Monoisoamyl meso-2,3-Dimercaptosuccinate as a Delayed Treatment for Mercury Removal in Rats

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Monoisoamyl meso-2,3-dimercaptosuccinate (Mi-ADMS) was found to be superior to meso-2,3-dimercaptosuccinic acid (DMSA) in decreasing the body burden of 203Hg in rats under conditions of early treatment. In this experiment Mi-ADMS was used as late treatment for mercury removal. Albino rats aged 6 weeks and 7-day-old sucklings received a single intraperitoneal injection of 203Hg (as nitrate). Two weeks later they were treated with DMSA or Mi-ADMS (0.25 mmole/kg bw) on two consecutive days. The radioactivity in the carcass (whole body after removal of the gastrointestinal tract; liver, kidneys and brain was determined by solid crystal gamma scintillation counting six days after chelation therapy administration (3 weeks after 203Hg application). Both chelators reduced the body burden of mercury compared to controls. The effect of Mi-ADMS was superior to DMSA treatment in older rats for decreasing carcass and kidney retention, and in suckling rats for decreasing carcass, liver, and kidney retention. They were equally effective in decreasing brain retention in older rats and had no effect on brain retention in sucklings. The efficiency of Mi-ADMS in reducing the body burden of mercury was generally higher than the efficiency of the DMSA treatment. Therefore, Mi-ADMS deserves further attention as a late treatment for mercury removal. — Environ Health Perspect 102(Suppl 3):309-311 (1994).

Key words: monoisoamyl meso-2,3-dimercaptosuccinate, delayed parenteral treatment; 203Hg retention, rats

Introduction

Chelation therapy, which decreases the body burden of metals sometime after metal entry into the body, is of great interest for practical reasons. The efficiency of chelating agents is known to depend on the time delay between metal intoxication and administration of the chelating agent. The best therapeutic results are usually obtained by early chelation administration (1).

We have recently shown that treatment with monoalkyl esters of meso-2,3-dimercaptosuccinic acid (Mi-ADMS) is superior to treatment with dimercaptosuccinic acid (DMSA) in decreasing mercury retention in rats (2). The efficiency increased with the number of carbon atoms in the alkyl group of the DMSA monoester, and the best results were obtained with Mi-ADMS. Since treatment with DMSA is an accepted therapeutic means for reducing the body burden of mercury (3,4), the newer and more efficient monoesters—especially Mi-ADMS—deserve further attention.

Our previous results were obtained with early (immediate) treatment with DMSA and its monoesters after intraperitoneal administration of 203Hg in rats (2). In our present experiment, DMSA and Mi-ADMS were administered two weeks after 203Hg administration. The experiments were performed on suckling and older rats; exposure to mercury in the youngest age group might be of special concern due to higher retention and higher toxicity of mercury at this period of development (5). Our results show that treatment with Mi-ADMS is superior to DMSA in reducing mercury body burden in both age groups of rats.

Materials and Methods

The experiment was performed on two age groups of albino Wistar-derived rats from the Institute's breeding farm in Zagreb, Croatia. At the beginning of the experiment, the animals were 6 weeks (females, average weight 130 g) and 7-day-old (sucklings of both sexes, average weight 12 g). Suckling rats were kept with their mothers in clear plastic (polycarbonate) individual cages, in litters of six per dam (reduced to this number at birth) till weaning at the age of 3 weeks. The numbers of 6-week and 7-day-old animals were 29 and 42, respectively. Each age group was divided into three subgroups (control, DMSA, and Mi-ADMS). Older rats were kept in groups of 9 to 10 in proper polycarbonate cages during the experiment.

Each animal received a single intraperitoneal injection of 203Hg as nitrate (New England Nuclear, Boston, MA); specific activity 21 GBq/g = 0.56 Ci/g). Older rats received 92.5 kBq (2.5 μCi) in a volume of 0.5 ml, and sucklings 55.5 kBq (1.5 μCi) in a volume of 0.03 ml.

Two weeks later, chelation therapy with DMSA or Mi-ADMS began. Both chelators were stored under nitrogen (to avoid oxidation), and solutions for injection were prepared immediately prior to application. Chelators were dissolved in aqueous 5% NaHCO3 and were administered intraperitoneally at a dose of 0.25 mmole/kg bw in a volume of 0.5 ml in older, and 0.2 ml in younger rats on two consecutive days, i.e., on days 14 and 15 after 203Hg administration.

