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Effect of Oral Therapy with Monoisoamyl Meso-2,3-dimercaptosuccinate on ^{203}Hg Retention in Rats

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Monoesters of DMSA (meso-2,3-dimercaptosuccinic acid), especially the higher analogues, have been found more efficient in reducing Cd (Jones at al. 1992) and Hg (Kostial et al. 1993) retention than DMSA. Since DMSA is known to be a very efficient and nontoxic agent to reduce the body burden of several metals, such as Pb, Hg, As (Graziano 1986, Jones 1991), the new monoesters of DMSA are of great interest as agents for chelation therapy in heavy metal poisoning.

In previous experiments we evaluated the efficiency of Mi-ADMS (monoisoamyl meso-2,3-dimercaptosuccinate), the most promising analogue of DMSA, in decreasing ^{203}Hg retention in rats after intraperitoneal administration (Kostial et al. 1993). In our present experiments we administered the same monoester, i.e., Mi-ADMS orally and evaluated its efficacy in decreasing ^{203}Hg retention after oral or intraperitoneal administration. For practical reasons oral chelation therapy has many advantages as compared to parenteral treatment. The present experiments performed in rats after oral or intraperitoneal administration of ^{203}Hg indicate that oral therapy with Mi-ADMS was superior to therapy with DMSA.

MATERIALS AND METHODS

Experiments were performed on six wk old female Wistar rats (body wt by about 140 g) from the breeding colony of the Institute for Medical Research and Occupational Health, University of Zagreb. Animals received ^{203}Hg as mercuric nitrate purchased from New England Nuclear Du Pont (specific activity 370 GBq or 10 Ci/g) orally or intraperitoneally. Chelating agents DMSA (meso-2,3-dimercaptosuccinic acid, Mol. wt 252) and Mi-ADMS (monoisoamyl meso-2,3-dimercaptosuccinate, Mol. wt 182)

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were used. Preparative method for Mi-ADMS was described earlier by Jones et al. (1992). Chelators were stored under nitrogen to avoid oxidation. Solutions for application were prepared in 5% aqueous $NaHCO_3$ solution and administered twice orally or intraperitoneally at a dose of 0.25 mM/kg (total dose 0.5 mM/kg).

In Experiment 1 (E1) both ^{203}Hg and chelator were administered orally by gavage (0.5 mL per animal). DMSA or Mi-ADMS was administered twice on two consecutive days, 24 + 48 hr after a single oral administration of 4 µCi ^{203}Hg (148 kBq). Controls received ^{203}Hg in the same way.

In Experiment 2 (E2) ^{203}Hg was administered by intraperitoneal injection. DMSA or Mi-ADMS was administered orally twice on two consecutive days, 48 + 72 hr after a single application of 1.2 µCi ^{203}Hg (44 kBq) in a volume of 0.5 mL per animal. Controls received ^{203}Hg in the same way.

At the end of both experiments, six days after ^{203}Hg administration, rats were killed under ether anaesthesia by cardiac exsanguination. The whole body (WB) radioactivity was determined in a double crystal scintillation counter (Tobor, Nuclear Chicago). Organs, i.e., liver (L), both kidneys (K) and brain (B), were dissected and radioactivity was determined in an automatic well type gamma scintillation counter (Tobor, Nuclear Chicago). The results were corrected for radioactive decay and the geometry of the samples by adjusting a fraction of the administered dose to volume and shape of biological samples in containers for measurements.

Results are expressed as percentage of the radioactive dose, and presented as arithmetic mean and standard deviation. The results of the treatment are expressed as percentage reduction to control in ^{203}Hg retention. Differences between groups were evaluated by one way analysis of variance followed by Duncan's multiple range test analysis.

RESULTS AND DISCUSSION

The effect of DMSA or Mi-ADMS treatment on retention of ingested ^{203}Hg (E1) is shown in Table 1 (upper part). Treatment with DMSA caused a reduction in whole body and organ retention ranging from 66% to 87% of control values, but only reduction in liver retention was statistically significant ($P<0.05$). Treatment with Mi-ADMS caused a reduction in retention in whole body and organs ranging from 29% to 44% of control values, and all

Table 1. Effect of oral therapy with DMSA or Mi-ADMS on retention of ^{203}Hg after oral or intraperitoneal administration in rats.

	CONTROL	D M S A		M i - A D M S	
	Retention after ingestion of ^{203}Hg (Experiment 1)				
	% p.o. dose (9)	% p.o. dose (9)	DMSA/ CON. %	% p.o. dose (9)	Mi-ADMS/ CON. %
WB	2.77 ±0.72	2.20 ±0.54	79	1.20 ±0.17	43
L	0.351 ±0.117	0.233 ±0.081	66	0.140 ±0.084	40
K	1.49 ±0.29	1.29 ±0.37	87	0.427 ±0.192	29
B	0.009 ±0.003	0.007 ±0.003	78	0.004 ±0.003	44
	Retention after intraperitoneal ^{203}Hg administration (Experiment 2)				
	% i.p. dose (8)	% i.p. dose (8)	DMSA/ CON. %	% i.p. dose (6)	Mi-ADMS/ CON. %
WB	62.7 ±4.61	52.1 ±4.16	83	24.9 ±2.47	40
L	6.29 ±1.70	4.69 ±1.98	75	2.75 ±0.56	44
K	58.2 ±4.95	44.5 ±2.46	76	14.8 ±1.37	25
B	0.144 ±0.023	0.141 ±0.020	98	0.109 ±0.012	76

