Inorganic Mercury Exposure, Mercury–Copper Interaction, and DMPS Treatment in Rats

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The aim of this study was to evaluate the efficiency of oral treatment with sodium 2,3-dimercaptopropane-1-sulfonate (DMPS) on reducing mercury deposits in rat kidney after chronic exposure to inorganic mercury. The effect on kidney copper levels was also evaluated. The results showed that after two months of exposure to 50 ppm of mercury (as mercuric chloride) the concentration of mercury in the kidney was 124 μg/g wet tissue. At the same time copper concentration rose from 11 to 77 μg/g. DMPS treatment caused 2- and almost 4-fold reduction of mercury and copper, respectively. This study demonstrates that chronic exposure to inorganic mercury may alter metabolism of copper and that DMPS is an effective means for reduction of both mercury and copper. — Environ Health Perspect 102(Suppl 3):305–307 (1994).

Key words: mercury–copper interaction, DMPS, sodium 2,3-dimercaptopropane-1-sulfonate, kidney, rat

Introduction

The most important mercury-binding protein in the kidney is metallothionein (MT), which can bind other divalent essential metals as well (1). A sequence reflecting the divalent-binding affinities of MT is, in decreasing order, Hg>Cu>Cd>Zn (2). It has been found that association of mercury with MT in kidneys resulted in altered concentration of copper and zinc (3). Since MT has a high affinity for certain essential metals, it plays an important role both in homeostasis of essential metals and in detoxification of nonessential metals (4).

Sodium 2,3-dimercaptopropane-1-sulfonate (DMPS), presently used as an antidote in mercury intoxication, has a significant advantage over other chelating agents. It is water soluble, it has intestinal absorption of 30 to 50%, and it has much lower toxicity than 2,3-dimercaptopropanol (BAL) (5). The effectiveness of DMPS for enhancing mercury elimination from the kidneys of rats has been reported both in animals (6) and in man (7).

Mobilization of mercury bound to MT after chelation with DMPS resulted in removal of mercury from MT, and enhancement of mercury elimination from the body. It was proposed that chelators like DMPS, whose complexes are not lipid soluble, mainly mobilize mercury stored in the kidney and favor urinary excretion (8).

Although a mercury-copper interaction was previously found (9), there was no information available on the effect of DMPS on copper homeostasis in chronic mercury intoxication.

The purpose of this study was to determine the efficiency of DMPS in reducing mercury deposits in organs after long-term exposure to inorganic mercury and its effect on copper levels. We found that DMPS efficiently eliminates both copper and mercury from kidney stones.

Materials and Methods

Animals

The experiments were performed on 70 female, Wistar-derived rats from the Institute’s breeding farm. Rats were 6 weeks old at the beginning of the experiment with an average body weight of 127 g. They were divided into seven groups and housed in plastic Macolon cages with five animals in each cage. Standard commercial laboratory diet and drink were provided ad libitum. Control animals were kept in the same room with the exposed groups. Food and drinking water as well as body weights were recorded weekly. All exposed groups were given 50 ppm of mercury (as mercuric chloride, pa. Kemika, Zagreb, Croatia) in drinking water. One group was given mercury during 1 month (treatment 1), two groups were given mercury during two months (treatment 2) and two groups were given mercury during one month and distilled water during another month thereafter (treatment 3). Respective control groups received distilled water during the same period. DMPS (Sigma Chemical Co, St. Louis, MO) was given orally by stomach tube to mercury-exposed groups in treatments 2 and 3, in a dose of 0.44 mmole/kg body weight during four consecutive days before the end of the experiment. At the end of each treatment, animals were sacrificed and kidneys dissected. Left and right kidneys were used for mercury and copper analysis, respectively.

Determination of Mercury and Copper

Analysis of mercury was performed using the cold-vapor atomic absorption spectrophotometry method (CVAAS) (10) using a Mercury Monitor LDC (Milton Roy, Riviera Beach, FL). The composite samples of left kidneys from each group were used for mercury analysis. Pooled samples were homogenized and about 1 g of each sample was wet digested with concentrated nitric acid at 80°C in closed ampules. Mercury analysis was carried out 7 to 18 times in each composite digested sample.

The detection limit of the method was 0.4 ng/ml for analyte solution, i.e., 4 ng/g wet weight of tissue. The accuracy of the method was checked by analyzing reference materials: horse kidney H-8 (IAEA) and pig kidney CRM-186 (BCR) with reference values of 910±40 and 1970±40 ng/g (mean±SEM). Our values obtained for reference samples were 1010±28 and 1994±40 ng/g, respectively.

Copper was determined by flame atomic absorption spectrophotometry (AAS; Varian AA375) with deuterium background correction. Samples of right
Table 1. Mercury and copper concentrations in rat kidneys exposed to 50 ppm of mercury (as mercuric chloride) in drinking water and the effect of DMPS treatment.

| Mercury (μg/g w/w) | Treatment 1 | Treatment 2 | Treatment 3 |
|-------------------|-------------|-------------|-------------|
| Control           | 0.170 ± 0.007 | 0.373 ± 0.010 | 1.14 ± 0.02 |
| Hg-exposed        | 122 ± 3.2 | 124 ± 7.4 | 75.3 ± 1.7 |
| Hg+DMPS<sup>a</sup> | -50.3 ± 1.3<sup>c</sup> | 27.9 ± 0.7<sup>c</sup> | 1.8 ± 0.1<sup>c</sup> |
| Copper (μg/g w/w) | 10.7 ± 1.1 | 14.6 ± 0.8 | 1.31 ± 0.04 |
| Control           | 80.0 ± 3.9 | 77.0 ± 4.6 | 63.9 ± 3.8 |
| Hg-exposed        | -21.7 ± 1.7<sup>c</sup> | 10.9 ± 0.7<sup>c</sup> | 1.66 ± 0.04 |

<sup>a</sup>Treatments: 1, exposure to mercury during one month; 2, exposure to mercury during two months; 3, exposure to mercury during one month with an additional recovery period of one month. DMPS treatment was performed orally for four consecutive days at the end of treatments 2 and 3. Significantly different from respective mercury-exposed groups at the level of p<0.001. Each value represents the arithmetic mean ± SE of the mean of 7 to 18 determinations of the composite samples for mercury analysis, and of 10 determinations of individual samples for copper analysis. Control animals received distilled water.

