Meso-2,3-DIMERCAPTOSUCCINIC ACID IN THE TREATMENT OF OCCUPATIONALLY EXPOSED LEAD WORKERS

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Received: 9 March 1998

The aim of this study was to evaluate the efficacy of *meso*-2,3-dimercaptosuccinic acid (DMSA) treatment in workers with increased lead absorption and no overt symptoms of lead poisoning. Seven occupationally lead exposed male workers with blood lead concentrations (PbB) exceeding 50 µg/100 ml and a positive calcium disodium ethylenediaminetetraacetate (EDTA) lead mobilization test were treated with DMSA for 19 days. Individual doses were 700 mg DMSA, three times a day from day one to five, and twice a day from day six to 19. The treatment intensified urinary lead excretion, most rapidly during the first five days. The increased elimination was followed by a decline of mean PbB to 15% of the pretreatment values. However, 15 days after the treatment, the PbB concentrations rebounded, yet kept below the baseline values and did not exceed 40 µg/100 ml. After repeated EDTA lead mobilization test, urine lead was 23–68% of that before DMSA treatment. It can be concluded that DMSA can effectively reduce chelatable lead in occupationally exposed workers.

Key words:
blood, calcium, CaNa$_2$EDTA, chelation therapy, copper, iron, magnesium, mobilisation test, urine, zinc

Chelating agents such as calcium disodium ethylenediaminetetraacetate (CaNa$_2$EDTA), 2,3-dimercapto-1-propanol (BAL), and d-penicillamine have been widely used in the treatment of acute and chronic lead poisoning. In more than 40 years of use, extensive experience has defined their advantages as well as the disadvantages and side effects (1–3). *Meso*-2,3-dimercaptosuccinic acid (DMSA) has been used in China as a chelator in lead poisoning since 1965 (4). In the USA, it was first tested in late 70s (5) and its efficacy and safety were soon confirmed (6, 7). Today, DMSA is considered the most promising new oral chelating drug, whose effectiveness in metal mobilization is particularly evident in the therapy of lead poisoning in children (8, 9). In Croatia, however, DMSA has not yet been applied as a drug, either in children or in adults.

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There are only a few reports on DMSA chelation in occupationally exposed workers (10, 11). The therapeutic benefit of chelation in chronic lead poisoning, especially in cases with fewer overt subclinical forms of lead toxicity, is still a subject of discussion (12).

The aim of this study was to evaluate the efficacy of DMSA treatment in occupationally lead exposed workers with increased chelatable lead and without overt clinical symptoms of lead intoxication.

SUBJECTS AND METHODS

The study included seven lead exposed male workers employed in manufacture of lead batteries. Two workers aged 25 and 27 had been exposed to lead for four years, other two aged 35 and 36 for 10 years, and three workers aged 38, 51, and 45 for 11, 12, and 23 years, respectively. Patients were socioeconomically comparable and had had similar personal habits including alcohol intake. The following criteria were used for the inclusion in the study: a) occupational lead exposure, b) blood lead levels (PbB) exceeding 50 μg/100ml (2.41 μmol/L) determined in a periodic examination that had taken place approximately one month before the study, and c) positive EDTA lead mobilization test.

Having given informed consent, the subjects were hospitalized for the entire study, which secured cessation of exposure and appropriate observation. All procedures were carried out according to the ethical standards approved by the Ethic Committee of the Institute for Medical Research and Occupational Health. The entire therapeutic protocol was approved by the Croatian Drug Committee.

The EDTA lead mobilization test was done by measuring 24-hour lead excretion in urine (PbU) after administration of 1 g CaNa$_2$EDTA (LEDCLAIR ampules, Sinclair, UK) in 250 ml of 5% glucose i.v. over one hour. An EDTA test was considered positive if PbU excretion exceeded 600 μg/24 hrs (13, 14), which was consistent with the increase in chelatable lead (15).

The treatment began six days after the EDTA test. Capsules of DMSA (CHEMET, McNeil, USA) were donated by a co-manufacturer Cilag, Switzerland. The treatment protocol and the inclusion criteria for the study (PbB 50 μg/100 ml) followed the manufacturer's instructions. Oral DMSA treatment consisted of 700 mg administered three times a day from days 1–5, and twice a day from days 6–19.

