Provocative chelation with DMSA and EDTA: evidence for differential access to lead storage sites

Byung-Kook Lee, Brian S Schwartz, Walter Stewart, Kyu-Dong Ahn

Abstract

***Objectives*—To validate a provocative chelation test with 2,3-dimercaptosuccinic acid (DMSA) by direct comparison with the standard ethylene diamine tetraacetic acid (EDTA) test in the same subjects; and to compare and contrast the predictors of lead excretion after DMSA with those after EDTA. A metal chelating agent given orally, DMSA may mobilise and enhance the excretion of lead from the storage sites in the body that are most directly relevant to the health effects of lead. A provocative chelation test with DMSA could thus have wide potential application in clinical care and epidemiological studies.**

***Methods*—34 male lead workers in the Republic of Korea were given a single oral dose of 10 mg/kg DMSA, urine was collected over the next eight to 24 hours, and urine volume and urinary lead concentration determined at 0, 2, 4, 6, 8, and 24 hours. Either two weeks before or two weeks after the dose of DMSA 17 of these workers also received 1 g intravenous EDTA followed by an eight hour urine collection with fractionation at 0, 2, 4, 6, and 8 hours.**

***Results*—Urinary lead concentration peaked at two hours after DMSA and four hours after EDTA. Lead excretion after DMSA was less than after EDTA, and cumulative excretion after DMSA plateaued at six to eight hours. The two hour and four hour cumulative lead excretions after DMSA were highly correlated with the eight hour total ($r = 0{\cdot}76$ and $0{\cdot}95$). In multiple linear regression analyses, blood lead was found to be an important predictor of EDTA-chelatable lead, whereas urinary aminolevulinic acid (ALAU) was associated with DMSA-chelatable lead. Notably, lead excretion after DMSA was greatly increased if EDTA was given first. An earlier dose of EDTA also modified the relation between ALAU and DMSA-chelatable lead in that workers who received EDTA before DMSA showed a much steeper dose-response relation between these two measures.**

***Conclusions*—The predictors of lead excretion after DMSA and EDTA are different and an earlier dose of EDTA may increase lead excretion after a subsequent dose of DMSA. The results suggest that two hour or four hour cumulative lead excretion after DMSA may provide an estimate of lead in storage sites that are most directly relevant to the health effects of lead.**

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Human exposure to lead is ubiquitous and its absorption can be assessed by different measures thought to reflect several definable lead storage compartments.[1–3] Blood lead and zinc protoporphyrin (ZPP) are the two most common measures used to identify people at risk of excess exposure or ill health caused by lead. A limitation of both of these measures is that they are poor predictors of such ill health, do not necessarily reflect recent exposure, and are generally thought to be inadequate measures of cumulative lead absorption.[4] Blood lead concentrations are influenced by recent exposure, bioavailable internal stores, and differences between individuals in lead toxicokinetics.[5] The interpretation of ZPP, an early biological intermediary in the haematopoietic system, is complicated by differences between people in the kinetics of lead, the kinetics of the multiple steps in the haem synthetic pathway, and the kinetics of red blood cells.[5]

The limitations of blood lead and ZPP have led to the development of other biological measures of lead absorption. As 90–95% of the total body burden of lead resides in bone,[6] in at least two definable compartments—a relatively inert cortical bone storage pool and a more bioavailable pool in trabecular bone—*x* ray fluorescence has emerged as a technique for measurement of bone lead.[7–10] Although *x* ray fluorescence of cortical bone lead probably best estimates cumulative lead absorption, few studies have validated this as a predictor of health effects. It can be hypothesised that because much of the bone lead compartment is biologically inert, with lead deep in cortical bone, *x* ray fluorescent measurements of cortical bone lead may be less relevant to long term changes in health than biological measures that estimate the bioavailable lead pool. Such measures may include *x* ray fluorescence of trabecular bone lead and chelatable lead.

