

## CADMIUM EXCRETION IN MICE GIVEN DIMERCAPTOPROPANESULPHONATE AND SOME OTHER COMPLEXING THIOLS

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### ABSTRACT

The toxic cadmium ion accumulates in mammalian organisms and has been reported difficult to remove from animal tissues by use of traditional chelating agents such as BAL (dimercaptopropanol) or EDTA (ethylenediamine tetraacetate).

In this study mice were given 5  $\mu\text{mol CdCl}_2/\text{kg}$  body weight intravenously. The control group excreted about 30 nmol during the subsequent seven-day-period. Oral treatment was instituted on the first day after the injection and was repeated daily during this period with D-penicillamine, N-acetyl-DL-penicillamine, mercaptosuccinate, dimercaptosuccinate (DMS) or dimercaptopropanesulphonate (Unithiol) (10 mmol SH/kg daily). This treatment did not reduce cadmium levels in the liver, kidneys, brain or blood, although DMS and Unithiol did increase the urinary excretion on the first day from 0.3 to 3-5 nmol Cd. An immediately given intravenous injection of DMS or Unithiol (1 mmol SH/kg) was an effective treatment, raising the urinary excretion on the first day to 40-50 nmol Cd, and lowering the liver and kidney levels to about 50% of controls. However, a treatment delay of only half an hour considerably reduced the effect. No effect was observed when the intravenous treatment was delayed six hours or more.

Cadmium is a highly toxic element which, in mammalian organisms has a long biological half-life (25-100 days in mice and many years in man)<sup>13,15</sup>. The effects of exposure to  $\text{Cd}^{++}$  include damage of renal tubuli and liver cells. Cadmium is also considered as an important causal factor in the Itai-Itai disease.

Previous attempts to increase the excretion of  $\text{Cd}^{++}$  by use of chelation therapy have been disappointing. In fact, the use of complexing agents in the treatment of cadmium poisoning in man has been discouraged<sup>4,5</sup>. Both BAL (2,3-dimercaptopropanol) and EDTA (ethylene diaminetetraacetate) as well as penicillamine appear to enhance the nephrotoxicity of cadmium in animals<sup>5,7,8,10,11,14</sup>. This effect of  $\text{Cd}^{++}$ -complexing agents may partially be due to their ability to increase the transfer of the cation from the liver to the kidney without increasing the urinary excretion<sup>6,11,14</sup>. Another polycarboxylate

derivative NTA (nitrilotriacetate), has been claimed to have protective effects, although other reports state that NTA enhances the teratogenicity and lethality of  $\text{CdCl}_2$  in rats<sup>12</sup>.

In recent years some derivatives of succinic acid and of BAL have been shown to be effective chelating agents toward mercury compounds<sup>1</sup>. These derivatives include mercaptosuccinic acid, 2,3-dimercaptosuccinic acid (DMS) and 2,3-dimercaptopropane-1-sulphonate (Unithiol). Furthermore, *in vitro* studies indicate that mercaptosuccinic acid, DMS and Unithiol are more effective than D-penicillamine and N-acetyl-DL-penicillamine to remove  $\text{Cd}^{++}$  from cadmium-containing epithelial cells cultured *in vitro*<sup>3</sup>. The toxicity of these three most effective agents was lower than that of BAL judged by the effect on cultured cells<sup>3</sup>.

The promising effects *in vitro* of Unithiol and of the succinate derivatives precipitated the present animal studies, in which the  $\text{Cd}^{++}$ -mobilizing effects of the same agents are investigated in mice.

#### MATERIALS AND METHODS

Radioactive cadmium chloride ( $^{109}\text{CdCl}_2$ ), a gamma emitter, was purchased from Radiochemical Centre, Amersham. The physical half-life of this isotope is 453 days. N-acetyl-DL-penicillamine, D-penicillamine and mercaptosuccinic acid were products of Koch-Light Ltd., England. Unithiol was obtained from Heyl & Co., Berlin. DMS was kindly donated by Dr. Friedheim, Geneva. Mercaptodextran was synthesized from dextran with average molecular weight 20 000 as previously described<sup>9</sup>.