Five ml of blood from cubital vein was collected for PbB analysis. Electrothermal atomic absorption spectrometry (ET-AAS) was applied after deproteinization with nitric acid according to a modified Stoeppler method (16, 17). Measurements were performed on Perkin Elmer, Model 5100 with Zeeman background correction. The method has constantly been verified by participation in the United Kingdom National External Quality Assessment Scheme programme (coefficient of variation, CV<1%).

Initial PbB concentrations were determined before the EDTA test. During DMSA treatment, PbB was measured on day 5, 12, and 19 and again 15 days after the treatment. Simultaneous laboratory tests included blood count and renal and liver function tests, i.e. concentrations of electrolytes, uric acid, serum creatinine, serum transaminases, bilirubin, alkaline phosphatase, and creatinine clearance.

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A baseline 24-hour urine lead was obtained before the EDTA test (PbU_0). The 24-hour urine samples for urine lead were collected repeatedly during the first five days, and then again on day 12 and day 19 of DMSA treatment. Urine lead concentrations were determined by ET-AAS (Perkin-Elmer, 5100; CV<5%) (18).

Other elements, that is, zinc (Zn), copper (Cu), iron (Fe), calcium (Ca), and magnesium (Mg) excretion in urine were analyzed in samples collected on days 0, 5, and 19. Ten ml of urine was dried, ashed at 450 ^{0}C in quartz crucibles, and dissolved in 10 ml of 2% HNO_3. The analyses were carried out by flame atomic absorption spectrometry on a Varian, Model AA375, with deuterium background correction (CV<5%) (19).

The EDTA lead mobilization test was repeated 15 days after the DMSA treatment to evaluate the efficacy of DMSA in reducing lead accumulation.

Since the employers' policy required workers to return to work immediately after normalization of PbB (<40 μg/dl), the follow-up did not extend over the 15 days after the cessation of the treatment.

The statistical difference between the results of EDTA lead tests and PbB concentrations obtained before and after the treatment was analyzed by Student's *t*-test for dependent samples using the CSS Biostatistica program (Statsoft 1991 package, release 3.1).

RESULTS

The EDTA lead mobilization test showed urinary lead excretion reaching up to 150% above the threshold level of 600 μg/24 h (range 953–1634 μg Pb/24 h), which was consistent with an increase of chelatable lead of PbB≥40 μg/dl in the seven workers (Table 1).

Table 1 *Urinary lead excretion (µg Pb/24 h) after each EDTA mobilization test**

EDTA lead mobilization test			
Patient	Before DMSA	After DMSA	Reduction (%)
1	1120	630	44
2	953	–	–
3	1345	629	53
4	1395	770	45
5	1634	379	77
6	1014	689	32
7	1041	256	75
Mean ± SD	1214 ± 243	559 ±198[†]	54 ± 18

* The first EDTA lead mobilization test was done six days before the start, and the second 15 days after the end of DMSA treatment by intravenous infusion of one gram $CaNa_2EDTA$ in 250 ml 5% glucose solution, administered during one hour (referent values <600 µg Pb/24 h urine).
[†] Statistically different from values before treatment at P<0.01

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The DMSA treatment significantly increased urine lead, especially on days 1–5 (Table 2). Six workers manifested a decline in urine lead after EDTA to 54±18% of pretreatment PbU (P<0.01) (Table 1).

Table 2 *Urinary lead (PbU) excretion (µg/24 h) before and during the treatment with DMSA* in workers occupationally exposed to lead.*

Patient	PbU_0	Days of DMSA treatment						
		1.	2.	3.	4.	5.	12.	19.
1	98	1570	1369	1156	916	924	306	329
2	83	1142	931	545	631	642	241	154
3	75	1333	967	841	577	616	277	137
4	66	1204	952	890	596	692	322	199
5	66	2058	1416	913	723	661	283	177
6	57	1597	1663	1113	1230	740	458	294
7	57	588	1040	949	836	850	487	218

* DMSA treatment lasted 19 days, with individual doses of 700 mg three times a day from days 1–5, and twice a day from days 6–19. A baseline 24–hour urine lead (PbU_0) was obtained six days before the start of DMSA treatment and before the first EDTA lead mobilization test.