Provocative chelation with 1 g of intravenous calcium disodium ethylene diamine tetraacetic acid (EDTA) followed by a six to 24 hour urine collection for measurement of

lead has long been used to estimate the chelatable lead burden, thought to be one estimate of the bioavailable lead pool. Several studies have found that EDTA-chelatable lead correlated with renal dysfunction,[11 14] neurobehavioral dysfunction,[15] or declines in function of the peripheral nervous system.[16] No studies have directly compared measures of cumulative lead exposure, blood lead, cortical bone lead, trabecular bone lead, and chelatable lead as predictors of health effects related to lead. It is thus not possible to conclude whether EDTA-chelatable lead is a better predictor of health effects than blood lead simply because it provides a better estimate of cumulative absorption.

An obstacle to the large scale epidemiological use of EDTA-chelatable lead is that EDTA needs to be given intravenously followed by at least a six to eight hour urine collection. In contrast, 2,3-dimercaptosuccinic acid (DMSA; succimer, Chemet) is a chelating agent that is given orally and has several advantages compared with EDTA. Firstly, it is more specific for lead than EDTA, resulting in less loss of such important minerals as zinc. Secondly, in studies in animals it does not result in increased gastrointestinal absorption of lead or increased brain lead concentrations as has been reported with EDTA.[17 18] During therapeutic chelation DMSA is generally well tolerated with occasional gastrointestinal (nausea, diarrhoea) or dermatological (rash) side effects. A provocative chelation test with DMSA would be more convenient and logistically feasible for estimation of the chelatable lead pool. Although DMSA has been extensively used for the treatment of lead intoxication in children and adults,[19 21] no previous studies have directly compared lead excretion after a dose of EDTA with that after a dose of DMSA in humans, nor identified predictors of cumulative lead excretion after a single oral dose of DMSA in currently exposed lead workers. We present the results of such a study in general industrial lead workers in the Republic of Korea.

Materials and methods

STUDY POPULATION AND DESIGN

Study subjects were recruited from four factories that use lead in the Republic of Korea. Participation was voluntary. All 34 workers exposed to lead were men, with a mean (SD) age of 39·6 (9·8) years and a mean (SD) work duration of 7·1 (6·1) years. In this study, we were interested in identifying predictors of DMSA-chelatable lead as well as comparing lead excretion after a dose of EDTA to that after DMSA in the same subjects. Subjects were divided into two groups because of constraints on resources. A total of 17 workers from a single secondary smelting facility received DMSA followed by an eight hour urine collection (DMSA only group). An additional 17 workers each received DMSA and EDTA two weeks apart in randomised order. In these workers, urine was collected for 24 hours after DMSA and eight hours after EDTA (DMSA *v* EDTA group). These 17 workers were recruited from a lead storage battery factory (n = 5), a litharge manufacturing factory (n = 6), and a polyvinyl chloride stabiliser manufacturing factory (n = 6). Finally, five non-exposed men each received DMSA orally followed by a 24 hour urine collection. These subjects had a mean (SD) age of 32·2 (9·0) years and a mean blood lead concentration of 5·7 (1·3) μg/dl. The study protocol was approved by the Institutional Review Board of the Johns Hopkins School of Hygiene and Public Health.

DATA COLLECTION

DMSA only group

A total of 17 lead workers received a single oral dose of 10 mg/kg of DMSA followed by a urine collection with urinary volume and lead concentration measured at about 0, 2, 4, 6, and 8 hours. These data were combined with DMSA data from the 17 workers who also received EDTA and were used to estimate cumulative lead excretion over time after DMSA and to identify the predictors of lead excretion after DMSA.

DMSA v EDTA group

Another 17 lead workers received a single oral dose of 10 mg/kg DMSA followed by a 24 hour urine collection with urinary volume and lead concentration measurements at about 0, 2, 4, 6, 8, and 24 hours (three workers had only an eight hour collection). These workers also received 1 g intravenous EDTA in 5% dextrose over one hour followed by an eight hour urine collection with urinary volume and lead concentration measurements at about 0, 2, 4, 6, and 8 hours. Workers each received the two chelating agents two weeks apart, with eight workers randomly assigned to receiving DMSA first and nine to EDTA first.

