Effect of kidney damage on the mobilisation of mercury by thiol-complexing agents

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ABSTRACT Mobilisation of mercury by thiol-complexing agents is the accepted treatment for chronic mercury intoxication. The success of such treatment is judged by the urinary excretion of mercury which might be modified by existing kidney damage caused either by the mercury itself or by other factors. In the present work the ability of three thiol-complexing agents, D-penicillamine, N-acetyl-D, L-penicillamine, and 2,3-dimercaptosuccinic acid (DMSA) to remove mercury from a damaged kidney and to increase the urinary excretion of mercury were studied. Kidney damage was induced by the injection of 20 mg/kg sodium chromate three days before the injection of 2.5 μ moles/kg HgCl_2 or three and seven days before the administration of a similar dose of mercury complexed with D-penicillamine.

It was shown that both renal uptake and urinary excretion of mercury were decreased in animals with damaged kidneys. This effect lessened with time between the induction of damage and the injection of mercury-penicillamine.

Each of the three chelating agents was given 48 and 54 hours after the administration of HgCl_2 in equivalent (400 μ moles/kg) doses. All were able to remove mercury from the kidneys, but DMSA was far the most effective and only DMSA increased the urinary excretion of mercury. The amount of mercury removed from the kidneys by the chelators was less in animals with renal damage than in controls, but the difference was insignificant if renal mercury depletion was related to the initial renal mercury content.

Except after exposure to short chain alkyl mercurials, mercury in the body is always in the divalent (Hg^{2+}) form that concentrates mainly in the kidneys.¹ This enables the chelation of Hg^{2+} after the administration of HgCl_2 to be used as a model for mercury mobilisation by thiol-chelating agents irrespective of whether Hg^{2+} is derived from exposure to mercury vapour or to phenyl- or methoxyethylmercury.

Though the main target in chronic mercury intoxication in man is the central nervous system,^{1,2} proteinuria has been occasionally observed in exposed workers.³⁻⁶ Moreover, an exposed worker may have kidney damage caused by factors unrelated to mercury. Whether the kidney is damaged by mercury or by other factors, signs and symptoms of mercury intoxication might make chelation

treatment desirable. As both the access of the chelator to the renal deposits of mercury and the renal excretion of mercury might be influenced by the condition of the kidneys, the present work is aimed at investigating the effect of three chelating agents on the renal uptake, mobilisation, and excretion of mercury by damaged kidneys. To be able to distinguish between the effects of renal damage and the degree of saturation of the high affinity binding sites for Hg^{2+} on the mobilisation of mercury by the chelator, the dose of HgCl_2 was kept constant, and renal damage was induced by a known non-mercurial nephrotoxin, sodium chromate, which affects the same part of the proximal tubules as HgCl_2 .^{8,9} The three chelating agents selected were D-penicillamine, N-acetyl-D,L-penicillamine, and dimercaptosuccinic acid (DMSA). Of these three chelating agents, only D-penicillamine is a registered drug.

Both penicillamines have a life-saving effect against HgCl_2 if given shortly after ingestion¹⁰ but only N-acetyl-D,L-penicillamine^{11,12} and not

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D-penicillamine¹³ increases the urinary excretion of mercury in chronic mercury vapour intoxication. DMSA is a water-soluble dithiol, which has been shown in animal experiments to be more effective than the penicillamines in increasing the urinary excretion of mercury.^{14,15} In clinical practice its metal-chelating effect has been tested only for lead.¹⁶

Material and methods

Male white rats of the Porton Wistar strain of 200 ± 10 g body weight and about 6 weeks old were used. Renal damage was induced by the subcutaneous administration of 20 mg/kg sodium chromate in a volume of 4 ml/kg; control animals were given an equal volume of saline. Mercury was given either as HgCl_2 or as mercury-penicillamine. The HgCl_2 was injected intraperitoneally in a dose of $2.5 \mu\text{mole/kg}$ three days after pretreatment, and mercury complexed with D-penicillamine in a dose of $2.5 \mu\text{mole/kg}$ mercury with $7.5 \mu\text{mole/kg}$ D-penicillamine three or seven days after pretreatment. Each dose was in the same volume as the sodium chromate solution. Mercury was labelled with $^{203}\text{HgCl}_2$ to the extent of 0.9 to $1.1 \mu\text{Ci/mg Hg}^{2+}$.

Half the animals treated with HgCl_2 were given intramuscular injections of saline after intervals of 48 and 54 hours. After the same intervals of time, the remaining animals were given intramuscular injections of D-penicillamine or N-acetyl-D,L-penicillamine or meso-dimercaptosuccinic acid (DMSA) in a dose of $400 \mu\text{mole/kg}$.

