Arch Toxicol (1986) 59: 16–20

Archives of
Toxicology

Kidney concentrations and urinary excretion of mercury, zinc and copper following the administration of mercuric chloride and sodium selenite to rats

Jadwiga Chmielnicka, Elżbieta Brzeźnicka, and Andrzej Śniady

Department of Toxicological Chemistry, Institute of Environmental Research and Bioanalysis, Medical Academy of Łódź, Narutowicza 120a, 90–145 Łódź, Poland

Abstract. The effect of single (SC) administration of mercuric chloride (1 mg Hg/kg) alone or jointly with (PO) sodium selenite (0.39 mg Se/kg) on kidney disposition of mercury (Hg) and metallothionein (MT) and urinary excretion of Hg, zinc (Zn) and copper (Cu) has been studied in the female rat. The excretion of Hg and essential metals was determined every day following exposure. Daily excretion of endogenous Cu and Zn the Hg-exposed group was about threefold and fourfold, respectively, in comparison with control groups of rats. Sodium selenite prevented the urinary excretion of endogenous Cu and partly of Zn.

Key words: Interaction – Kidney – Mercury – Selenium – Metallothionein – Endogenous copper – Zinc – Urinary excretion – Metals.

Introduction

Despite numerous studies of the effects of mercury (Hg) on the rat kidney, many important questions concerning the pathogenesis of renal cell death induced by this toxin remained unanswered (Ganote et al. 1974; Oken 1981; Kluwe 1981). The results of Kyle et al. (1983) illustrate the importance of using a combination of biochemical and functional tests to elucidate the sequence of events in kidney following toxic injury. Bogden et al. (1980) suggest that the classical nephrotoxic effects of inorganic Hg may be due in part to the associated elevated copper levels. A significant increase in kidney copper (Cu) was found in the animals exposed to inorganic Hg (Szymańska and Żelazowski 1979; Bogden et al. 1980) and in those intoxicated with alkylmercurials (Bogden et al. 1980; Brzeźnicka and Chmielnicka 1985).

Selenite is well known to give protection against the toxicity of mercuric compounds (Magos and Webb 1980). Simultaneously administered selenium (Se) decreases the accumulation of Hg and metallothionein (MT) in the kidneys (Komsta-Szumska and Chmielnicka 1977; Chmielnicka and Brzeźnicka 1978). The mechanism by which Hg toxicity is mitigated by the coexistence of Se is not yet clear, but it is generally thought that it may involve a direct or indirect binding of Se with Hg and endogenous Cu and zinc (Zn) Komsta-Szumska and Chmielnicka 1983; Chmielnicka et al. 1983).

The present work investigates the effect of mercury accumulation in the kidneys on the level of MT and the kidney concentrations and urinary excretion of the essential Zn and Cu in rats exposed to mercuric chloride alone or jointly with Na_2SeO_3.

Offprint requests to: J. Chmielnicka

Material and methods

Ninety-six white female rats of Wistar strain weighing about 200 g were free fed commercial rat ration (LSM) and water. Animals were allowed to adjust to laboratory environment for 1 week. They were then given a single dose (SC) of $HgCl_2$ (1 mg Hg/kg) – group I, single dose (SC) of $^{203}HgCl_2$ (1 mg Hg/kg) – group Ia, single dose (PO) of Na_2SeO_3 (0.39 mg Se/kg) – group II, single doses of $^{203}HgCl_2$ (1 mg Hg/kg) and Na_2SeO_3 (0.39 mg Se/kg) – group III, and single dose of $HgCl_2$ (1 mg Hg/kg) and Na_2SeO_3 (0.39 mg Se/kg) – group IIIa. Control rats received saline injections – group IV.

On days 1, 3 and 7 the animals of groups Ia, II and III (four rats each) were kept individually in metabolic cages and urine samples were collected for 7–8 h in beakers kept in an ice bath.

