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Effects of Selenium on Metabolism of Mercuric Chloride

Yasuhiro Yamane, Hideki Fukino, Yukio Aida, and Masayoshi Imagawa

Faculty of Pharmaceutical Sciences, University of Chiba[1]

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The distribution of mercury and selenium in subcellular fractions was examined to see effects of selenium on metabolism of mercuric chloride in rats. The subcellular distribution of mercury and selenium was changed greatly with the concurrent administration of them and decreased in soluble fraction and increased in nuclei and debris fractions. We found an interesting result that 56% of mercury existed in soluble fraction with the administration of mercury alone and only 1% of mercury with the concurrent administration of mercury and selenium. In this point, the molar ratio of mercury to selenium was about 1:1 in nuclei and debris, mitochondria and microsomes fractions, respectively.

Amount of mercury in nuclear fraction prepared by the method of Chanda and others with the concurrent administration of mercury and selenium, was 9 times that with the administration of mercury alone.

Furthermore, insoluble nonhistone protein fraction from nuclear fraction had 60% of mercury and in this fraction, amount of mercury per mg protein with the concurrent administration of mercury and selenium was about 13 times that with the administration of mercury alone.

The behavior of mercury and selenium was also examined in the plasma. It was considered that mercury and selenium accumulated with the ratio of 1:1 in the plasma. We found that the gel filtration pattern of mercury in plasma protein on Sephadex G-200 chromatography was changed greatly and most of mercury was accumulated in some protein of high molecular fraction with the concurrent administration of mercury and selenium.

Keywords——mercury; selenium; metabolism; distribution; supernatant; nuclear nonhistone; molar ratio

Introduction

Our preceding report[2] suggested that inhibitory effects of selenium on the toxicity of mercuric chloride were very marked and that both mercury and selenium were accumulated remarkably in the liver when these were administered concurrently, and molar ratio of mercury to selenium was about 1.0.

In the present work, subcellular distribution of mercury and selenium in the liver and amount of mercury and selenium in plasma protein were examined in order to discuss effects of selenium on metabolism of mercuric chloride in rats and then, to study the mechanism of inhibitory effects of selenium on the toxicity of mercuric chloride.

Materials and Methods

1. **Animals**——Male rats of the Wistar strain aged 5 weeks, were purchased and fed solid diets (CE-2) which were obtained from CLEA Japan Inc., Tokyo. The animals aged 12 weeks were employed throughout the present experiment.

2. **Chemicals**——All reagents were analytical grade products.

1) Location: *1–33, Yayoi-cho, Chiba.*

2) Y. Yamane, H. Fukino, Y. Aida, and M. Imagawa, *Yakugaku Zasshi*, **97**, 667 (1977).

3. **Determination of Mercury and Selenium**——Mercury was analyzed by flameless atomic absorption spectrophotometry[3] of vaporized mercury after digesting the tissues in sulfuric-nitric acid[4] and selenium was determined by fluorometry[5] of selenium-2,3-diaminonaphthalene complex, after digesting the tissues according to the method of Watkinson.[6]

4. **Subcellular Distribution of Mercury and Selenium in the Liver**——Rats were devided into 3 groups. Each group consisted of 3 animals. The animals in group I were administered with mercuric chloride (0.010 mmol/kg/day) subcutaneously once a day for 4 days. The animals in group II were administered with mercuric chloride (0.010 mmol/kg/day) and sodium selenate (0.026 mmol/kg/day), subcutaneously at the same time once a day for 4 days. The animals in group III were administered with sodium selenate (0.026 mmol/kg/day) subcutaneously once a day for 4 days. The animals were killed and the livers were used for examination 24 hr after the last treatment. Namely, the livers which were obtained from 3 rats treated with mercury and/or selenium, were perfused and put together. Then, they were homogenized in 1.15% KCl using an all glass homogenizer to yield 20% (w/v) homogenates. Nuclei and debris, mitochondria, microsomes, and soluble fractions were obtained by differential centrifugation according to the method of Hogeboom[7] using a Tominaga 90-UV centrifuge and a Hitachi 65P ultracentrifuge.