Kidneys were treated individually by wet digestion with 10 ml of concentrated nitric acid in a digestion system (DS-40, Tecator, Höganäs, Sweden).

Results are presented as arithmetic mean ± SEM. Differences obtained by Student’s t-test at the 1% confidence level were denoted as statistically significant.

Results

Body weights of the animals did not differ significantly in any of the treatments at the end of the experiments. The consumption of drinking water in control and exposed groups was about 30 and 20 ml, respectively. The average daily food intake for all groups was about 15 g. The kidneys of rats exposed to mercury during one (treatment 1) or two (treatment 2) months had mean wet weights of 1.50 ± 0.03 and 1.66 ± 0.04 g compared to control groups for the same period of 1.23 ± 0.03 and 1.31 ± 0.04 g, respectively. This difference was statistically significant (p<0.01). Kidney weights after treatment 3 were 1.42 ± 0.05 g, which was not significantly different from the control.

Table 1 shows the concentrations of mercury and copper in the kidneys of mercury-exposed rats for the various treatment protocols. These data are compared with those obtained with DMPS chelation treatments. The results showed that after one or two months of exposure to 50 ppm of mercury, the kidney and mercury concentrations were 122 and 124 μg/g, respectively. At the same time, copper rose from 10.7 in control groups to 80.0 and 77.0 μg/g in exposed groups, respectively. In treatment 3, after one month of recovery, mercury concentrations lowered spontaneously to 75 μg/g, while copper levels remained high at 64 μg/g. Chelation with DMPS (treatment 2) decreased the concentration of mercury and copper 2.1- and 3.5-fold, respectively. Mercury and copper concentrations in rat kidneys after treatment 3 plus chelation with DMPS decreased 2.7- and 5.8-fold, respectively. These results show that only copper concentrations were returned to control values after treatment 3 with DMPS.

Discussion

Higher kidney weights but no change in body weights were observed after one and two months of exposure to mercury in our experiments. These data are in agreement with previously found higher kidney weights even at a lower mercury concentration in drinking water, i.e., 20 ppm (1). The kidney weights of recovered animals (treatment 3) did not differ significantly from the controls.

The concentration of mercury after one or two months of exposure to mercuric chloride did not differ significantly (p>0.01). This suggests that the mercury concentration reaches a steady-state in the rat kidney even after one month of exposure to inorganic mercury. Similar mercury kidney levels were found at different mercury exposure concentrations and lengths of mercury exposure (3,11). In this study, the steady-state mercury concentration obtained in the kidneys may be due to a balance between enhanced production and excretion of MT from the kidney cytosol in urine (2,8,11).

The kidney copper levels in mercury-intoxicated rats were found to be about six times higher than in controls and remained at the same level after one or two months of mercury exposure. This indicates that copper also reaches a plateau. The effect of inorganic mercury on the accumulation of copper in rat kidneys has been reported previously (9,12). Bogden and Boscolo found a 5-fold higher mean kidney copper concentration following the exposure to 20 and 50 ppm of mercury in drinking water during 44 and 350 days, respectively. Marked increases of copper levels in mercury-intoxicated rats could be explained by induction of MT biosynthesis, which can strongly bind copper along with mercury.

Although the mercury-copper interaction in rat kidneys has previously been reported, the influence of the chelating agent DMPS on the level of copper in mercury-exposed rats had not been studied until now. DMPS was found to be very efficient in reducing mercury accumulation in the rat kidneys, where most of body burden of mercury is located (6,13). The efficiency of DMPS treatment was found to be dependent on age, dose, time, and route of administration of both mercury and chelating agent. Higher doses of DMPS in delayed treatment were more effective particularly in reducing mercury accumulation in the kidneys of older rats (14,15). Mercury and copper concentrations in treatment 2 after chelation with DMPS were 2.1 and 3.5 times lower than in respective controls. In treatment 3, after DMPS chelation, these reduction factors were even higher, i.e., 2.7 and 5.8, respectively.

We have previously observed that DMPS, if given orally immediately after administration of 200 μg Hg in rats, increases mercury retention, and we concluded that DMPS should not be administered orally while mercury is still in the gastrointestinal tract (15). In the current experiment DMPS was administered orally while the animals were still being treated with mercury in drinking water. Under these conditions of exposure, DMPS caused a 2-fold reduction of mercury in the kidney. Although we cannot eliminate the possibility that DMPS caused increased gastrointestinal absorption of mercury during the 4 days of treatment, the 2-fold decrease of kidney mercury indicates that DMPS was able to mobilize the metal. This mobilization was very effective even after 1 month of recovery from the mercury exposure (Treatment 3) which indicates that DMPS can be used as late therapy for elimination of mercury.

This study demonstrates that chronic exposure to 50 ppm of mercury induces changes in the levels of copper in rat kidneys, and that DMPS is an effective antagonist for reduction of both mercury and copper in this organ. Finally, the protective effect of DMPS against mercury-induced toxicity suggests the need to explore its therapeutic potential as well as its effect on decreasing copper levels in diseases unrelated to mercury overload.
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