Results are presented as arithmetic means ± SD (number of animals in parentheses).
DMSA and Mi-ADMS were administered orally at a dose of 0.25 mM/kg twice: in Experiment 1 24 and 48 hr after oral ^{203}Hg administration, in Experiment 2 48 and 72 hr after intraperitoneal ^{203}Hg administration.
WB - Whole Body; L - Liver; K - Kidneys; B - Brain.

values in whole body, liver, kidneys, and brain were significantly lower than in controls ($P<0.001$, $<.01$, $<.001$, and $<.01$, respectively). Differences between the efficiency of Mi-ADMS and DMSA treatment show the advantage of Mi-ADMS over DMSA in decreasing the body burden of ingested ^{203}Hg. Treatment with Mi-ADMS caused a higher reduction in whole body ($P<0.001$), kidney ($P<0.001$), and brain retention ($P<0.01$) (not in the liver) compared to DMSA.

Treatment with DMSA or Mi-ADMS on retention of intraperitoneally administered ^{203}Hg (E2) is presented in Table 1 (lower part). DMSA caused a reduction in whole body and organ retention ranging from 75% to 98% of control values, but only reduction in the whole body and kidneys was statistically significant (at level $P<0.001$). Treatment with Mi-ADMS caused statistically significant reduction to 40%, 44%, 25% of control values in the whole body, liver, kidneys (at level $P<0.001$), and in brain 76% of control ($P<0.05$). Differences between the efficiency of Mi-ADMS and DMSA show the advantage of Mi-ADMS over DMSA in decreasing the body burden of intraperitoneally administered ^{203}Hg. Mi-ADMS was more efficient than DMSA in whole body and all organs; whole body and kidneys $P<0.001$, liver $P<0.02$, and brain $P<0.01$.

Results on Table 2 present Duncan's multiple range test analysis of results from Table 1. The value of $P<0.05$ was taken as significance level. Significant difference is indicated by "plus" and a difference which is not significant by "minus". The significances are listed for each pairwise comparison in order: whole body / liver / kidneys / brain. By presenting data in this way the advantage of Mi-ADMS over DMSA treatment after oral or parenteral administration of ^{203}Hg is very obvious.

Jones et al. (1992) found a similar efficiency in decreasing intracellular Cd deposits by Mi-ADMS after oral or parenteral treatment. However, they used much higher oral doses of Mi-ADMS (2x1.0 mM/kg) than we used for decreasing Hg retention (2x0.25 mM/kg) in present experiments.

The Hg to chelator ratio in our experiment E2 was 1 : $7.5x10^3$, and in experiment E1 1 : $1.8x10^3$. Results of our experiments in progress show the same differences between the efficiency of DMSA and Mi-ADMS if additional carrier is added to ^{203}Hg (0.5 mM/kg Hg as mercuric chloride) modifying the Hg to chelator ratio to 1 : $1x10^2$. This indicates that differences in this ratio have no effect on the results obtained. When comparing the efficiency of DMSA to Mi-ADMS a comment should be given to their comparative toxicity since both factors should be considered before recommending a new chelating agent for

Table 2. Duncan's multiple range test analysis.

	DMSA WB/L/K/B	Mi-ADMS WB/L/K/B
Retention after p.o. ^{203}Hg (Experiment 1)		
CONTROL	-/+/-/-	+/+/+/+
DMSA		+/-/+/+
Retention after i.p. ^{203}Hg (Experiment 2)		
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: Whole Body/Liver/Kidneys/Brain.

therapeutic use. It is assumed that the ability of the new monoesters of DMSA (including Mi-ADMS) to cross cell membranes may account for their superiority in mobilizing metals from target organs. It was therefore expected that the toxicity of Mi-ADMS would be higher than that of DMSA, since it is known that the toxicity of a chelating agent is proportional to the possibility of its entering into the cells. The i.p. LD_{50} value in mice for DMSA is reported to be 16 mM/kg, for Mi-ADMS 3 mM/kg and DMPS (2,3-dimercaptopropane-1-sulphonate) 1.1 mM/kg (Walker et al. 1992). Therefore, the toxicity of Mi-ADMS seems to be in between the toxicity values for DMSA and DMPS which are presently used as chelating agents for treatment in humans.

Recently, we also evaluated the interaction of DMSA and Mi-ADMS with some essential trace elements (Fe, Zn, Cu), since many aspects of the toxicity of chelators are related to their interaction with endogenous trace elements. We found that both chelators administered intraperitoneally over 10 days in rats (daily dose 0.25 mM/kg) had a similar effect on trace elements, causing only increased elimination of Cu (Blanuša et al. 1993). This effect on Cu was observed earlier for DMSA by several authors both in humans and animals (Aposhian and Aposhian, 1990). Since oral therapy with Mi-ADMS seems to be far superior to DMSA in decreasing the body burden of ingested and intraperitoneally administered Hg, we consider that further studies with this new chelator deserve attention.

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