Non-exposed subjects

The five non-exposed subjects each received 10 mg/kg oral DMSA followed by a 24 hour urine collection fractionated at 0, 2, 4, 6, 8, and 24 hours.

Other measures of interest included blood lead concentration, zinc protoporphyrin (ZPP), aminolevulinic acid (ALA) in the urine (ALAU), baseline urinary lead concentration before the chelating agent, duration of employment in the lead industry, age, weight, urinary specific gravity, and order of doses of DMSA and EDTA. Blood lead, ZPP, ALAU, and baseline urinary lead concentration were all obtained before each chelating agent was given.

LABORATORY ANALYSES

All laboratory analyses were performed at Soonchunhyang University in the Republic of Korea. This laboratory participates in Korean and Japanese quality assurance and control programs and is a reference laboratory for the analysis of lead in Korea. Blood lead was measured in duplicate by flameless atomic absorption spectrophotometry (AAS; Hitachi-Zeeman 8100, Japan) by a standard addition

method. The ZPP was measured by haematofluorometry (Aviv-206 portable haematofluorometer). Urinary lead was assayed by flameless AAS (Hitachi-Zeeman 8100) by a standard addition method. The ALAU was measured with the method of Tomokuni and Ogata by spectrophotometry (Spectronic 21, USA).[22] Urine specific gravity was measured with a refractometer (NOW, Japan).

For statistical analyses of cumulative lead excretion, urinary lead concentration was adjusted for a standard urine specific gravity of 1·020.[23] Urinary lead concentrations after a dose of DMSA, adjusted for urine specific gravity, correlated highly with the unadjusted measures ($r = 0{\cdot}69$, $P < 0{\cdot}001$). After EDTA was given, the correlation of the adjusted and unadjusted urinary lead concentrations was lower ($r = 0{\cdot}39$, $P = 0{\cdot}001$). Statistical analyses were performed with both unadjusted and adjusted urinary lead concentrations. Only the results of analyses with unadjusted urinary lead concentrations are presented.

DATA ANALYSIS

Data were analysed with the BMDP (BMDP Statistical Software, Los Angeles, CA) and SAS (SAS Institute, Cary, NC) statistical software programs. Detailed frequency distributions and summary statistics were examined for all study variables. Relations between variables were assessed by correlation and multiple linear regression.

Data on all 34 subjects exposed to lead who received DMSA were used to identify predictors of urinary lead excretion after a dose of DMSA. Linear regressions modeled cumulative lead excretion at eight hours after DMSA for order of chelating agent, blood lead, ZPP, ALAU, age, weight, work duration, and baseline urinary lead concentration. Blood lead, ZPP, and ALAU were each entered separately in the model because of small numbers of study subjects. Modification of the effect by the order of chelating agent was evaluated by including a cross product term of the order variable with each of blood lead, ZPP, or ALAU in the regression model.

Cumulative lead excretion at eight hours after EDTA was similarly modelled by multiple linear regression with data from the 17 subjects who received EDTA, as a function of the order of chelating agent, blood lead, ZPP, ALAU, age, weight, work duration, and baseline urinary lead concentration.

Results

The 34 lead workers had moderate to high exposure to lead as shown by a mean (range) blood lead concentration of almost 56 (29–77) μg/dl (table 1). Mean ZPP, ALAU, and urinary lead concentrations at baseline were similarly high (table 1). Non-exposed subjects all excreted <100 μg of lead in eight hours after DMSA, compared with a mean of 1369 μg for the 34 lead workers (table 1). In the 17 subjects who received both DMSA and EDTA, urinary lead excretion was always higher after EDTA, and the mean lead excretion eight hours after EDTA was almost three times higher than that after DMSA (table 1). Although the mean cumulative lead excretion at 24 hours after DMSA had increased by an average of 36% above the eight hour total, the eight hour and 24 hour values were perfectly correlated ($r = 1{\cdot}00$, $P < 0{\cdot}001$, $n = 14$ subjects). Hereafter, only results with the eight hour values are reported.

