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SUPERIORITY OF Mi-ADMS TO DMSA AS PARENTERAL TREATMENT FOR DECREASING MERCURY (^{203}Hg) BODY BURDEN IN RATS

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The efficiency of meso-2,3-dimercaptosuccinic acid (DMSA) and the monoisoamyl ester of meso-2,3-dimercaptosuccinic acid (Mi-ADMS) in decreasing ^{203}Hg retention was evaluated in rats in relation to age and time of treatment. The experiments were performed on six-week- and seven- day-old Wistar rats, which received ^{203}Hg by intraperitoneal administration. The chelators DMSA or Mi-ADMS were also administered intraperitoneally, twice, on two consecutive days, in doses of 0.25 mmol/kg body weight as early (0.5 and 24 h) or delayed treatment (24 and 48 h, or 48 and 72 h) after ^{203}Hg administration. The retention of ^{203}Hg was determined in the carcass, liver, kidneys and brain six days after administration using gamma scintillation counters (double crystal, well type). In all experimental conditions, regardless of the animals' age and time of chelation therapy, Mi-ADMS was found to be superior to DMSA in reducing the body burden of ^{203}Hg in whole body and organs. Mi-ADMS therefore seems to be a very promising chelator in the treatment of mercury poisoning.

Key terms: age, chelation therapy, delayed treatment, immediate treatment, mercury poisoning, radioactiviy determination, radionuclide administration

DMSA (meso-2,3-dimercaptosuccinic acid) is known to be an efficient antidote against mercury poisoning (1, 2). Recently, its role in the treatment of other metal poisonings, including lead, mercury and arsenic, has been emphasized because it appears to be more effective and less toxic than other agents for reducing metal retention (3, 4). New evidence indicates that DMSA monoesters might be more efficacious than DMSA for this purpose (5, 6). *Jones and co-workers* (6) tested the efficacy of nine DMSA monoesters to mobilize intracellular cadmium and found that higher analogues were definitely superior to DMSA. Recently, we have tested the efficiency of the same nine DMSA monoesters for reducing ^{203}Hg retention in acute experiments on rats (7). The reduction of mercury retention by these monoesters was higher than by DMSA and Mi-ADMS (monoisoamyl ester of meso-2,3-dimercaptosuccinic acid) was found to be the best chelator for the treatment.

The purpose of the present experiments was to establish whether Mi-ADMS was also superior to DMSA if administered at later time intervals after ^{203}Hg administration (in our previous experiments the chelators were administered almost immediately after ^{203}Hg). We also wanted to compare the efficacy of Mi-ADMS and DMSA in two age groups of rats (sucklings and older rats) as the age factor needs to be considered in evaluating the effectiveness of chelation therapy (8).

We found that Mi-ADMS was superior to DMSA in reducing the body burden of mercury regardless of the time of administration of the chelating agent and the age of the animals.

MATERIAL AND METHODS

Animals

The experiment was performed on Wistar rats from the Institute's own breeding colony. The animals were seven-day-old sucklings of both sexes and six-week-old female rats (average weight 12 and 130 g, respectively). The suckling rats were kept with their mothers in litters of six in the individual polycarbonate cages. Two sucklings from each litter were used as controls (untreated), and the other four as experimental animals (receiving chelation therapy). Sucklings were returned to their mothers each day after the treatment with chelator.

The six-week-old female rats were kept in groups of six to eight animals per cage. All animals were provided standard rat feed and tap water *ad libitum*.

Radionuclide administration

All rats received ^{203}Hg (as nitrate), specific activity 21 GBq/g (0.56 Ci/g) (New England Nuclear, Du Pont) by intraperitoneal injection in a dose of 44.4 kBq (1.2 μCi, 2 μg Hg^{2+}) in a volume of 0.03 (sucklings) or 0.5 ml (older rats) of distilled water.

Chelation therapy

The chelating agents were meso-2,3-dimercaptosuccinic acid (DMSA) and the monoisoamyl ester of DMSA (Mi-ADMS). Preparative methods for the monoester were described earlier by Jones and co-workers (6). Both chelators were stored under nitrogen to avoid oxidation. Solutions for injection were prepared immediately before use in 5 % $NaHCO_3$. Doses of 0.25 mmol/kg body weight in a volume of 0.03 ml in sucklings, and 0.5 ml in older rats were administered intraperitoneally on two consecutive days as early treatment (Experiment 1, E1 = 0.5 and 24 h) or delayed treatments (Experiment 2, E2 = 24 and 48 h; Experiment 3, E3 = 48 and 72 h). The sucklings received only E1 and E2 treatments.

Radioactivity determination

Six days after ^{203}Hg administration rats were killed by cardiac exsanguination under ether anaesthesia. The radioactivity was first determined in the carcass (whole body after removal of the total gastrointestinal tract) in a double crystal scintillation counter (Nuclear Chicago). The liver, both kidneys, and brain were dissected and radioactivity was determined in an automatic well-type gamma scintillation counter (Nuclear Chicago).