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In each litter at least one rat was used as control, and two experimental animals received Mi-ADMS or DMSA treatment. After each treatment the suckling rats were returned to the dams.

At the end of the experiment, i.e., three weeks after $^{203}$Hg administration, rats were sacrificed under ether anesthesia by cardiac exsanguination. The carcass (whole body after removal of the total gastrointestinal tract), liver, both kidneys, and brain were dissected for radioactivity determination. The radioactivity of the carcass was measured in a twin crystal whole body scintillation counter (Tobor, Nuclear Chicago, Des Plains, IL), and the radioactivity in the organs (liver, kidneys, brain) was measured in an automatic well-type gamma scintillation counter (Nuclear, Des Plains, IL). The results were corrected for radioactive decay and the geometry of the samples.

The results were expressed as a percentage of the radioactive dose, and data are presented as the arithmetic mean ± SEM. The results of the treatment were expressed as percentage reduction to control for $^{203}$Hg retention. Differences between the groups were evaluated by the two-tailed Student’s $t$-test and by one way analysis of variance followed by Duncan’s multiple range test analysis.

Results

In older rats DMSA caused a significant reduction in carcass, kidney, and brain retention (percent reduction of control was 21, 21, and 27, respectively), and had lower effect on liver retention (Table 1). In younger rats treatment with DMSA showed a similar efficiency as in older rats, in reducing carcass and kidney retention (20 and 28%, respectively), but caused also a decrease in liver retention (26%). In these rats DMSA was without effect on brain retention.

Treatment with Mi-ADMS in older rats caused a significant reduction in all body compartments (43% in carcass, 32% in liver, 46% in kidneys, and 25% in brain). In younger rats the treatment was even more efficient in reducing carcass (56%), liver (54%), and kidney retention (79%) than in older rats, but was without effect on brain retention (Table 1). The efficiency of Mi-ADMS treatment was therefore generally higher than the efficiency of DMSA therapy.

The statistical analysis of these results is presented in Table 2, showing the comparative efficiency of both treatments. In older rats Mi-ADMS was superior to DMSA in decreasing carcass and kidney retention, and in younger rats in decreasing carcass, liver, and kidney retention. The lack of efficiency in reducing brain retention was similar for both chelators.

Discussion

It has been known that DMSA can be used as late treatment for mercury removal, but it is a new and interesting finding that its monoester Mi-ADMS is superior to DMSA for this purpose. The reduction in the body burden of mercury by Mi-ADMS administration was much higher than by DMSA treatment. An exception is the brain, where no differences between the efficiency of the two chelators were observed in older rats, and no effect was obtained in sucklings by either chelator. This indicates that mercury incorporated into the brain during the suckling period is difficult to remove by late administration of chelators, even with Mi-ADMS, which was shown to be far superior to DMSA in reducing brain retention of mercury in suckling rats under conditions of early treatment (6).

The efficiency of DMSA in decreasing the body burden in the carcass, liver, and kidney was similar for both age groups of rats while for Mi-ADMS the efficiency of the treatment was even higher in younger than in older rats. Most mercury chelators were found previously to be less efficient in enhancing metal elimination in the young as compared to older animals (7). In explanation of these results, the immaturity of the excretory system and differences in metal-ligand binding were mentioned. However, these arguments cannot be used to interpret our present results, since chelators were administered at the age of three weeks when—according to toxicokinetic data—absorption, distribution, and elimination of most metals are similar to those in older age groups of rats (8). The higher efficiency of Mi-ADMS in inducing a decrease in the body burden of mercury in the young remains to be explained.

In conclusion, Mi-ADMS is not only superior to DMSA as an agent to decrease the body burden of mercury under conditions of immediate parenteral treatment, as found earlier, but also under conditions of late parenteral administration some time after mercury incorporation. In other words, Mi-ADMS is more suitable for removal of mercury than DMSA, which is commonly used in the current therapy of mercurial intoxication.

Furthermore, Mi-ADMS was also found to be superior to DMSA in mobilizing “aged” cadmium deposits in mice (9). This indicates that further experiments with Mi-ADMS are justified, since this chelator might be the agent of choice in the treatment of both mercury and cadmium intoxication.