There was no difference in age between the 17 subjects who received DMSA only and the 17 subjects who received both DMSA and EDTA (table 1). The mean blood lead, ZPP,

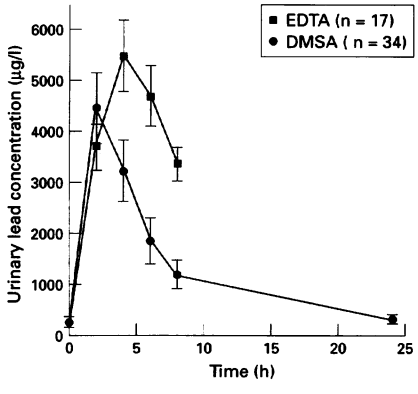

Figure 1 Estimated urinary lead concentration over time after 1 g of intravenous EDTA to 17 lead workers or 10 mg/kg of oral DMSA to 34 lead workers in the Republic of Korea. Error bars represent SEMs.

Table 1 Summary of statistics for selected study variables in various groups of workers exposed and not exposed to lead

Study variable	*All workers (n = 34) Mean (SD; range)*	*Specific groups (mean (SD))*		
		DMSA only (n = 17)	*DMSA v EDTA (n = 17)**	*P value†*
Age (y)	39·6 (9·8)	38·8 (10·3)	42·6 (8·7)	0·25
Work duration	7·1 (6·1)	3·9 (5·6)	10·2 (4·9)	<0·01
PbB (μg/dl)	55·6 (12·5; 29–77)	51·3 (14·2)	60·0 (9·0)	0·04
ZPP (μg/dl)	133·4 (65·7; 18–265)	97·5 (58·4)	169·4 (52·3)	<0·01
ALAU (mg/l)	8·2 (6·1; 1–32)	8·7 (8·2)	7·7 (3·0)	0·65
PbU at baseline (μg/l)	149 (89; 42–360)	171·6 (76·7)	126·2 (95·8)	0·14
Pb excretion after DMSA (μg):				
Non-exposed subjects at 8 h (n = 5)	61 (26; 30–97)	—	—	—
Exposed subjects at 8 h	1369 (798; 387–3666)	928 (453)	1811 (833)	<0·01
Exposed subjects at 24 h (n = 14)	1867 (862; 768–3742)	—	—	—
8 h PbU after EDTA (μg)	—	—	3916 (1463)	—

*Nine workers received EDTA first then DMSA, and eight workers received DMSA first then EDTA.
†Comparing means in DMSA only and DMSA *v* EDTA groups.
‡These five subjects are the non-exposed controls.
Pb = lead; PbB = blood lead; PbU = urinary lead.

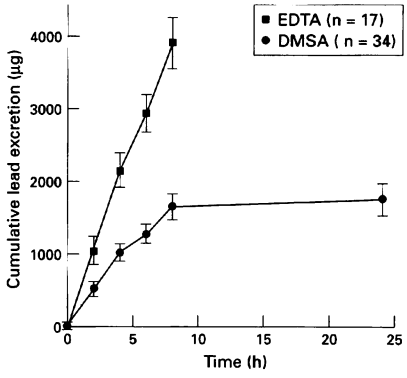

Figure 2 Cumulative lead excretion over time after 1 g intravenous EDTA to 17 lead workers or 10 mg/kg oral DMSA to 34 lead workers in the Republic of Korea. Error bars represent SEMs.

and work duration were higher in the DMSA *v* EDTA group, whereas ALAU and baseline urinary lead were higher in the DMSA only group (table 1).

On average, urinary lead concentration peaked at about two hours after DMSA and at four hours after EDTA (fig 1). Urinary lead concentrations decreased rapidly after DMSA, returning to baseline by no later than 24 hours. After EDTA, urinary lead concentrations reached a higher peak and by eight hours the mean urinary lead concentration in 17 workers was still over 10 times the mean baseline concentration. Cumulative lead excretion after DMSA plateaued by six to eight hours, whereas it was still rapidly increasing at eight hours after EDTA (fig 2). Figure 1 shows that cumulative lead excretion after EDTA would begin to plateau at about 10 hours.