Female mice of NMRI strain, weighing  $20 \pm 1$  g, were given a single intravenous injection of  $5 \mu\text{mol CdCl}_2/\text{kg}$  (about 100 nmol Cd and  $1 \mu\text{Ci}$  per animal). The mice were housed in metabolic cages as previously described<sup>2</sup>. Oral treatment with 10 mmol SH/kg of a complexing thiol was given to four mice for a 7-day-period from the first day after the cadmium injection. The thiols studied were as follows: D-penicillamine, N-acetyl-DL-penicillamine, Unithiol, mercaptosuccinate and DMS. The thiols were mixed into the food which the mice consumed daily.

Another series of experiments were designed to study the effect of intravenous treatment on cadmium excretion. One group of cadmium-injected mice was kept as non-treated controls while others were given Unithiol, DMS (1 mmol/kg) or Mercaptodextran (0.25 mmol/kg) intravenously. The thiols were either administered immediately (1–2 minutes after the cadmium-injection) or after a delay of half an hour or 6 hours after the  $^{109}\text{CdCl}_2$ -injection.

The radioactivity in organs and blood of the animals was measured by means of 0.88 MeV irradiation top of the isotope in a Packard spectrometer 3001. The radioactivity in the faeces, urine as well as in the whole animal was determined in a whole body counter. By simultaneous counting of a reference standard with a known amount of radioactivity and with the same specific activity as the injected solution the cadmium content in the specimens could be calculated.

## RESULTS AND DISCUSSION

## Oral treatment

In the control animals injected with 5  $\mu\text{mol Cd/kg}$  the urinary excretion was only 0.3 nmol (range: 0.1–0.4 nmol) on the first day after the injection, while 10 nmol (range: 8–11 nmol) was excreted in the faeces. After 7 days the cumulative excretion was about 0.7 nmol in the urine and 32 nmol in the faeces. Oral treatment with D-penicillamine, N-acetyl-DL-penicillamine or mercaptosuccinate had no effect on the urinary or faecal excretion of cadmium. The treatment with Unithiol or DMS (10 mmol SH/kg daily) increased the urinary excretion of cadmium the first day after the metal injection (Fig. 1), but had no effect on the cadmium excretion the following days.

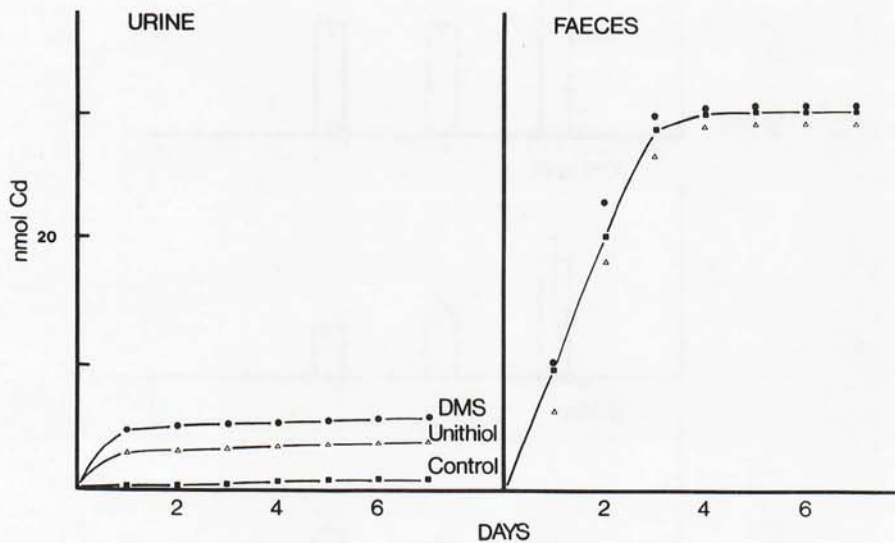


FIG. 1 – Cumulative urinary and faecal excretion of cadmium during a 7-day-period after injection of  $\text{CdCl}_2$  (5  $\mu\text{mol/kg}$ ) to mice. Mean values are given for three groups of mice. One group was given 10 mmol SH/kg of Unithiol daily (represented by triangles), the other received dimercaptosuccinate (DMS) treatment (represented by circles) and the third group remained untreated and served as control group (represented by squares). Each value represents the mean of four mice.