### Table 1. Effect of late chelation therapy on $^{203}$Hg retention in rats.

|          | Control | 1.0 mg DMSA | Reduction to control | Mi-ADMS | Reduction to control |
|----------|---------|-------------|---------------------|---------|---------------------|
| 6-week-old rats |         |             |                     |         |                     |
| Carcass  | 34.7 ± 0.81 | 27.4 ± 0.51 | 21                  | 19.6 ± 1.01 | 43                  |
| Liver    | 0.572 ± 0.082 | 0.499 ± 0.059 | 13                  | 0.389 ± 0.042 | 33                  |
| Kidney   | 32.8 ± 1.08  | 25.9 ± 0.52  | 21                  | 17.6 ± 0.88 | 46                  |
| Brain    | 0.032 ± 0.004 | 0.023 ± 0.001 | 27                  | 0.024 ± 0.001 | 25                  |
| 7-day-old rats |         |             |                     |         |                     |
| Carcass  | 48.6 ± 1.37  | 39.0 ± 1.14  | 20                  | 21.3 ± 0.98 | 56                  |
| Liver    | 7.51 ± 0.32   | 5.53 ± 0.52   | 26                  | 3.49 ± 0.17 | 54                  |
| Kidney   | 28.9 ± 0.86   | 29.8 ± 0.89   | 26                  | 5.97 ± 0.15 | 79                  |
| Brain    | 0.512 ± 0.003 | 0.506 ± 0.017 | 1                   | 0.471 ± 0.012 | 8                   |

Results are expressed as arithmetic mean ± SEM (number of animals in parentheses). DMSA and Mi-ADMS were administered intraperitoneally (0.25 mmole/kg) as delayed treatments on days 14 and 15 after a single intraperitoneal administration of $^{203}$Hg.

### Table 2. Duncan’s multiple range test analysis of results from Table 1.

|          | DMSA CA/K/B | Mi-ADMS CA/K/B |
|----------|-------------|----------------|
| 6-week-old rats |             |                |
| Control     | €/€/+      | €+/-/+      |
| DMSA        | €/€/€      | €/€/€      |
| 7-day-old rats |             |                |
| Control     | €/+/-/-    | +/+/+/-     |
| DMSA        | €/+/-/-    | +/+/+/-    |

A significant difference with $p<0.05$ is indicated by “+” and a difference which is not significant by “€”. The significances are listed for each pairwise comparison in order: Carcass/Liver/Kidneys/Brain.
REFERENCES

1. Catsch A, Harmuth-Hoene A-E. Pharmacology and therapeutic applications of agents used in heavy metal poisoning. In: The Chelation of Heavy Metals, International Encyclopedia of Pharmacology and Therapeutics (Levine WG, ed). New York: Pergamon Press, 1979:107–224.
2. Koštal K, Blanuša M, Simonović I, Jones MM, Singh PK. Decreasing 203Hg retention by intraperitoneal treatment with monoalkyl esters of meso-2,3-dimercaptosuccinic acid in rats. J Appl Toxicol 13:321–325 (1993).
3. Graziano JM. Role of 2,3-dimercaptosuccinic acid in the treatment of heavy metal poisoning. Med Toxicol 1:155–162 (1986).
4. Jones MM. New developments in therapeutic chelating agents as antidotes for metal poisoning. Crit Rev Toxicol 21:209–233 (1991).
5. Sandstead HH, Doherty RA, Mahaffey KA. Effect and metabolism of toxic trace metals in the neonatal period. In: Reproductive and Developmental Toxicity of Metals (Clarkson TW, Nordberg GF, Sager PR, eds). New York: Plenum Press, 1983:205–224.
6. Koštal K, Blanuša M, Simonović I, Jones MM, Singh PK. Superiority of Mi-ADMS to DMSA as parenteral treatment for decreasing mercury (203Hg) body burden in rats. Arh Hig Rada Toksiol 44:217–222 (1993).
7. Koštal K, Kargačin B, Arežina R, Landeka M, Simonović I. Factors influencing the efficacy of chelation therapy. J Hyg Epidemiol Microbiol Immunol 35:337–350 (1991).
8. Koštal K, Jugo S, Rabar I, Maljković T. The influence of age on metal metabolism and toxicity. Environ Health Perspect 25:81–86 (1978).
9. Jones MM, Singh PK, Gale GR, Smith AB, Atkins LM. Cadmium mobilisation in vivo by intraperitoneal or oral administration of monoalkyl esters of meso-2,3-dimercaptosuccinic acid in mouse. Pharmacol Toxicol 70:336–343 (1992).