The duration of the urine collection after a chelating agent has been given can be an obstacle to the epidemiological use of these measures of chelatable lead. Notably, the two and four hour cumulative lead excretions after DMSA correlated highly with the eight hour total in the 34 subjects ($r = 0{\cdot}76$ for two hour *v* eight hour and $r = 0{\cdot}95$ for four hour *v* eight hour), which suggests that a two or four hour urine collection would be adequate to estimate DMSA-chelatable lead. The eight hour cumulative lead excretions after DMSA and EDTA showed a modest correlation ($r = 0{\cdot}44$, $P = 0{\cdot}08$, $n = 17$); this value increased to $r = 0{\cdot}83$ ($P < 0{\cdot}001$) after the removal of two outliers. One of these subjects, who had DMSA first, had an eight hour lead excretion of 4417 µg after EDTA but only 768 µg after DMSA. The other, who received EDTA first, had an eight hour lead excretion of 6853 µg after EDTA but only 915 µg after DMSA.

In bivariate correlations, age was not associated with eight hour lead excretion after either DMSA or EDTA (table 2). Weight was significantly correlated with eight hour urinary lead excretion after EDTA but not after DMSA, whereas work duration was correlated with lead excretion after DMSA but not after EDTA. Blood lead and ZPP each showed different relations with DMSA—and EDTA-chelatable lead. Consistent with earlier research, blood lead was significantly correlated with EDTA-chelatable lead ($r = 0{\cdot}55$) but was not associated with DMSA-chelatable lead. An early biological intermediary in the haem synthetic pathway, ZPP, was correlated only with DMSA-chelatable lead ($r = 0{\cdot}50$). Neither ALAU nor baseline urinary lead concentration were associated with lead excretion after either chelating agent.

A unique finding in this study was that cumulative lead excretion after DMSA was greater if EDTA was given first (table 3). In linear regression analyses, lead excretion after DMSA and EDTA were modelled with a variable indicating the order in which the chelating agent was given. For example, after controlling for blood lead and weight, workers who received EDTA before DMSA excreted, on average, 1068 µg more lead after DMSA than did workers who did not receive EDTA before the DMSA ($P = 0{\cdot}0002$). The data showed that an earlier dose of EDTA caused an interesting modification of effect on the relation between ALAU, measured before the dose of DMSA, and eight hour urinary lead excretion after DMSA (table 3, model 3, and fig 3). Workers who received EDTA before DMSA showed a much stronger association—that is, steeper dose-response curve—between ALAU and lead excretion after DMSA. For each 1 mg/l increase in ALAU, urinary lead excretion after DMSA was estimated to increase by 156 µg in workers who received EDTA before DMSA, but only 22 µg if EDTA was not given first. The same modification of effect was not found with blood lead

Table 2 Correlations of independent variables with cumulative lead excretion eight hours after DMSA and EDTA in lead workers in the Republic of Korea

	Pearson's correlation coefficient r (P-value)	
*Variable**	*8 h DMSA (n = 34)**	*8 h EDTA (n = 17)**
Age	0·19 (0·28)	−0·40 (0·11)
Weight	0·19 (0·28)	0·50 (0·04)
Work duration in lead industry	0·38 (0·03)	0·03 (0·91)
PbB at time of chelation (µg/dl)	0·15 (0·41)	0·55 (0·02)
ZPP at time of chelation (µg/dl)	0·50 (0·003)	0·23 (0·37)
ALAU at time of chelation (mg/l)	0·23 (0·19)	0·33 (0·20)
PbU at baseline at time of chelation (µg/l)	−0·003 (0·99)	−0·17 (0·51)

*8 h DMSA (or EDTA) = 8 h cumulative lead excretion after 10 mg/kg oral DMSA (or 1 g intravenous EDTA) (µg). Abbreviations as for table 1.