5. **Distribution of Mercury in the Rat Liver Nuclear Fraction**——Nuclear fraction was obtained from the liver and fractionated by the method of Chanda and others.[8,9] That is, after washing the nuclear fraction with 0.14 M NaCl in order to remove the globulin fraction, the residue was solubilized in 0.6 M NaCl, 6 M urea, 0.05 M $NaHSO_3$ solution (pH 7.6). The solution was then dialyzed against distilled water overnight at 4° to bring down the concentration of NaCl to 0.14 M and then centrifuged at 3000 rpm (Kubota KC-70). The supernatant was called soluble nonhistone protein ($NHP_{sol.}$). Histones were extracted from the residue with 0.2 N HCl, then centrifuged. The residue was suspended in 5% trichloroacetic acid and treated at 95° for 15 min instead of treatment with DNase and RNase, and centrifuged to obtain nucleic acid fraction. After removing lipids from this residue by extracting with ethanol: ether (3: 1), it was dissolved in 0.2 M phosphate buffer (pH 7.4) containing 6 M urea, 0.05 M $NaHSO_3$ and 10% sodium dodecyl sulfate and then centrifuged. The supernatant was called insoluble nonhistone protein ($NHP_{ins.}$). Amount of mercury in these fractions was determined by flameless atomic absorption spectrophotometry after wet digestion. Protein was estimated by the method of Lowry.[10]

6. **Amount of Mercury and Selenium in Rat Plasma**——Rats were treated with the same methods as described in **4**, and these were injected with heparin intraperitoneally. Blood samples were collected from carotid using heparinized tubes. Then, plasma was obtained for the determination of mercury and selenium by centrifugation.

7. **Elution Profiles of Mercury in Rat Plasma Protein on Sephadex G-200 Chromatography**——Plasma obtained in **6** was gel filtered over Sephadex G-200 column (1.8 × 60 cm) eluted with 0.01 M Tris–HCl buffer, pH 7.4 at 4°. Fraction of 5 ml was collected for the determination of mercury, and the absorption at 280 nm was measured for determination of protein by photoelectric spectrophotometer (Hitachi 139).

Results

Subcellular Distribution of Mercury and Selenium in the Liver

As shown in Table I, the amount of mercury in the liver from group II, was 2 times or more that from group I and then, 56% of mercury existed in soluble fraction from group I, while in soluble fraction from group II did only 1% of mercury. Percentage of mercury in nuclei and debris fractions increased markedly to result in 76.5% of mercury.

The amount of selenium in the liver from group II, increased markedly by the concurrent administration of mercury and selenium. The subcellular distribution of selenium in the liver, represented the same tendency as that of mercury. Namely, the amount of selenium in soluble fraction from group II decreased, but increased remarkably in nuclei and debris fractions as compared with group III.

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4) "Standard Methods of Analysis for Hygienic Chemists with Commentary," ed. by the Pharmaceutical Society of Japan, Kanehara Press Inc., Tokyo, 1973, pp. 275—276.
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Table I. Subcellular Distribution of Mercury and Selenium in the Liver

Treatment	μg/g tissue		Subcellular distribution (%)							
			Nuclei and debris		Mitochondria		Microsomes		Soluble fraction	
	Hg	Se	Hg	Se	Hg	Se	Hg	Se	Hg	Se
Control (untreated)	—	1.63	—	49.6	—	14.7	—	11.6	—	24.1
Hg (group I)	15.7	—	32.1	—	8.8	—	3.1	—	56.0	—
Hg–Se (group II)	39.2	11.30	76.5	73.8	16.3	15.7	6.2	6.1	1.0	4.4
Se (group III)	—	2.98	—	53.9	—	11.2	—	10.3	—	24.6

Control (untreated): 0.9% NaCl *s.c.* once a day for 4 days, Hg (group I): $HgCl_2$. (0.010 mmol/kg/day) *s.c.* once a day for 4 days, Hg-Se (group II): $HgCl_2$. (0.010 mmol/kg/day) and Na_2SeO_4 (0.026 mmol/kg/day) *s.c.* at the same time once a day for 4 days, Se (group III): Na_2SeO_4 (0.026 mmol/kg/day) *s.c.* once a day for 4 days, ——: This means that we did not analyze.

As the amount of both mercury and selenium increased markedly in each fraction in group II, as compared with group I and group III, the molar ratio of mercury to selenium was examined in each fraction. The results were shown in Table II. The molar ratios in nuclei and debris, mitochondria and microsomes fractions were 1.03, 1.02 and 1.04, respectively. Consequently, it was suggested that mercury and selenium were accumulated by the ratio of 1: 1 in each fraction.

Table II. Molar Ratio of Mercury to Selenium in the Liver Subcellular Fractions

Treatment	Subcellular fraction	Nuclei and debris	Mitochondria	Microsomes	Soluble fraction
Hg–Se (group II)	Hg μmol/g tissue	0.117	0.025	0.010	trace
	Se μmol/g tissue	0.121	0.026	0.010	0.007
	Molar ratio	1.03	1.02	1.04	—

Experimental conditions are as given in Table I and experimental groups are all the same in Table I.

