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Simultaneous administration of sodium selenite and mercuric chloride decreases efficacy of DMSA and DMPS in mercury elimination in rats

Dijana Jureša*, Maja Blanuša, Krista Kostial

Mineral Metabolism Unit, Institute for Medical Research and Occupational Health, P.O. Box 291, HR-10001 Zagreb, Croatia

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Abstract

Two chelating agents *meso*-2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercapto-propane-1-sulphonate (DMPS) were tested for their efficiency in mercury removal from the body of rats in the presence and in the absence of selenium. Female Wistar rats were given a single intraperitoneal injection of mercuric chloride or an equimolar mixture of mercuric chloride and sodium selenite (1.5 μmol/kg body weight). The chelating agents were given orally, in excess (500 μmol DMSA/kg body weight; 300 μmol DMPS/kg body weight), 30 min after the administration of mercury and selenium. The animals were euthanized 24 h after the treatment and mercury in the kidney, liver, and 24 h urine was determined using cold vapour atomic absorption spectrometry (CV-AAS). The simultaneous administration of mercuric chloride and sodium selenite led to a redistribution of mercury in the organs, so that accumulation of mercury in the kidneys was decreased and in the liver increased. Selenite also caused decrease in the level of urinary mercury excretion. Both chelating agents were effective in mercury removal from the body, by increasing its urinary excretion. However, when animals were simultaneously treated with mercury and selenite, the rise of mercury excreted in the urine due to the treatment with chelating agents was lower when compared to animals receiving mercury without selenite. It is concluded that sodium selenite decreases the efficiency of DMSA and DMPS in mercury removal from the body of rats.

Keywords: Mercuric chloride; Sodium selenite; Interaction; Chelation therapy; *Meso*-2,3-dimercaptosuccinic acid; 2,3-Dimercapto-propane-1-sulphonate

1. Introduction

The interaction between mercury and selenium in the body of mammals has been known for more than three decades. Since Parizek and Ostadalova (1967)

* Corresponding author. Tel.: +385 1 4673188; fax: +385 1 4673303.
E-mail address: djuresa@imi.hr (D. Jureša).

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found that the toxicity of inorganic mercury was decreased by simultaneous injection of selenite, many studies have been carried out to examine the role of selenium in the detoxification of mercury, which have led to many hypotheses about the mechanism of this interaction (reviewed by Cuvin-Aralar and Furness (1991)). Although the complete mechanism of mercury–selenium interaction is still unsolved, there is evidence that selenium in plasma forms a complex with mercury, which then binds to selenoprotein P (Sasakura and Suzuki, 1998; Gailer et al., 2000). Formation of mercury–selenium complex with selenoprotein P causes redistribution of mercury in the organism. In the presence of selenium, the accumulation of mercury in the target organ, kidney is reduced, but body retention of mercury on the whole is increased, especially in the liver (Cuvin-Aralar and Furness, 1991). Selenium also affects mercury elimination, by reducing urinary and fecal excretion (Magos and Webb, 1976; Fang, 1977). Therefore, body load of mercury remains high despite of lower toxicity noticed in the presence of selenium.

Urine is a main pathway for the elimination of inorganic mercury from the body. A method for the detoxification of mercury that is widely recommended is its transformation into a chelate complex (Jones, 1994), which has a water solubility greater than that of the mercury alone and therefore is readily excreted in the urine. In this manner, body levels of mercury are reduced to a less dangerous level. Metal binds to two or more atoms of the chelating agent and forms a complex which is, in general, soluble and less toxic than the metal compound from which it is derived. The chelating agent competes with the biological binding sites for the metal ion through the process of ligand exchange, and usually has a greater affinity for the metal ion than biological ligands. Thus, chelating agents decrease metal toxicity caused by the binding of the metal ion to biologically important molecules. *Meso*-2,3-dimercaptosuccinic acid (DMSA) and sodium 2,3-dimercapto-propane-1-sulphonate (DMPS) are chelating agents verified to be effective for the treatment of mercury intoxication in animals and humans (Aposhian et al., 1995). DMPS is often used as challenge test for the diagnoses of mercury exposure, especially after long term exposure from dental amalgams (Torres-Alanis et al., 2000; Vamnes et al., 2000).