The cadmium levels in the liver and kidney of untreated controls 7 days after the cadmium injection were 47 nmol/g (38–58 nmol/g) and 23 nmol/g (19–26 nmol/g), respectively. These levels were not significantly changed by treatment with the complexing thiols tested here. The brain level, 0.18 nmol/g (0.15–0.20 nmol/g), also remained unchanged.

## Intravenous treatment

Immediate intravenous treatment with DMS as well as with Unithiol (1 mmol/kg) increased the urinary excretion the first day to 43 nmol (35–60 nmol)

and 49 nmol (41–65nmol), respectively. The faecal, as well as the urinary, excretion from the second day on after the cadmium injection was unchanged as compared to untreated controls. Two days after the  $\text{CdCl}_2$ -injection the cadmium levels in the liver and kidney were significantly reduced as compared to control values (Fig. 2). The cadmium content in the blood and brain, however, was not changed by such treatment.

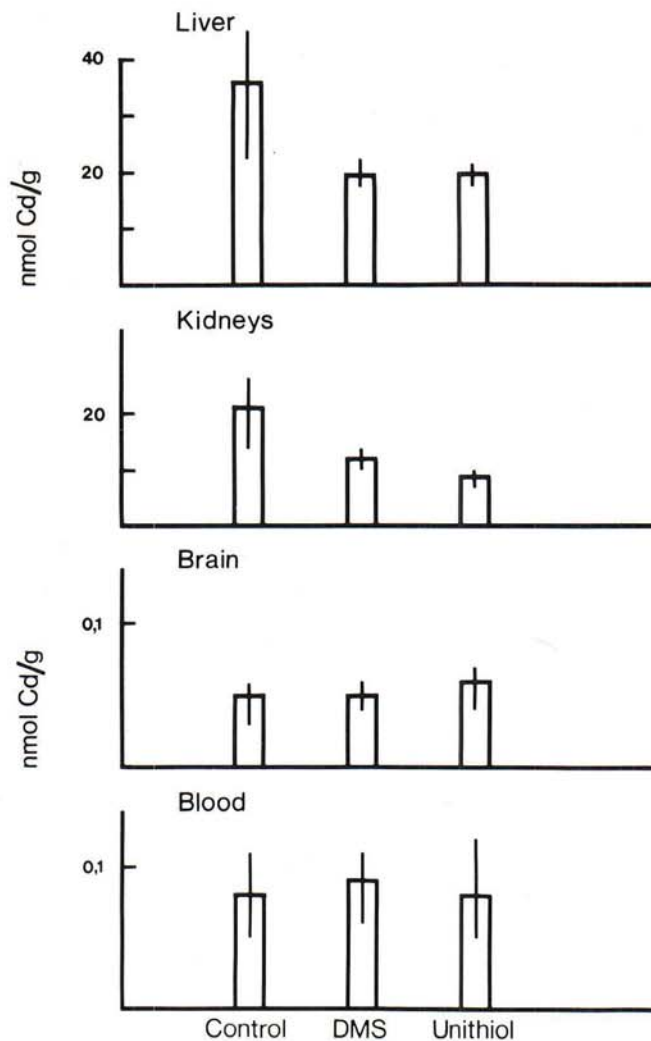


FIG. 2 – Effect of immediate intravenous treatment on the concentration of cadmium in the liver, kidney, brain and blood 2 days after an intravenous injection of  $5 \mu\text{mol/kg}$  of  $\text{CdCl}_2$  to mice. Mean and range of four animals are given for a control group, for a group treated with DMS (1 mmol SH/kg) and for a group treated with Unithiol.