Mean PbB (Table 3) significantly dropped (P<0.01) to 25% of the baseline concentrations determined before the EDTA test. Fifteen days after the cessation of treatment (i.e. on day 34) PbB values were down to 51% of the initial value (P<0.01)

Table 3 *Lead in blood (µg Pb/dl) before, during, and after treatment with DMSA*

Patient	Treatment[a]					Post-treatment reduction (%)[b]	Rebound (%)[c]
	Before	During			After		
		Day 5	Day 12	Day 19			
1	41.4	13.2	10.1	8.9	24.2	78.5	36.9
2	39.5	12.2	7.66	4.76	–	88.0	–
3	43.5	11.8	8.69	5.38	18.8	87.6	30.9
4	45.5	12.4	9.32	6.67	25.9	85.5	42.2
5	54.9	14.3	9.73	7.87	19.9	85.7	21.9
6	59.0	14.9	11.2	10.6	30.4	82.0	33.6
7	33.3	7.25	4.55	4.97	19.2	85.1	42.7
Mean ± SD	45.3 ± 8.9	12.3 ±2.5	8.75 ± 2.15	7.02 ± 2.20[†]	23.1 ± 4.6[†‡]	84.6 ± 3.3	34.7 ± 7.8

[a] DMSA treatment lasted 19 days, with individual doses of 700 mg three times a day from days 1–5, and twice a day from days 6–19. The first PbB was analyzed six days before the start, and the last 15 days after the end of DMSA treatment.
[b] Post-treatment reduction (at day 19) was calculated as percent reduction of PbB value obtained before the treatment.
[c] Rebound was calculated as per cent of: $PbB_{After} - PbB_{Day\,19} / PbB_{Before}$
[†] Statistically different from before treatment and from Day 19 at P<0.01, respectively.

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and did not exceed the threshold level for the occupationally exposed workers of 40 μg/100 ml (20).

In contrast to the early excretion of lead, the urinary excretion of trace elements increased with the duration of DMSA treatment (Figures 1 and 2).
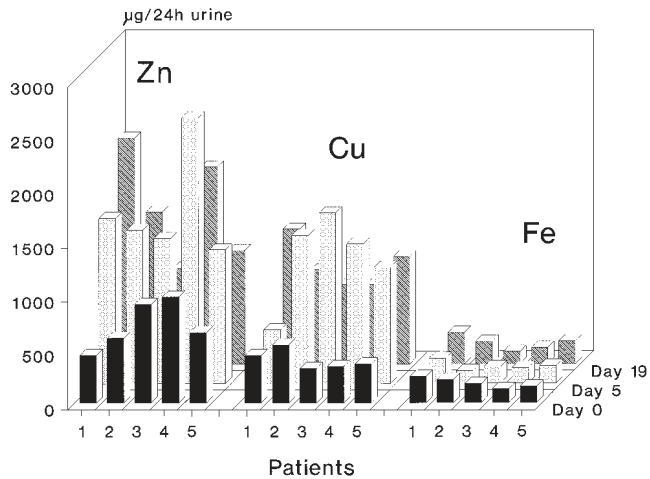

Figure 1 *Zinc (Zn), copper (Cu), and iron (Fe) in 24-hour urine before (day 0), during (day 5) and after (day 19) DMSA treatment*
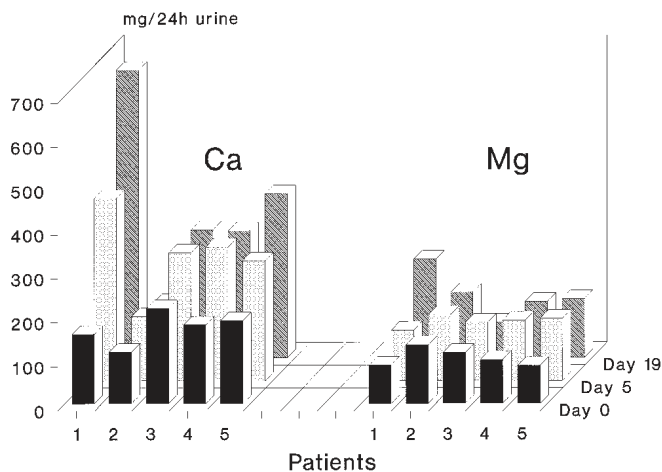

Figure 2 *Calcium (Ca) and magnesium (Mg) in 24-hour urine before (day 0), during (day 5) and after (day 19) DMSA treatment*

There were no clinically significant changes in the blood count. Clinical chemistry changes were limited to a mild and transient rise in alanine transaminase in three of the seven subjects.

DISCUSSION

This study of DMSA chelation in seven workers occupationally exposed to lead for several years with increased chelatable lead on EDTA lead mobilization test was prompted by the emerging evidence that toxic effects of lead exposure may occur at PbB≤40 μg/dl (21).