The purpose of this study was to evaluate the effectiveness of DMSA and DMPS for the removal of mercury from the body of rats when mercury is simultaneously administered with selenium. The hypothesis was that the formation of mercury complex with selenium and selenoprotein P, which causes redistribution of mercury in the organism and decreases mercury excretion, could affect the efficiency of chelating agents in mercury removal.

2. Materials and methods

2.1. Experimental design

Two experiments with the same protocol were carried out. Influence of selenium on the effectiveness of the chelating agents DMSA (Experiment 1) and DMPS (Experiment 2) was tested. In each experiment female Wistar rats (from the Laboratory Animals Unit of the Institute for Medical Research and Occupational Health, Zagreb, Croatia), weighing at the beginning of the experiments approximately 170 g, were randomly divided into four groups of 9 or 10 animals in each. Animals received mercuric chloride or a mixture of mercuric chloride and sodium selenite intraperitoneally (ip), followed by an oral administration of the chelating agent. The groups were assigned: Hg; Hg + chelating agent; Hg + Se; Hg + Se + chelating agent. Chelating agents were administered 30 min after mercury or mercury and selenium administration. After the treatment, all animals were placed individually in cylindrical aluminium metabolic cages (described by Howells et al. (1964)) for 24 h for urine collection. Animals had ad libitum access to water, but no food. Light:dark cycle was 12:12 h (07:30–19:30 h; light) and the room temperature was maintained at 22–24 °C. After 24 h animals were euthanized by exsanguination from the abdominal aorta in light ether anaesthesia and liver and left kidney were removed for further analyses.

All procedures with animals were carried out in accordance with guidelines on the protection of animal welfare and were approved by the Croatian Ministry of Agriculture and Forestry.

2.2. Administered compounds

Mercury, as mercuric chloride ($HgCl_2$, pro analysi grade, Kemika, Croatia), was given in an ip injection

at a dose of 1.5 μmol Hg/kg body weight in a volume of 0.5 ml deionised water. The applied dose represented approximately 10% of LD_{50} for mercuric chloride (Maljković, 1983).

Selenium, as sodium selenite (Na_2SeO_3, pro analysi grade, Sigma, Japan), was given mixed with mercury in a single ip injection at an equimolar dose of 1.5 μmol/kg body weight in a volume of 0.5 ml deionised water.

The chelating agents were given orally by stomach tube. In Experiment 1, DMSA (Aldrich Chemical Co., USA) was given at a dose of 500 μmol DMSA/kg body weight in a volume of 0.5 ml, dissolved in 5% $NaHCO_3$. Groups not receiving DMSA were given the same volume of 5% $NaHCO_3$. In Experiment 2 a volume of 1.35 ml of sodium DMPS (solution for injection, Dimaval®, Heyl, Germany) per kg body weight was given, which made a dose of 300 μmol DMPS/kg body weight. Groups not receiving DMPS were given the same volume of physiological saline (0.9% NaCl). Since DMPS is more toxic than DMSA, a lower dose of DMPS was given. Both doses represented less than 5% of LD_{50} (determined in mice) (Aposhian et al., 1995), for each chelating agent.

2.3. Analysis of mercury in urine and organs

Mercury in collected samples was determined according to a modified Farant's method (Farant et al., 1981; Prester et al., 1998) by cold vapour atomic absorption spectrometry in a Mercury Monitor (LDC/Milton Roy, FL, USA) after the samples were digested in a closed glass tubes with nitric acid at 80 °C. Approximately 1 g of liver, 2 ml of urine and whole kidney were digested with 2 ml of nitric acid. To check the accuracy of determination, the certified reference material horse kidney H8 from the International Atomic Energy Agency (IAEA) was used. Preparation and measurements for the reference material were identical as for experimental samples. Measured values in the reference material (0.96 ± 0.66 μg Hg/g dry weight; n = 20; mean and standard deviation) fell within the range of certified values (0.91 ± 0.70 μg Hg/g dry weight; mean and 95% confidence limits).

2.4. Statistical analysis

Statistical evaluation of the data was performed with the program "Statistica for Windows" (StatSoft 1995 package, release 5.0). The results are presented as absolute values of arithmetic means and standard deviations. Mercury content in microgram in whole organs and in 24 h urine is given. The statistical differences between groups were analysed by one-way analysis of variance (ANOVA) followed by an F-test, used to evaluate the following planned comparisons (at the level of significance of $P < 0.05$): Hg + Se versus Hg; Hg + chelating agent versus Hg; Hg + Se + chelating agent versus Hg + Se. To eliminate the heterogeneity of variances (tested by the Levene test), data were logarithmically transformed before the analyses.