The animals of groups I, II and IIIa (four rats each) were killed on the 1st, 3 and 7th days by heart puncture under light ether narcosis and kidneys were removed and weighed. Zinc and Cu in the kidneys and in the urine were determined by atomic absorption spectrophotometry (Pye-Unicam SP-192). Mercury was determined in the homogenized kidney and in the urine by gamma counting in a USB-2 scintillation counter. The counting time was 100 s, precision amounting to ±4%. Metallothionein in the homogenates of rat kidneys was determined by the radiochemical method (Żelazowski and Piotrowski 1977). Urinary creatinine was determined by the method of Bartels and Böhmer (1971).

The significance of the differences between control and the treated groups was evaluated by Student's *t*-test.

Results

1) Effects of Hg and Se on the concentrations of MT, Hg and trace elements in kidney

Following a single dose of 1 mg Hg/kg the kidneys contained 18.8, 26.2 and 34% of the administered dose at 1, 3 and 7 days, respectively (Table 1). Similarly, there was a considerable increase in the concentration of MT in the kidneys. The renal concentrations of (endogenous) Cu and

Table 1. Kidney concentration of Hg expressed μg Hg/g and MT (mg/g) as mean ± SD following administration of $HgCl_2$ (1 mg Hg/kg) alone or jointly with Na_2SeO_3 (0.39 Se mg/kg)

Groups	Kind of exposure	Day after administration		
		1	3	7
		μg Hg/g tissue		
Ia	Hg	26.62 ± 2.68 [b]	34.66 ± 3.70 [b,c]	42.85 ± 5.00 [b,c]
IIIa	Se + Hg	6.05 ± 1.06	13.80 ± 2.98 [c]	24.67 ± 4.33 [c]
		mg metallothionein/g tissue		
I	Hg	0.81 ± 0.24 [a]	1.61 ± 0.17 [a,b,c]	1.52 ± 0.19 [a,b,c]
III	Se + Hg	0.59 ± 0.05 [a]	0.92 ± 0.22 [a,c]	1.07 ± 0.13 [a,c]
II	Se	0.46 ± 0.02 [a,b]	0.62 ± 0.06 [b]	0.67 ± 0.12 [b]
IV	Control	0.38 ± 0.04	0.54 ± 0.11	0.48 ± 0.08

[a] Significantly different from control ($p < 0.05$)
[b] Significantly different from group (Se + Hg)
[c] Significantly different from 1st day

Table 2. The level of endogenous Cu and Zn in μg/g as mean ± SD in the kidneys of control rats and rats exposed to $HgCl_2$ alone or jointly with Na_2-SeO_3

Groups	Kind of exposure	Day after administration		
		1	3	7
		μg Cu/g tissue		
I	Hg	33.35 ± 4.28 [a,b]	53.14 ± 2.46 [a,b,c]	59.50 ± 6.13 [a,b,c]
III	Se + Hg	20.32 ± 2.52	27.86 ± 2.66 [a]	38.47 ± 2.99 [a,c]
II	Se	22.13 ± 4.26	25.56 ± 1.38 [a]	29.12 ± 3.04 [a,b]
IV	Control	14.30 ± 4.00	16.44 ± 2.30	15.50 ± 3.72
		μg Zn/g tissue		
I	Hg	32.60 ± 3.04 [a,b]	34.15 ± 2.64 [a,b]	35.62 ± 1.64 [a,b]
III	Se + Hg	24.05 ± 1.46 [a]	25.44 ± 2.85 [a]	27.05 ± 1.82 [a]
II	Se	26.38 ± 2.19 [a]	25.08 ± 1.89 [a]	27.31 ± 3.19 [a]
IV	Control	19.38 ± 1.90	19.88 ± 1.47	19.96 ± 2.18

[a] Significantly different from control ($p < 0.05$)
[b] Significantly different from group (Se + Hg)
[c] Significantly different from 1st day

Table 3. Molar ratio of Zn to Cu in kidneys at 1, 3 and 7 days after administration of $HgCl_2$ alone or jointly with Na_2 SeO_3

Groups	Kind of exposure	Day after administration		
		1	3	7
I	Hg	0.950	0.624	0.576
IIIa	Se + Hg	1.150	0.915	0.682
II	Se	1.158	0.952	0.910
IV	Control	1.315	1.178	1.255

Zn also were increased markedly during the first 3 days after the administration of Hg (Table 2). The kidney concentration of Hg was decreased when $HgCl_2$ was administered together with an equimolar dose of Na_2SeO_3. The concentrations of Cu, Zn and MT in this tissue were also decreased in comparison with the group exposed to Hg alone. In animals exposed to Se alone an augmentation in the Cu and Zn concentrations was observed in the kidneys in comparison with the control group.