Mercaptodextran (0.25 mmol SH/kg), given intravenously, also increased the urinary excretion on the first day to 25 nmol (21–29 nmol), which was reflected by a marginal lowering of the liver and kidney levels of cadmium.

When the chelation treatment was given 30 min after the injection of  $\text{CdCl}_2$  its effect on urinary excretion was much smaller than that produced by immediate treatment. The urinary excretion the first day after such delayed Unithiol treatment was 9 nmol (7–11 nmol) and the treatment brought about a marginal decrease of the liver and kidney levels of cadmium (Fig. 3). When the treatment with Unithiol was given 6 hours after the injection of  $\text{CdCl}_2$  no effect was observed on the excretion or distribution of the metal.

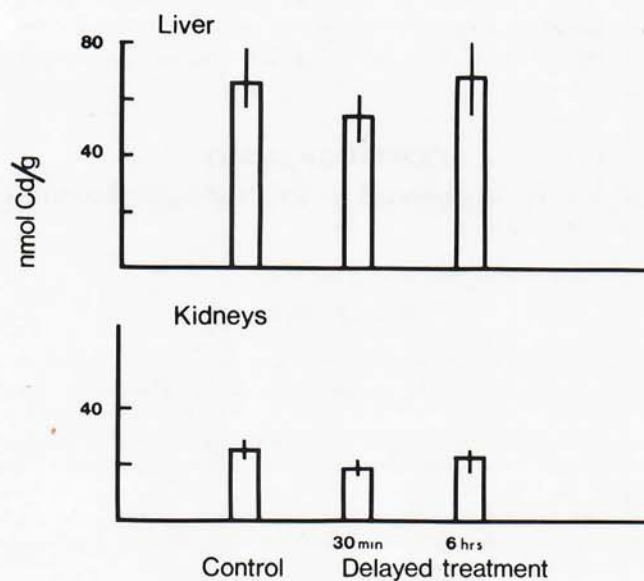


FIG. 3 – Effect of delayed treatment with Unithiol (1 mmol SH/kg) on concentration of cadmium in the liver and kidneys 3 days after an intravenous injection of 5  $\mu\text{mol/kg}$  of  $\text{CdCl}_2$  to mice. The thiol was given intravenously to mice either 30 min. or 6 hours after the cadmium injection, and mean and range of four animals are given in the figure.

#### DISCUSSION

Immediately applied intravenous chelation therapy is supposed to bring about a transfer of  $\text{Cd}^{++}$  from serum proteins to the chelating agent. The  $\text{Cd}^{++}$ -DMS and the  $\text{Cd}^{++}$ -Unithiol-chelate, as well as  $\text{Cd}^{++}$ -Mercaptodextran appear in our study to be rapidly excreted in the urine. After the removal of the metal from serum proteins, the uptake of cadmium in the liver and kidney was reduced. Oral treatment with the dithiols DMS or Unithiol also had some effect on urinary cadmium excretion when given early after the exposure, whereas monothiols such as penicillamine seemed to lack cadmium-chelating effect *in vivo*.

Circulating cadmium is known to be rapidly taken up by blood cells and liver cells<sup>12</sup>. It is also taken up by other tissues, particularly the kidney. Intracellularly deposited cadmium is known to be tightly fixed by low molecular weight cytoplasmic proteins, the metallothioneins<sup>3,12</sup>.

Previous *in vitro* studies<sup>3</sup> have shown that mercaptosuccinates as well as Unithiol can partly remove cadmium from the epithelial cells containing cadmium-metallothionein. The present study shows that the treatment with the same agents *in vivo* is ineffective when cadmium is bound by the tissue cells. Apparently the fixation of cadmium in the liver and kidney cells is too tight to be reversed even by relatively high levels of Unithiol in the circulation.

This study indicates that the practical usefulness of chelation therapy in cadmium poisoning is still of limited value. Whether or not immediate intravenous treatment with Unithiol or DMS can reduce cadmium toxicity requires further studies.

#### ACKNOWLEDGEMENT

This work was partly supported by The Norwegian Research Council for Science and the Humanities.

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