The animals were housed individually in metabolic cages and their urine and faeces were collected. They

were decapitated 48 or 96 hours after the administration of the mercury; blood was collected from the cervical vein, and the kidneys and liver were removed. The whole body burden of ^{203}Hg was estimated from the γ radiation in live animals placed in a $14 \times 6 \times 6.5$ cm plexiglass box between two NaI crystal pairs facing each other with a counting efficiency of 9.6%. Faeces, urine, blood, kidneys, and liver were counted in a well-shaped NaI crystal (diameter 13 cm, depth 11 cm) with an efficiency of 43%.

Results

Pretreatment with sodium chromate decreased the kidney content of mercury and increased the liver and blood contents of mercury at 48 and 96 hours after the injection of HgCl_2 (table 1). Treatment with chelating agents significantly decreased the renal content of mercury in both chromate pretreated and control rats. The order of the efficiency of chelating agents to remove mercury from the kidney was the same in both groups: D-penicillamine < N-acetyl-penicillamine < DMSA.

In the chromate pretreated rats, chelation also decreased the blood mercury concentration. Mobilisation of mercury from the kidney did not necessarily increase the urinary excretion of mercury. D-penicillamine and N-acetyl-penicillamine had no effect on the urinary excretion of mercury in either of the two groups, but DMSA increased the urinary mercury excretion by 4.1 times in controls, and by 3.5 times in the pretreated rats (table 2). By contrast, the faecal excretion of mercury was higher in the pretreated rats than in the controls both during the

Table 1 Effect of renal damage on kidney, liver, and blood concentrations of mercury before and after chelation treatment*

Pretreatment	Chelating agent†	No	Hg^{2+} contents in % of dose (mean $\pm$ SEM)					
			Kidneys		Liver		Blood	
			48 h	96 h	48 h	96 h	48 h	96 h
Saline	—	15	18.1 ± 0.79	20.2 ± 0.62	6.6 ± 0.49	3.3 ± 0.37	5.3 ± 0.40	1.4 ± 0.14
Saline	D-penicillamine	5	—	$14.6 \pm 1.08§$	—	4.1 ± 0.64	—	1.0 ± 0.25
Saline	N-acetyl-D,L-penicillamine	5	—	$11.3 \pm 1.08§$	—	5.0 ± 0.64	—	1.4 ± 0.25
Saline	DMSA	5	—	$10.4 \pm 1.08§$	—	3.3 ± 0.64	—	1.2 ± 0.25
Sodium chromate	—	15	$7.7 \pm 0.85‡$	$9.9 \pm 0.62‡$	$9.5 \pm 0.87‡$	$5.3 \pm 0.37‡$	5.3 ± 0.36	$2.3 \pm 0.14‡$
Sodium chromate	D-penicillamine	5	—	7.2 ± 1.08	—	4.6 ± 0.64	—	$1.2 \pm 0.25§$
Sodium chromate	N-acetyl-D,L-penicillamine	5	—	$6.0 \pm 1.08§$	—	5.8 ± 0.64	—	$1.6 \pm 0.25§$
Sodium chromate	DMSA	5	—	$4.1 \pm 1.08‡§$	—	$6.7 \pm 0.64‡$	—	1.8 ± 0.25

*Kidney damage was produced by subcutaneous administration of 20 mg sodium chromate/kg body weight given three days before administration of HgCl_2 in an intraperitoneal dose of $2.5 \mu\text{moles/g}$.

†Chelating agents were given 48 and 54 h after HgCl_2 in doses of $400 \mu\text{moles/kg}$.

‡Significantly different ($p < 0.05$) from the corresponding control (without chromate pretreatment).

§Significantly different ($p < 0.05$) from the mean of animals which were not given any of the chelators.

Table 2 Effect of renal damage on the excretion of mercury before and after chelation treatment*

Pretreatment	Chelating agent†	Excretion of Hg ²⁺ in % of dose (mean ± SEM)			
		In urine		In faeces	
		0-48 h	48-96 h	0-48 h	48-96 h
Saline	—	19.4 ± 0.61 (n = 29)	4.7 ± 0.73 (n = 15)	17.9 ± 0.92 (n = 29)	11.8 ± 0.71 (n = 15)
Saline	D-penicillamine	—	5.2 ± 1.41 (n = 4)	—	14.2 ± 1.38 (n = 4)
Saline	N-acetyl-D,L-penicillamine	—	3.0 ± 1.26 (n = 5)	—	17.9 ± 1.23§ (n = 5)
Saline	DMSA	—	19.4 ± 1.26§ (n = 5)	—	12.1 ± 1.23 (n = 5)
Sodium chromate	—	8.8 ± 0.50‡ (n = 29)	3.6 ± 0.73 (n = 15)	24.3 ± 1.05‡ (n = 29)	16.3 ± 0.71‡ (n = 15)
Sodium chromate	D-penicillamine	—	4.9 ± 1.26 (n = 5)	—	16.7 ± 1.23 (n = 5)
Sodium chromate	N-acetyl-D,L-penicillamine	—	4.0 ± 1.26 (n = 5)	—	19.6 ± 1.23§ (n = 5)
Sodium chromate	DMSA	—	12.8 ± 1.26‡§ (n = 5)	—	12.5 ± 1.23§ (n = 5)