All results were corrected for radioactive decay and geometry of the samples. Results are expressed as percentages of the radioactive dose and presented as arithmetic mean and standard error of the mean. They are also presented as percentage reduction of ^{203}Hg retention of treated to control animals.

Statistical treatment

Differences between groups were evaluated by the one way analysis of variance followed by Duncan's multiple range test.

RESULTS

Table 1 shows the results for the older group of rats and Table 2 for suckling rats as percentage retention of the ^{203}Hg dose, and as percentage reduction of retention in the treated animals in respect to controls.

Table 1. *The effect of chelation therapy on ^{203}Hg retention in six-week-old rats.*

	Control	DMSA		Mi-ADMS	
	% dose	% dose	% RC	% dose	% RC
Experiment 1		(6)		(8)	
Carcass		26.0±1.11[a]	57	6.02±041[ab]	90
Liver		1.17±0.06[a]	57	0.46±0.05[ab]	83
Kidneys		15.7±0.70[a]	65	2.79±0.23[ab]	94
Brain		0.068±0.002[a]	38	0.018±0.002[ab]	84
Experiment 2	(16)	(8)		(8)	
Carcass	60.9±1.57	44.3±1.66[a]	27	18.6±0.65[ab]	70
Liver	2.72±0.24	3.34±0.32	–	2.22±0.16[b]	20
Kidneys	44.1±0.94	27.7±0.67[a]	37	9.50±0.82[ab]	78
Brain	0.109±0.003	0.095±0.007	13	0.68±0.002[ab]	37
Experiment 3		(8)			
Carcass		45.0±1.00[a]	29	20.9±0.45[ab]	63
Liver		3.23±0.32	–	2.64±0.32	3
Kidneys		30.3±1.04[a]	31	10.0±0.61[ab]	77
Brain		0.104±0.006	4	0.079±0.003[ab]	29

Values are presented as arithmetic mean ± SEM (number of animals in parentheses).
RC – reduction to control.
Statistical differences (Duncan's multiple range test, $P<0.05$) between control and experimental groups (a) and between two experimental groups (b).

In six-week-old rats (Table 1) both chelators caused a reduction of ^{203}Hg retention in the carcass and organs under conditions of early (E1) treatment. The percentage reduction in respect to controls in different organs ranged for DMSA from 38 to 65, and for Mi-ADMS from 83 to 94. Delayed treatments by both chelators were also effective in reducing ^{203}Hg retention in the carcass and kidneys and had no effects on liver retention at both delayed

intervals (E2 and E3). Mi-ADMS reduced brain retention at the delayed intervals (E2 and E3), while DMSA failed to produce an effect on brain retention.

Table 2. *The effect of chelation therapy on ^{203}Hg retention in seven-day-old rats.*

	Control	DMSA		Mi-ADMS	
	% dose	% dose	RC	% dose	% RC
Experiment 1					
	(9)	(9)		(9)	
Carcass	81.5±1.64	47.9±1.11	41	16.48±0.81	80
Liver	16.6±0.44	10.8±0.53	35	5.11±0.29	69
Kidneys	26.9±1.17	12.5±0.43	53	2.62±0.20	90
Brain	0.676±0.047	0.450±0.012	33	0.220±0.13	68
Experiment 2					
		(9)		(9)	
Carcass		54.97±1.79	33	22.29±0.74	73
Liver		13.85±0.48	17	5.59±0.18	66
Kidneys		14.11±0.77	47	3.29±0.13	88
Brain		0.533±0.027	21	0.329±0.022	51

Values are presented as arithmetic mean ± SEM (number of animals in parentheses).
RC – reduction to control.
Statistical differences (Duncan's multiple range test, $P<0.05$) between control and experimental groups (a) and between two experimental groups (b).

In seven-day-old sucklings (Table 2) both chelators caused a significant reduction in ^{203}Hg body retention after early (E1) and delayed (E2) treatments with the exception of DMSA which had no effect on brain retention in conditions of delayed treatment (E2). The efficiency of Mi-ADMS to reduce the ^{203}Hg retention was higher than that of DMSA at both treatment intervals.

For both chelators the effectiveness of the delayed therapy (E2, E3) was lower than that of the immediate treatment (E1). In older animals, the greatest decrease in effectiveness occurred between the early (E1) and the first delayed treatments (E2), while differences in effectiveness between the first (E2) and second (E3) delayed treatments were less pronounced. In sucklings the decrease in effectiveness between immediate (E1) and the delayed treatments (E2) was much lower than in older rats. The decrease in effectiveness in reducing ^{203}Hg retention with time after chelator administration (E1 as compared to E2 and E3 treatments) was always lower for Mi-ADMS than for DMSA in both age groups of animals.