Table 3 presents the molar ratios of Zn to Cu in the kidneys of control and exposed rats. In all groups of exposed animals the Cu concentration in the kidney increased, leading to a decrease in the value of the Zn/Cu ratio.

2) Effect of Hg and Se on the urinary excretion of Hg and trace elements

Urinary and faecal excretion of ^{203}Hg were decreased by the simultaneous administration of Na_2SeO_3 (Fig. 1).

The urinary excretion of Hg was increased at 48 h in the Hg-exposed group. The amounts of Cu and Zn excreted daily in the Hg-treated groups were about 3-fold and 4-fold greater than in the corresponding control groups (Fig. 2) and even 7 days after the injection of Hg the urinary excretion of these metals was elevated.

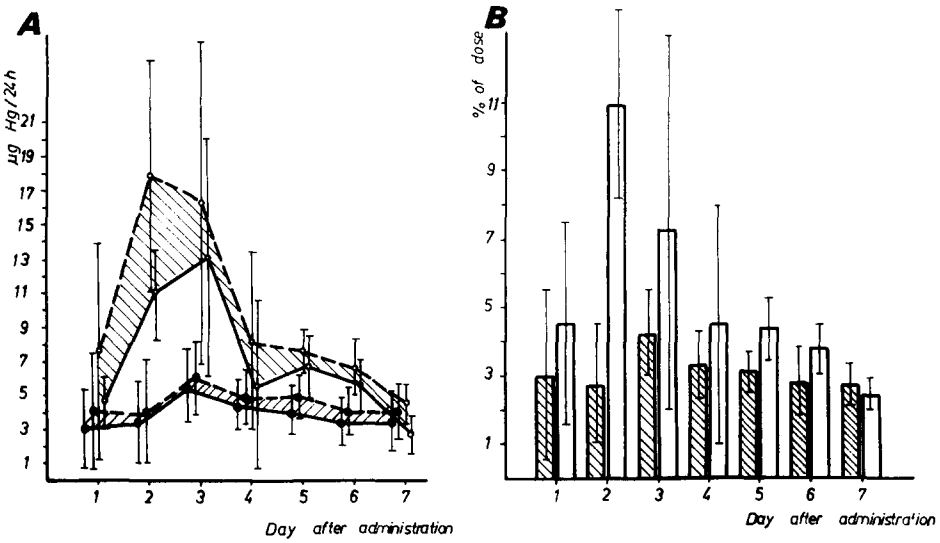

Fig. 1. Changes in amounts of urinary Hg (———) and urinary and faeces (– – –) mercury after injections of $HgCl_2$ alone (– O –) or jointly with Na_2SeO_3 (– ● –). Urine was collected for 24 h and the amounts of Hg are expressed µg Hg as mean ± SD of four samples (**A**) or percent of dose (**B**), □ $^{203}HgCl_2$, ▨ $^{203}HgCl_2 + Na_2SeO_3$