* † ‡ § See footnotes to table 1.

Effects of pretreatment of rats with 20 mg/kg sodium chromate on concentration of mercury in the kidney, liver, and blood in rats treated intraperitoneally with mercury-penicillamine (2.5 μ moles HgCl₂ + 7.5 μ moles D-penicillamine/kg body weight) three days (triangles) or seven days (squares) after chromate. Circles show values for control rats.

first and the second 48-hour collection period. The faecal excretion of mercury was affected only by N-acetyl-penicillamine in both the control and pretreated rats.

The difference between the renal and urinary concentrations of mercury in the control and pretreated rats indicates that all the mercury removed by DMSA from the kidneys was excreted in the urine. Mercury removed from the kidneys of the D-penicillamine- and N-acetyl-penicillamine-treated rats was not excreted into the urine and therefore if mercury was removed from the kidneys into the tubules by these penicillamines, these complexes of mercury were duly reabsorbed and redistributed.

To establish how the kidneys handle mercury-penicillamine when given as a single dose, rats were given a single injection of HgCl₂ complexed with D-penicillamine. In the first 180 minutes (see fig) the renal uptake of mercury was inhibited and the mercury content of the liver and the blood was increased by chromate pretreatment. These effects were more pronounced in the rats given mercury three days after chromate pretreatment than in those with a seven-day interval between the administration of the two nephrotoxic agents. In some animals 24-hour samples of urine and faeces were collected between 0-24 or 24-48 hours. At the end of each collection period the body burden and the mercury concentration in the three selected tissues were also estimated. Table 3 shows that pretreatment influenced body burden, urinary and faecal excretion, and the concentration of mercury in the kidney, liver, and blood. At 48 h this change was not

Table 3 Effect of renal damage on the organ contents and excretion of mercury after the administration of mercury-penicillamine*

Pretreatment	Days between pretreatment and mercury injection	1st 24 h period						2nd 24 h period					
		Hg ²⁺ in % of dose											
		Body burden	Urine	Faeces	Kidneys	Liver	Blood	Body burden	Urine	Faeces	Kidneys	Liver	Blood
Saline	3	70.9	18.9	10.0	24.4	9.1	6.6	55.0	1.4	8.6	17.5	7.2	5.5
Chromate	3	69.8	7.0†	17.7†	17.0†	9.6	5.3	60.7	2.1	17.5†	11.9†	8.2	4.3
Saline	7	73.5	17.7	7.8	26.9	11.1	6.3	61.4	1.7	7.6	16.9	9.5	5.2
Chromate	7	61.1†	21.8†	15.0†	18.0†	6.5†	3.2†	42.9†	6.2†	11.3†	16.6	4.8†	2.1†
Effect of time (days between pretreatment and HgCl ₂ administration)		‡	‡	—	—	—	—	—	‡	‡	—	—	‡
Interaction between pretreatment and time		‡	‡	—	—	—	—	‡	‡	‡	—	—	—

*Kidney damage was produced by subcutaneous administration of 20 mg/kg sodium chromate/kg body weight given three or seven days before intraperitoneal injection of HgCl₂ (2.5 μ moles/kg) mixed with D-penicillamine (7.5 μ moles/kg).

†Significantly different ($p < 0.05$) from saline pretreated rats by the least significant difference method.

‡Significant difference ($p < 0.05$) with the two-way analysis of variance.

significant for liver because the variance was too high. Table 3 also shows that some of the parameters were significantly influenced by the time interval between pretreatment and administration of mercury. There was a significant interaction between pretreatment and time in relation to body burden and urinary mercury excretion at 24 h and body burden and urinary and faecal mercury excretion at 48 h.