The PbB declined rapidly in the first five days of DMSA treatment. On day 19, the PbB concentrations dropped to approximately 15% of the pretreatment values. Like in other studies (10, 11), the decline in PbB was associated with increased PbU.

Experimental investigations show that DMSA reduces lead concentrations in blood, brain, kidney, and liver, even in the face of continued Pb exposure (22, 23). A major question is whether DMSA mobilizes skeletal lead and redistributes it to target organs, especially the brain. *Cory-Slechta* (24) reported that DMSA did not produce any observable depletion in the bone lead concentration. There was a dose-related increase in bone lead and it was proposed that chelation therapy redistributed lead from the soft tissue compartments to the bone. Similar results were described by *Smith and Flegal* (25). However, some authors showed a significant decrease of bone lead due to DMSA chelation (23, 26). *Blanuša and co-workers* (26) treated suckling rats with DMSA and a monoisoamyl ester of DMSA after loading them with lead and found that both chelators caused a significant decrease of lead concentration in the brain.

Consistently with previous human and animal studies (10, 27), we found a twofold to threefold increase in the excretion of zinc and copper during DMSA treatment, along with slight variations in the excretion of iron, calcium, and magnesium.

The rebound of PbB after cessation of DMSA treatment was similar to that in other studies (10, 28) and was attributed to re-equilibration of lead from bone stores into the blood (29). A significant post-chelation rebound may indicate a preponderance of lead in slow pools and a small impact of chronic, heavy exposure on the total body stores. However, *Grandjean and co-workers* (11) treated a chronically lead poisoned patient with neurologic symptoms (headache, fatigue, vertigo, decreased memory and concentration) with five courses of DMSA treatment. Despite a rebound of blood lead after each treatment course, a significant improvement in patient's mental status occurred at the end of treatment. The clinical significance of redistribution and rebounding of lead remains unclear.

The justifiable use of chelation in occupationally lead exposed workers is controversial. The recommended therapy for industrial lead intoxication is to cease exposure until blood lead decreases to 1.95 μmol/L (40.4 μg/100 ml). Chelation therapy should be reserved for those with significant symptoms or signs of toxic reactions (30). However, there are serious gaps in the knowledge of the chronic, subclinical toxicity of lead and dose-response relationships are still largely uncertain (31, 32) as is the role of chelation therapy.

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Sažetak

PRIMJENA *mezo*-2,3-DIMERKAPTOJANTARNE KISELINE U RADNIKA PROFESIONALNO IZLOŽENIH OLOVU

Svrha ovog istraživanja bila je ustanoviti učinkovitost *mezo*-2,3-dimerkaptojantarne kiseline (DMSA) u radnika s povećanom apsorpcijom olova, a bez znakova trovanja olovom. Danas se DMSA smatra jednim od najboljih kelatirajućih sredstava, koje se pokazalo učinkovitim posebno u djece otrovane olovom. U Hrvatskoj, međutim, do sada još nije bilo primjenjivano.

Sedam radnika profesionalno izloženih olovu bilo je tretirano s DMSA. Radnici nisu imali kliničkih simptoma otrovanja olovom, koncentracija olova u krvi bila je viša od 50 µg/100 ml, a mobilizacijski test s kalcij dinatrij etilendiamintetraacetatom (EDTA) bio je pozitivan. Postupak je trajao 19 dana davanjem pojedinačnih doza od 700 mg DMSA tri puta na dan od prvog do petog dana i dva puta na dan od šestog do devetnaestog dana.

Rezultati su pokazali da je izlučivanje olova urinom za vrijeme primjene DMSA bilo povišeno, i to najviše prvih pet dana. Nakon toga se smanjilo do 15% od početne vrijednosti. Koncentracije olova u krvi, međutim, povisile su se 15 dana nakon završetka tretmana, ali nisu premašile vrijednost od 40 µg/100 ml. Nakon ponovljenog testa mobilizacije

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EDTA-om, olovo u urinu iznosilo je 23–68% od one vrijednosti koja je dobivena prije primjene DMSA. Izlučivanje cinka i bakra urinom bilo je za vrijeme postupka povišeno 2–3 puta, a nije bilo promjena u izlučivanju željeza, kalcija i magnezija.

Može se zaključiti da DMSA učinkovito smanjuje koncentracije olova u radnika profesionalno izloženih tom toksičnom metalu.

Ključne riječi:
bakar, CaNa$_2$EDTA, cink, kalcij, krv, kelatirajuća sredstva, magnezij, mobilizacijski test, urin, željezo

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