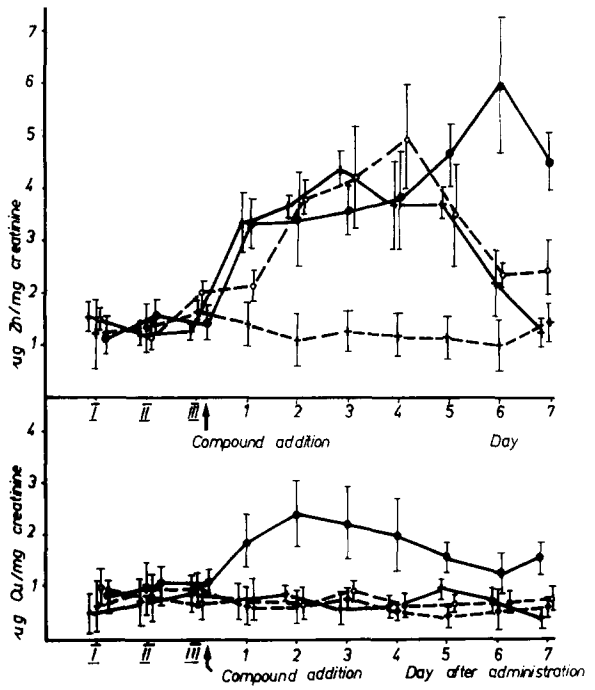

Fig. 2. Changes in amounts of urinary Zn and Cu (µg metal/mg creatinine). Urine was collected as in Fig. 1, (– + – + –) control group, (– O – O) $HgCl_2 + Na_2SeO_3$, (– ● – ● –) $HgCl_2$, (– × – × –) Na_2SeO_3 administration

Sodium selenite prevented the urinary excretion of endogenous Cu. The administration of Na_2SeO_3 jointly with Hg did not prevent the urinary excretion of endogenous Zn after 5 days of injection of Hg. Selenium administered to rats alone resulted in the higher excretion in urine of Zn in comparison with control rats (Fig. 2).

Discussion

Morphological alterations in the proximal tubular epithelium of kidneys in $HgCl_2$ exposed animals paralelled the changes in enzyme excretion with respect to time of onset and dose-effect (Kemspon et al. 1977; Kyle et al. 1983).

Urinary levels of renal brush border enzymes were most sensitive among the indices of $HgCl_2$ acute nephrotoxicity (Stroo and Hook 1977; Planas-Bohne 1977; Braun et al. 1978; Senger et al. 1979; Price 1982). However, enzymes activities were dramatically elevated at 24 h and back to normal about 72 h after 1 mg Hg/kg (Kyle et al. 1983).

The most interesting findings in our study are the increases in urinary Zn and Cu in rats exposed to the above dose of Hg. The levels of these endogenous metals in urine are still significantly higher than their respective controls even at 7 days post-injection of $HgCl_2$ (Fig. 2). It follows therefore that the increases in the urinary levels of these metals are more sensitive indicators of acute $HgCl_2$ nephrotoxicity than are the urinary concentrations, or activities of Zn-dependent enzymes. Zinc deficiency may be seen in patients with chronic renal disease (Prasad 1979) and may be related to the increased urinary zinc losses (Reimold 1980; Mahajan et al. 1983). The marked increase in kidney Cu and Zn after Hg treatment reported in the present study (Table 2) is consistent with the results of other investigators (Bogden et al. 1980; Lee et al. 1983) and is presumably due to the association of those metals with the renal MT (Table 1).

Lee et al. (1983) have observed that urinary MT level was elevated in response to inorganic Hg. As the result of renal damage a large increase in the urinary excretion of Hg, Cu and Zn has occurred (Fig. 1, Fig. 2). In copetition for ligands in biological systems, Hg may effect the deposition and homeostasis of these essential elements. Metallothionein provides a focal point for elucidating the pathways of Cu and Zn metabolism and is a buffering ligand to counteract the deleterious effect of toxic metals.

On the other hand Zn and Cu decreased the incorporation of Hg in MT in the kidney of rats exposed simultaneously to these metals (Komsta-Szumska and Chmielnicka 1983).

Selenium displaces Hg from the soluble kidney fraction bound mainly with metallothionein to the nonhistone

protein fraction of liver nuclei (Komsta-Szumska and Chmielnicka 1977) and results in an increase of the whole-body retention (Chmielnicka et al. 1978).

Selenium diminishes the affinity of Hg for the kidney and reduces the level of mercury bound by renal metallothionein-like proteins (Chmielnicka and Komsta-Szumska 1980). The complex formation in the blood in the presence of Hg and Se delays considerably the transfer of Hg from the blood and lowers its accumulation in the kidney. The major effect of co-administered Se is on the urinary, rather than faecal excretion of Hg is observed within the first 3 days (Fig. 1). The reduction in Hg and Se is most pronounced in urine compared to faeces. Evidence that the interaction between Hg and Se occurs shortly after their co-administration has been given by Kristensen and Hansen (1979, 1980). Because of this interaction, induction with MT by Hg is decreased in the kidneys and, in consequence, the amounts of MT-bound Zn and Cu also are diminished (Table 1, 2). The increase of MT and Cu in this organ took place when inorganic Hg liberated from alkylmercurials in the kidneys of rats exceeded 10 μg Hg/g and reached a plateau of about 40 μg Hg/g (Brézeźnicka and Chmielnicka, 1981, 1985).