3. Results

Body weights of animals were lower at the end of experiments due to deprived food during urine collection in metabolic cages. Organ weights and volume of urine excreted during 24 h were not different after different treatments.

Selenium administered simultaneously with mercury significantly altered the level of mercury in the examined organs of rats (comparison Hg + Se versus Hg) (Table 1). Mercury administered without selenium

Table 1
The effect of selenium and/or chelating agents on mercury content in whole liver and kidney in female rats 24 h after treatment

Experimental group	n	Liver (μg Hg)	Kidney (μg Hg)
Experiment 1			
Hg	10	3.10 ± 0.48	24.8 ± 4.2
Hg + DMSA	10	2.42 ± 0.26	5.83 ± 0.84*
Hg + Se	10	19.8 ± 2.0*	1.55 ± 0.29*
Hg + Se + DMSA	9	15.5 ± 5.3**	1.76 ± 0.22
Experiment 2			
Hg	9	2.74 ± 0.56	31.4 ± 5.0
Hg + DMPS	10	2.86 ± 0.54	5.66 ± 1.44*
Hg + Se	9	15.8 ± 1.9*	0.832 ± 0.135*
Hg + Se + DMPS	10	15.3 ± 1.5	1.29 ± 0.25**

Results are presented as arithmetic mean ± S.D. Mercuric chloride was administrated intraperitoneally (1.5 μmol Hg/kg body weight), either alone or mixed in a single injection with equimolar dose of sodium selenite. Chelating agents were applied orally by stomach tube (500 μmol DMSA/kg body weight; 300 μmol/DMPS kg body weight).

* Statistically significant difference ($P < 0.05$) for planned comparisons: Hg + chelating agent vs. Hg; Hg + Se vs. Hg.

** Statistically significant difference ($P < 0.05$) for planned comparison: Hg + Se + chelating agent vs. Hg + Se.

Table 2
The effect of selenium and/or chelating agents on mercury content in 24 h urine

Experimental group	*n*	Urine (μg Hg/24 h)
Experiment 1		
Hg	10	3.99 ± 0.57
Hg + DMSA	10	23.6 ± 6.0*
(Hg + Se)	10	1.64 ± 0.70*
(Hg + Se) + DMSA	9	6.18 ± 1.37**
Experiment 2		
Hg	9	3.17 ± 0.91
Hg + DMPS	10	25.2 ± 3.7*
(Hg + Se)	9	0.366 ± 0.175*
(Hg + Se) + DMPS	10	1.81 ± 0.50**

Results are presented as arithmetic mean ± S.D. For experimental design see Table 1.

* Statistically significant difference ($P < 0.05$) for planned comparisons: Hg + chelating agent vs. Hg; Hg + Se vs. Hg.

** Statistically significant difference ($P < 0.05$) for planned comparison: Hg + Se + chelating agent vs. Hg + Se.

was deposited mainly in the kidneys, with small amount in the liver. In simultaneous exposure to mercury and selenium the situation was reversed. A higher accumulation of mercury was found in the liver. The accumulation of mercury in the kidney of groups receiving mercury and selenium was decreased more than 94% compared to groups receiving mercury only, whereas accumulation in the liver was increased about six times. The excretion of mercury in the urine was also altered by selenium (Table 2). Simultaneous administration of mercury and selenium resulted in statistically significant decrease of mercury excreted in urine.

DMSA and DMPS increased urinary mercury excretion both when mercury was administered alone (comparison Hg + chelating agent versus Hg) and when it was administered together with selenium (comparison Hg + Se + chelating agent versus Hg + Se) (Table 2). However, in the presence of selenium the efficiency of both chelating agents was lower. When mercury was administered without selenium, DMSA and DMPS increased the level of urinary mercury excretion approximately six and eight times, while in the presence of selenium urinary mercury excretion was increased approximately four and five times, respectively.

When mercury was administered without selenium both chelating agents had no influence on liver mercury content (Table 1). In the presence of selenium, only DMSA decreased mercury in the liver, while DMPS had no significant effect. Both chelating agents significantly decreased mercury in the kidney when mercury was administered alone. When mercury was simultaneously administered with selenium, mercury content in the kidney was low and the chelating agents did not additionally decrease it. On the contrary, DMPS increased mercury in the kidney approximately 1.6 times. DMSA showed no effect on mercury content in the kidney in the presence of selenium.