Distribution of Mercury in the Rat Liver Nuclear Fraction

The amount of mercury in each fraction from nuclear fraction was examined. As shown in Table III, the distribution pattern of mercury in group II, was almost the same as that in group I, and the amount of mercury was accumulated mostly in $NHP_{ins.}$ from both group I and group II, but the amount of mercury per mg protein in $NHP_{ins.}$ from group II, was about 13 times that from group I.

Table III. Amount of Mercury in the Different Fractions of Rat Liver Nuclei

Treatment	Hg μg in nuclei /g tissue	Soluble nonhistone protein (%)	Histone protein (%)	DNA (%)	Insoluble nonhistone protein (%)
Hg (group I)	1.94	23.0	18.5	3.5	55.0
Hg–Se (group II)	17.76	17.0	19.5	2.0	61.5

Experimental conditions are as given in Table I.

Amount of Mercury and Selenium in Rat Plasma

As shown in Table IV, amount of mercury and selenium in the plasma from group II, increased markedly as compared with that from group I and group III. The molar ratio of mercury to selenium in the plasma from group II, was 1.15. It was assumed that mercury and selenium were accumulated with the ratio of 1 : 1 in the plasma as well as in the liver from group II.

TABLE IV. Amount of Mercury and Selenium in Rat Plasma

Treatment	Element	μmol $\times 10^{-2}$/ml plasma	Molar ratio
Hg (group I)	Hg	1.82±0.54	—
Hg–Se (group II)	Hg	9.20±0.62	1.15±0.08
	Se	7.99±1.35	
Se (group III)	Se	2.03±0.06	—

Experimental conditions are as given in Table I.
Each value represents the mean ±S.E. of 3 rats.

Elution Profiles of Mercury in Rat Plasma Protein on Sephadex G-200 Chromatography

Gel filtration pattern of mercury in rat plasma protein on Sephadex G-200 chromatography, was shown in Fig. 1. Four peaks obtained by gel filtration represented the absorptions of plasma protein at 280 nm in group I. If each peak was named peak 1, 2, 3 and 4 according to the order of elution, amount of mercury in group I existed in peak 1, 2 and 3. On the other hand, in group II, there was no great changes in the elution profiles of plasma protein but amount of mercury increased greatly in peak 1.

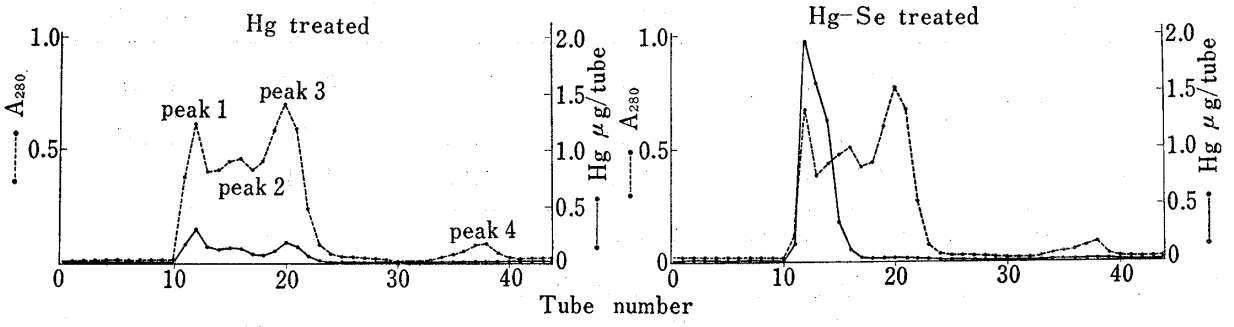

Fig. 1. Elution Profiles of Rat Plasma Protein on Sephadex G-200 Chromatography
Hg treated: $HgCl_2$ (0.010 mmol/kg/day) *s.c.* once a day for 4 days, Hg–Se treated: $HgCl_2$ (0.010 mmol/kg/day) and Na_2SeO_4 (0.026 mmol/kg/day) *s.c.* at the same time once a day for 4 days, Fractions (5 ml/tube) were collected and analysed for mercury and protein (A_{280}).