Sodium selenite prevents the increase of endogenous Cu in the urine (Fig. 2) which may be due to a decrease in the kidney concentration of Hg. We demonstrated previously a clear-cut interaction between exogenous Zn, endogenous Cu and Se in rats (Chmielnicka et al. 1983; Komsta-Szumska and Chmielnicka 1981).

The administration of cadmium to rats also results in dose-related of rats increases in the urinary Zn and Cu (Bonner et al. 1979; Suzuki et al. 1983) and MT (Lee et al. 1983) in rats. The disturbances in the metabolism of these endogenous metals in the kidneys of rats occur when the concentration of cadmium in this organ attains about 10 μg Cd/g (Chmielnicka et al. 1985).

In the light of our studies and of the suggestion of Bogden et al. (1980) the increase in kideny Cu and Zn may be general metabolic response to heavy metals which damage kidneys. The presence of Se considerably affects the metabolism of exogenous Hg and endogenous Zn and Cu in the rat.

Acknowledgements. This work was performed within the framework of Project MZ-IX 1.38 of the Ministry of Health, Polish People's Republic "Occupational Medicine".

References

Bartels H, Böhmer M (1971) Micro-determination of creatinine. Clin Chem Acta 32: 81–85

Bogden ID, Kemp FW, Troiano RA, Jortner BS, Timpone C, Giuliani D (1980) Effect of mercuric chloride and methylmercury chloride exposure on tissue concentrations of six essential minerals. Environ Res 21: 350–359

Bonner FW, King LJ, Parke DV (1979) The tissue disposition and urinary excretion of cadmium, zinc, copper and iron, following repeated parenteral administration of cadmium to rats. Chem Biol Interact 27: 334–351

Braun JP, Rico AG, Bernard P, Burgat-Sacare V, Eghbali B, Godfrain JC (1978) La gamma – glutamyl transferase urinaire en toxicologie renalel chez le rat. Bases de son utilisation-interxet lors de nephrite aique mercurielle. Toxicology 11: 73–82

Brzeźnicka EA, Chmielnicka J (1981) Interaction of alkylmercuric compounds with sodium selenite. I. Metabolism of ethylmercuric chloride administered alone and in combination with sodium selenite in rats. Environ Health Perspect 39: 131–142

Brzeźnicka EA, Chmielnicka J (1985) Interaction of alkylmercuric compounds with sodium selenite. III. Selenium-induced changes on the levels of metallothionein-like proteins and endogenous copper in some tissues of rats exposed to methyl- or ethylmercuric chloride. Environ Health Perspect 60: 423–431

Chmielnicka J, Brzeźnicka EA (1978) The influence of selenium on level of mercury and metallothionein in rat kidneys in prolonged exposure to different mercury compounds. Bull Environ Contam Toxicol 19: 183–190

Chmielnicka J, Hajdukiewicz Z, Komsta-Szumska E, Łukaszek S (1978) Whole-body retention of mercury and selenium and histopathological and morphological studies of kidneys and liver of rats exposed repeatedly to mercuric chloride and sodium selenite. Arch Toxicol 40: 189–199

Chmielnicka J, Komsta-Szumska E (1980) Variation of the level of mercury and metallothionein in the kidneys and liver of rats with time of exposure to sodium selenite. Biol Trace Elem Res 2: 109–120

Chmielnicka J, Komsta-Szumska E, Zaręba G (1983) Effect of interaction between ^{65}Zn, mercury and selenium in rats (retention, metallothionein, endogenous copper). Arch Toxicol 53: 165–175

Chmielnicka J, Bem E, Brzeźnicka E, Kasperek M (1985) The tissue disposition of zinc and copper following repeated administration of cadmium and selenium to rats. Environ Res 37: 419–424