Discussion

A short time after the injection of Hg²⁺ the concentration of mercury in the kidney and the urine can be used as a measure of renal uptake of mercury from the blood. Clearly from the data presented in this paper renal uptake was significantly impaired when mercury was given three days after the injection of 20 mg/kg sodium chromate—that is, in the acute necrotic phase when, according to unpublished observations, the urinary excretion of alkaline phosphatase, glutamic oxalacetic transaminase, and lactic dehydrogenase remained significantly raised. Impaired uptake was noticeable both in HgCl₂ (tables 1 and 2) and Hg(Pen)₂-treated rats (table 3). When mercury-penicillamine was given seven days after chromate—that is, in the regenerating phase when tubular cells without a brush border replace damaged cells (unpublished observation by Dr J P R Cabral)—the renal accumulation of mercury was slower but urinary excretion greater than in control rats. Despite the increased urinary excretion the kidney content of mercury hardly decreased from 24 to 48 h in the advanced phase of regeneration while the decrease in kidney mercury contents was 30% or more in control rats or in rats in the acute necrotic phase.

Despite the significant effect of acute renal damage on the renal uptake and the urinary excretion of mercury, the thiol-chelating agents removed mercury to the same extent from the kidneys in relation to the size of the renal mercury deposits in control and chromate-treated rats (table 1). Of the three compounds tested DMSA was the most efficient and was the only compound that increased the urinary excretion of mercury.

The results presented in this paper indicate that in relation to renal mercury concentrations, chelating agents have the same effect on the mobilisation of mercury from the kidney whether it is damaged or not. In the acute necrotic phase, however, the ability of kidneys to extract mercury both for renal storage and for urinary excretion is impaired, at least in the first 24 hours. With the progress of renal regeneration this effect becomes less pronounced. In the regenerating phase, compared with control kidneys, the accumulation of mercury is somewhat slower, but once mercury is stored in the kidneys its release is also slower.

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References

- Clarkson TW. The pharmacology of mercury compounds. *Ann Rev Pharmacol* 1972;12:375-406.
- Magos L. Mercury: an environmental hazard. *J Human Nutr* 1978;32:179-86.
- Friberg L, Hammarström S, Nyström A. Kidney injury after chronic exposure to inorganic mercury. *AMA Arch Indust Hyg* 1953;8:149-53.
- Joselow MM, Goldwater LJ. Absorption and excretion of mercury in man. XII. Relationship between urinary

- mercury and proteinuria. *Arch Environ Health* 1967; **15**:155-9.
- ⁵ Kazantzis G. Chronic mercury poisoning: clinical aspects. *Ann Occup Hyg* 1965; **8**:65-71.
- ⁶ Suzuki T. Plasma and urine mercury values in relation to hand tremor and proteinuria in workers exposed to mercury vapour. *Indust Health (Japan)* 1977; **15**:77-85.
- ⁷ Clarkson TW, Magos L. Studies on the binding of mercury in tissue homogenates. *Biochem J* 1966; **99**:62-70.
- ⁸ Balazs T, Hatch A, Zavidzka Z, Grice HC. Renal tests in toxicity studies on rats. *Toxicol Appl Pharmacol* 1963; **5**:661-74.
- ⁹ Evan AP, William GD. The effects of sodium chromate on the proximal tubules of the rat kidney. *Lab Invest* 1974; **30**:704-15.
- ¹⁰ Aposhian HV, Aposhian MM. N-acetyl-DL-penicillamine, a new oral protective agent against the lethal effects of mercuric chloride. *J Pharmacol Exp Ther* 1959; **126**:131-5.
- ¹¹ Pagnotto LA, Brugsch HG, Elkins HB. Treatment of chronic mercurialism with N-acetyl-penicillamine. *Am Ind Hyg Assoc J* 1960; **21**:419-22.
- ¹² Goldblatt D, Greenwood MR, Clarkson TW. Chronic metallic mercury poisoning treated with N-acetyl-penicillamine. *Neurology* 1971; **21**:439.
- ¹³ Teisinger K, Srbova J. Effect of D-penicillamine on the urinary excretion of mercury and lead. *Pracovni Lekar*, 1964; **16**:433-5.
- ¹⁴ Magos L. The effects of dimercaptosuccinic acid on the excretion and distribution of mercury in rats and mice treated with mercuric chloride and methylmercury chloride. *Br J Pharmacol* 1976; **56**:479-84.
- ¹⁵ Magos L, Stoytchev T. Combined effect of sodium maleate and some thiol compounds on mercury excretion and redistribution. *Br J Pharmacol* 1969; **35**:121-6.
- ¹⁶ Friedheim E, Graziano JH, Popovac D, Dragovic D, Kaul B. Treatment of lead poisoning by 2,3-dimercaptosuccinic acid. *Lancet* 1978; **2**:1234-5.
- ¹⁷ Snedecor GW, Cochran WG. *Statistical methods*. 6th ed. Ames: Iowa State College Press, 1967.