Discussion

It has been shown that selenium played some role in the mechanism of detoxication of inorganic mercury, since dramatic effect of selenium on the toxicity of inorganic mercury was reported by Parizek.[11] Namely, Parizek found that amount of mercury increased markedly in the blood with concurrent administration of mercury and selenium,[12] and excretion of

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12) J. Parizek, I. Benes, I. Ostadalova, A. Babicky, J. Benes, and J. Lener, *Physiol. Bohemoslov.*, **18**, 95 (1969).

selenium decreased with administration of mercuric chloride[13] and the accumulation of mercury and selenium in body increased.[14] Judging from these facts, Parizek suggested that mercury bound with selenium each other. Levander and others[15] reported that amount of selenium in the kidney, blood and spleen, increased by the concurrent administration of mercury and selenium. Potter and others[16] represented that amount of mercury in the liver increased by 10—20 times with the concurrent administration of mercury and selenium, as compared with the administration of mercury alone. Kosta and others[17] reported that the molar ratio of mercury to selenium in the organs from mine workers was approximately 1:1.

Our preceding report[2] suggested a possibility that both mercury and seelnium were accumulated remarkably in the liver when these were administered concurrently and molar ratio of mercury to selenium was about 1.0. But we did not find where they accumulated in subcellular fractions. Then, in the present work, the distribution of mercury and selenium was examined in subcellular fractions obtained from the liver. Namely, the subcellular distribution of mercury and selenium was changed greatly with the concurrent administration of them and decreased in soluble fraction and increased in nuclei and debris fraction. We found an interesting result that 56% of mercury existed in soluble fraction with the administration of mercury alone and only 1% of mercury, with the concurrent administration of mercury and selenium. In this point, the molar ratio of mercury to selenium was examined in each fraction and then, the ratio was about 1:1 in nuclei and debris, mitochondria and microsomes fraction, respectively. It was suggested that mercury and selenium were accumulated with the ratio of 1:1 in these fractions. Then, Chen and others[18] examined also the mercury content in various tissues and reported that in nuclei and debris, mitochondria, and microsomes fractions of the liver, the mercury content was increased by the concurrent administration of selenium and mercury, whereas the mercury content in soluble fraction was decreased. In their experiment, however, the mercury content did not decrease markedly in soluble fraction and not increase remarkably in nuclei and debris fractions by selenium as compared with our results presumably because experimental conditions were different and the liver was not perfused. As it was in nuclei and debris fractions that mercury and selenium were accumulated mostly, nuclear fraction was prepared by the method of Chanda and others, and mercury was determined. Consequently, amount of mercury in nuclear fraction with the concurrent administration of mercury and selenium, was about 9 times that with the administration of mercury alone and then, $NHP_{ins.}$ fraction from nuclear fraction had 60% of mercury and in this fraction amount of mercury per mg protein in the concurrent administration of mercury and selenium was 13 times that in the administration of mercury alone. As described just above, we found that the subcellular distribution of mercury changed greatly with the concurrent administration of mercury and selenium, and most of mercury was accumulated in $NHP_{ins.}$ fraction in nuclear fraction. This observation was in agreement with the result reported previously by Chanda and others.[9] But the acute toxicity of mercuric chloride was protected markedly by selenium.

Secondly, as Burk and others[19] reported that the gel filtration pattern of mercury in plasma protein was changed with the concurrent administration of mercury and selenium,

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18) R.W. Chen, P.D. Whanger, and S.C. Fang, *Pharm. Res. Commun.*, **6**, 571 (1974).
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as compared with the administration of mercury alone, the behavior of mercury and selenium was examined in the plasma and then, the molar ratio of mercury to selenium was about 1. Namely, it was considered that mercury and selenium also were accumulated with the ratio of 1:1 in the plasma. We found that the gel filtration pattern of mercury in rat plasma on Sephadex G-200 chromatography was changed greatly and most of mercury was accumulated in some protein of high molecular fraction (peak 1) by the concurrent administration of mercury and selenium. The molar ratio of mercury to selenium in rat plasma was in agreement with results reported previously by Chen and others[18] and Burk and others[19]. But furthermore, we need to examine about mercury binding protein by the concurrent administration of mercury and selenium, because considerable amount of mercury is also accumulated in a little lower molecular protein than peak 1 by the concurrent administration of mercury and selenium.

Judging from these results, it was considered that mercury and selenium reacted each other and were accumulated with the ratio of 1:1 in the plasma by the concurrent administration of them and then, mercury was accumulated mostly in $NHP_{ins.}$ fraction obtained from nuclear fraction of the liver by the method of Chanda and others.[8,9]