Ganote GE, Reimer KA, Jenning RB (1974) Acute mercuric chloride nephrotoxicity. An electron microscopic and metabolic study. Lab Invest 31: 633–647

Kempson SA, Ellis BG, Price RG (1977) Changes in rat renal cortex isolated plasma membranes and urinary enzymes following the injection of mercuric chloride. Chem Biol Interact 18: 217–234

Kluwe WM (1981) Renal function tests as indicators of kidney injury in subacute toxicity studies. Toxicol Appl Pharmacol 57: 414–424

Komsta-Szumska E, Chmielnicka J (1977) Binding of mercury and selenium in subcellular fractions of rat liver and kidneys following separate and joint administration. Arch Toxicol 38: 217–228

Komsta-Szumska E, Chmielnicka J (1981) Organ and subcellular distribution of mercury in rats in the presence of cadmium, zinc, copper and sodium selenite. Clin Toxicol 18: 1327–1334

Komsta-Szumska E, Chmielnicka J (1983) Effect of zinc, cadmium or copper on mercury distribution in rat tissues. Toxicol Lett 53: 349–354

Kristensen P, Hansen JC (1979) Wholebody elimination of $^{75}SeO_3^{2-}$ and $^{203}HgCl_2$ administered separately and simultaneously to mice. Toxicology 12: 101–109

Kristensen P, Hansen JC (1980) Urinary and fecal excretion of selenium ($Na_2{}^{75}SeO_3$) and mercury $^{203}HgCl_2$) administered separately and simultaneously to mice. Toxicology 16: 39–47

Kyle GM, Luthra R, Bruckner JV, Mackenzie WF (1983) Assessment of functional, morphological, and enzymatic tests for acute nephrotoxicity induced by mercuric chloride. J Toxicol Environ Health 12: 99–117

Lee YH, Shaikh ZA, Tohyama C (1983) Urinary metallothionein and tissue metal levels of rats injected with cadmium, lead, copper or zinc. Toxicology 27: 337–345

Mahajan SK, Speck J, Varghese G, Abu-Hamdan D, Migdal S, Briggs W, Prasad A, Mc Donald F (1983) Zinc metabolism in nephrotoxic syndrome. Abstracts Inter. Symp. Health Effects and Interactions of Essential and Toxic Elements, p 105, Lund, Sweden 13–18 June

Magos L, Webb M (1980) The interactions of selenium with cadmium and mercury. CRC Crit Rev Toxicol 8: 1–41

Oken DE (1981) Pathogenic mechanisms in acute renal failure. In: Hook JB (edn) Toxicology in the kidney, Raven Press, New York, pp 117–134

Planas-Bohne F (1977) The effect of mercuric chloride on the excretion of two urinary enzymes in the rat. Arch Toxicol 37: 219–225

Prasad AS (1979) Trace Elements: Biochemical and clinical effects of zinc and copper. Am J Hematol 6: 77–87

Price RG (1982) Urinary enzymes, nephrotoxicity and renal disease. Toxicology 23: 99–134

Reimold EW (1980) Changes of zinc metabolism during the course of the nephrotoxic syndrome. Am J Dis Child 134: 46–50

Senger S, Braun JP, Rico AG, Bernard P, Burgat-Sacate V (1979) Urine gamma-glutamyl transferase in rat kidney toxicology: nephropathy by repeated injections of mercuric chloride. Toxicology 12: 293–305

Stroo WE, Hook JE (1977) Enzymes of renal origin in urine as indicators of nephrotoxicity. Toxicol Appl Pharmacol 39: 423–424

Suzuki KT, Yaguchi K, Ohnuki R, Nishikawa M, Yamada YK (1983) Extent of cadmium accumulation and its effect on essential metals in liver, kidney, and body fluids. J Toxicol Environ Health 11: 713–726

Szymańska J, Żelazowski A (1979) Effect of cadmium, mercury and bismuth on the copper content in rat tissues. Environ Res 19: 121–126

Żelazowski A, Piotrowski JK (1977) A modified procedure for determination of metallothionein – like proteins in animal tissues. Acta Biochem Polon 24: 97–103

Received April 22, 1985/Accepted January 7, 1986