-1})		11.2		52.3	98.1	179
Recovery (%)		112		105	98.1	89.5

beings and animals, because of its impairment of enzyme functioning.[20] Conversely, the high affinity of mercury with –SH is one of the most important means to detoxify mercury, because the binding of Hg–SH in MT is one of the most important routes for mercury excretion.[23,24] The MT contents in each organ of the different rat groups shown in Table 3 indicate that large amounts of MT were induced in the liver and kidney of the cinnabar group IV and $HgCl_2$ group, in which the high contents of mercury in the two organs were observed in Table 2.

Moreover, an important role of MT is in the homeostasis of essential metals, such as copper and zinc. For example, the amounts of copper and zinc in the $HgCl_2$ group were 3.05 and 1.41 times respectively (for kidney) and nearly 1.2 and 1.5 times respectively (for liver) higher than in the control group, which is consistent with the high content of MT in the two tissues shown in Tables 2 and 3 respectively. MT in the control group was bound with essential metals such as copper and zinc, and MT would be rapidly bound with mercury to form mercurothionein because the affinity of mercury for MT is much greater than that for copper or zinc (affinity of the former is 3×10^4 times that of the latter two).[25] The mercurothionein, in which the toxicity of mercury did not present, was innocuously stored and phagocytosed by the lysosome in the cytosol. Then, the phagocytosed mercurothionein disintegrated slowly into lower molecules and was excreted into the nephritic tubule for further evacuation as uric mercury.[26]

Relationship between MT concentration and metal distribution

Use of MT as a biomarker for non-essential elements has been reported previously, but those studies were oriented mainly towards issues related to environmental pollution.[27–29] This study illustrates that MT could be used as an index to reflect the level of mercury assimilation in each tissue from oral intake of cinnabar. Figure 1 shows the results of the relationship between MT and mercury in rat liver and kidney for the same rat groups. The MT in both liver and kidney showed high positive correlation with mercury concentrations contained in the two tissues ($R^2 = 0.9299$, $P < 0.02$ for liver; $R^2 = 0.9923$, $P < 0.0008$ for kidney). The coefficients in the two correlation equations were different. The larger slope suggested that the hepatic MT inducement was more sensitive than nephritic MT inducement to mercury pollution. This is the reason why the mercury concentration is higher in the kidney than in the liver, but why the MT concentration is higher in the liver than in the kidney of rats treated with cinnabar. The MT was vastly induced for self-protection once the tissue was exposed to mercury. The positively high correlation of MT concentrations and mercury level in both liver and kidney validated that MT can be used as a biomarker for the toxicological study of cinnabar.

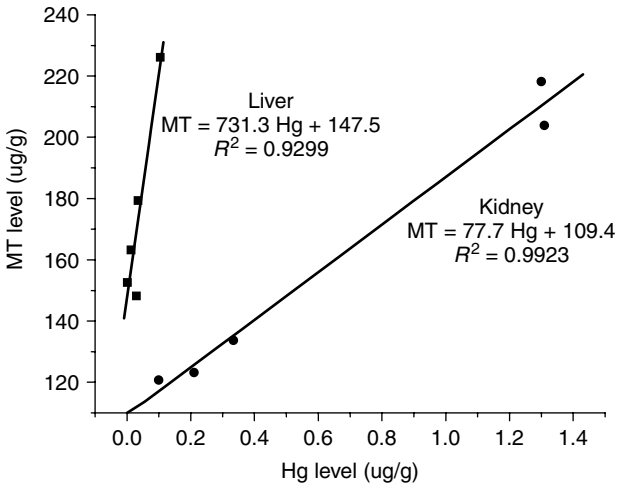

Figure 1. Relationship between MT and mercury in rat liver and kidney.

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