DISCUSSION AND CONCLUSIONS

DMSA has been increasingly applied in the treatment of lead, cadmium and other metal poisonings (3). Recently, new DMSA monoesters have been found superior to DMSA as late treatment for reducing intracellular cadmium deposits (6) and as early treatment for reducing mercury body burden in rats (7). Among the nine monoesters tested, Mi-ADMS was found to be the treatment of choice. A treatment that could decrease the body burden of several metals seems to be especially desirable.

In this work we found Mi-ADMS to be superior to DMSA in reducing the body burden of ^{203}Hg in all experimental conditions regardless of the animals' age or the time of administration of chelation therapy.

Although delayed treatment with chelating agents is known to be less efficacious than the early treatment, our present results show that the effectiveness of Mi-ADMS is less dependent upon the time of chelation therapy than that of DMSA. Another advantage of the Mi-ADMS treatment pertains to decreasing mercury retention in sucklings. It is known that parenteral administration of chelator for decreasing the body burden of several metals is less effective in sucklings than in older animals (8). However, in our present experiments, although early administration of Mi-ADMS was less efficacious in sucklings than in older rats (percentage reduction in respect to controls in Table 2), delayed (E2) treatment was more effective in sucklings than in older rats. The effectiveness of delayed treatment being very important for practical purposes the therapy with Mi-ADMS might also have advantages over DMSA for decreasing the body burden of mercury in the youngest age group, although the mechanism of this action is still unknown.

In summarizing our results we may conclude that Mi-ADMS has advantages over DMSA for the following reasons:

– the reduction of ^{203}Hg retention in all body compartments (including kidneys and brain essential for the effect of mercury) was higher than that of DMSA in conditions of early and delayed treatments;

– the fall in effectiveness in reducing ^{203}Hg retention between early and delayed treatments was lower than that of DMSA;

– the effectiveness of treatment in sucklings was also superior to that of DMSA especially in conditions of delayed administration.

Therefore, we believe that Mi-ADMS deserves further attention as a possibly successful chelator in decreasing the body burden of mercury.

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Sažetak

PREDNOST PARENTERALNE PRIMJENE Mi-ADMS PRED DMSA U SNIŽAVANJU TJELESNOG OPTEREĆENJA ŽIVOM (^{203}Hg) U ŠTAKORA

Procjenjivana je učinkovitost mezo-2,3-dimerkaptojantarne kiseline (DMSA) i monoizoamilnog estera mezo-2,3-dimerkaptojantarne kiseline (Mi-ADMS) u snižavanju opterećenja živom (^{203}Hg) u štakora u odnosu na dob i vrijeme primjene. Pokusi su provedeni na šestotjednim ženkama i sedmodnevnim sisajućim bijelim štakorima Wistar iz uzgoja Instituta u Zagrebu, kojima je intraperitonejski davana ^{203}Hg. Kelati DMSA i Mi-ADMS davani su, također intraperitonejski, dva dana zaredom u dozi od 0,25 mmol/kg tjelesne težine kao neposredna/rana primjena (E1 = 0,5 i 24 h) ili kao odgođena/kasna primjena (E2 = 24 i 48 h, E3 = 48 i 72 h) nakon davanja ^{203}Hg. Retencija ^{203}Hg mjerena je u karkasu (tijelo nakon odstranjenja probavila), u jetri, u bubrezima i u mozgu šest dana nakon primjene ^{203}Hg u odgovarajućim gamascintilacijskim brojačima. U šestotjednih životinja i DMSA i Mi-ADMS primijenjeni kao rana terapija (E1) snizili su retenciju ^{203}Hg u karkasu i u organima u odnosu na vrijednosti u kontrolnih (netretiranih) životinja. Odgođena primjena (E2 i E3) jednog i drugog kelata snizila je retencije ^{203}Hg u karkasu i bubrezima, nije imala učinka na retenciju u jetri, a retenciju u mozgu snizio je samo Mi-ADMS. U sedmodnevne sisančadi oba kelata značajno su snizila tjelesnu retenciju ^{203}Hg i nakon rane (E1) i nakon kasne primjene (E2), a jedino DMSA u kasnijoj primjeni (E2) nije imao učinka na retenciju ^{203}Hg u mozgu. U svim eksperimentalnim uvjetima neovisno o dobi životinja i vremenu kad je primijenjen kelat, terapija s Mi-ADMS bila je učinkovitija od DMSA u snižavanju tjelesnog opterećenja ^{203}Hg u tijelu i u organima. To je glavni nalaz ovog istraživanja. Čini se da je Mi-ADMS kelat koji puno obećava u budućnosti kao lijek izbora u terapiji otrovanja živom.

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Ključne riječi: dob, odgođena primjena, određivanje radioaktivnosti, otrovanje živom, primjena radionuklida, rana primjena, terapija kelatima.