4. Discussion

In this study we evaluated the effectiveness of chelating agents for mercury removal from the body of rats when distribution of mercury and its excretion was altered by the presence of selenium. The effect of mercury and selenium interaction in vivo depends on the molar ratio of these elements administered to animals. The maximal effect of selenium on mercury distribution and excretion is observed when selenium is given in excess or at least in equimolar doses with mercury (Fang, 1977; Kristensen and Hansen, 1979). Naganuma et al. (1984) showed that the interaction of mercury with selenium occurs to the greatest extent when both compounds are administered simultaneously. Therefore, in this study, equimolar doses of selenium and mercury and simultaneous intraperitoneal administration of compounds was selected to cause maximal effect of selenium on mercury distribution and excretion.

As described in numerous investigations (Magos and Webb, 1976; Fang, 1977; Cikrt and Bencko, 1989; Cuvin-Aralar and Furness, 1991), selenium decreased mercury content in the kidney not by increasing its excretion in urine, but on the contrary mercury urinary excretion was decreased and whole body retention was increased, but by redistribution to other organs.

Our results on mercury distribution and on the efficiency of the chelating agents when mercury was administered without selenium were in close agreement with published data. It has been well documented that DMSA and DMPS decrease mercury retention in the body by increasing its excretion in the urine (reviewed by Andersen (1999)).

There are no literature data on selenium interaction with chelating agents for mercury removal. This study showed that the presence of sodium selenite affected

the efficiency of the chelating agents in mercury removal. In the presence of selenium, the rise of mercury excreted in urine in the groups treated with DMSA or DMPS was lower when compared to groups receiving mercury without selenium. When mercury was administered without selenium, mercury deposited mainly in the kidney, what was in agreement with published data (WHO, 1991), and the rise in mercury excreted in the urine corresponded to decreased mercury content in the kidney (Planas-Bohne, 1981). In the presence of selenium, mercury content in the kidney was low. It represented less than 6% of mercury content in the kidney when mercury was administered without selenium. The chelating agents did not additionally decrease this low mercury content in the kidney. On the contrary, DMPS significantly increased it. This result is possibly explained by the fact that urinary excretion pathway passes through kidneys. In the presence of selenium mercury is bound in a high molecular weight complex with selenoprotein P, which seems to prevent mercury uptake by the kidneys (Yamamoto, 1985), and therefore mercury content in the kidneys is low. It appears likely that the chelating agent competes for mercury with high molecular weight biological ligands (selenoprotein P) in organs and tissues other than kidney and forms a chelate, a low molecular weight complex which is then easily taken up by the kidneys and excreted into urine. Buchet and Lauwerys (1989) reported that the reduction of mercury in liver is achieved only after a repeated chelation treatment. In this study when mercury was given without selenium the reduction of mercury content in the liver after a single chelating agent administration was not observed, what was in agreement with results also reported by Planas-Bohne (1981). In the presence of selenium, treatment with DMSA significantly decreased mercury content in the liver, while DMPS did not have a significant effect on mercury content in the liver. These results indicate that the increase in urinary mercury excretion due to the chelating agents treatment in the presence of selenium corresponds to decreased mercury in the liver and organs other than the kidneys, while when mercury was administered without selenium, the rise in urinary mercury mainly corresponded to a decrease in mercury content in the kidneys. Changes of mercury binding to plasma proteins and changed organ distribution of mercury in the presence of selenium had influence on dynamics of mercury chelation.

From the results of this work it could be concluded that the presence of selenium affects the efficiency of the chelating agents in mercury removal. Both investigated chelating agents decreased mercury body retention by increasing its urinary excretion, even in the presence of selenium, but in the presence of selenium the rise in urinary mercury excretion due to the treatment with chelating agents was lower. Selenium decreased the effectiveness of chelating agents for mercury removal from the body of rats. It is well known that selenium decreases mercury toxicity (Cuvin-Aralar and Furness, 1991), but it reduces mercury excretion and efficiency of the chelating agents in mercury removal. Therefore, before any conclusions on the benefits or disadvantages of combining selenium with chelating agents can be made, studies on toxic effects of the mercury after chelating agents treatment in the presence of selenium are needed.

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