*Table 3 Linear regression results in models of eight hours cumulative lead excretion after a single oral dose (10 mg/kg) of DMSA in 34 Korean lead workers**

Independent variable†	*β Coefficient*	*SEM β*	*P value*	*Total r²*
Model 1—with PbB:				0·43
PbB	7·975	9·030	0·38	
Weight	19·537	15·690	0·22	
EDTA before	1068·172	248·548	0·0002	
Model 2—with ZPP:				0·44
ZPP	2·779	2·073	0·19	
Weight	19·471	15·256	0·21	
EDTA before	847·428	304·077	0·009	
Model 3—with ALAU‡				0·43
ALAU	21·636	18·019	0·24	
Work duration	32·728	19·312	0·10	
EDTA before	−325·599	665·685	0·63	
EDTA before × ALAU§	134·568	66·940	0·05	

*Regressions were performed separately with PbB, ZPP, and ALAU. Half the workers also received 1 g intravenous EDTA, two weeks before or after DMSA.
†EDTA before = 1 if EDTA was given before DMSA.
‡This model is presented graphically in fig 3.
§Cross product term evaluated modification of effect of EDTA given first on association between ALAU and 8 h cumulative lead excretion after DMSA.

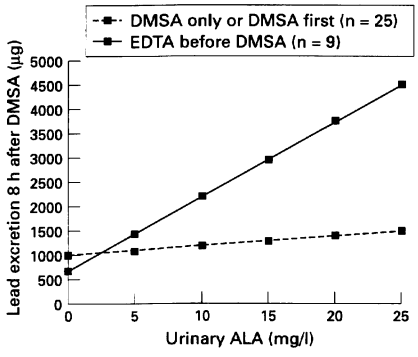

Figure 3 Modification effected by EDTA on the relation between ALAU and eight hour cumulative lead excretion after DMSA in 34 lead workers. These are the results of model 3 in table 3. The data indicate that the dose-response relation between ALAU and eight hour lead excretion after DMSA was much steeper if EDTA was given two weeks before DMSA.

and ZPP and either DMSA- or EDTA-chelatable lead. Notably, when the relation between ALAU and eight hour lead excretion after DMSA was evaluated in the 17 subjects who received only DMSA (lower line in fig 3), ALAU was directly related to lead excretion in these subjects ($\beta = 25{\cdot}66$, $P = 0{\cdot}04$, regression data not shown), but this association was not found after the elimination of one subject with a large value for ALAU. Lead excretion after EDTA seemed to be less if DMSA was given first, but none of the differences in any of the models were significant (all P values for DMSA first were variable and >0·05, table 4).

Lead excretion after DMSA and EDTA showed different relations with blood lead, ZPP, and ALAU in a manner consistent with the above findings. Specifically, ALAU was more strongly related to DMSA-chelatable lead than was either blood lead or ZPP (table 3). In contrast, blood lead at the time EDTA was given was more strongly related to cumulative lead excretion after EDTA than was either ZPP or ALAU (table 4). For each 1 µg/dl increase in blood lead, urinary lead excretion after EDTA increased by 98 µg. Age, work duration, and baseline urinary lead concentration were not consistently associated with cumulative lead excretion after either agent was given.

Discussion

To our knowledge, no previous studies have compared lead excretion after DMSA with that after EDTA in the same subjects, nor compared the predictors of such excretion with the two agents. Previous investigators have reported on the comparison of lead excretion after DMSA (270 mg three times, eight hours apart) and EDTA (1 g intravenously twice, 12 hours apart) in a single worker with an initial blood lead of 82 µg/dl.[24] In this worker, cumulative lead excretion after the two agents was similar. A provocative chelation test with DMSA could have broad applications in clinical settings and for use in epidemiological studies of the health effects of lead. Although XRF measurement of bone lead provides an estimate of cumulative lead absorption, much of the measured lead is not relevant to current health as it is quiescent in cortical bone. As will be discussed, DMSA may chelate lead from storage sites in the body that are directly relevant to changes in health over time, and thus DMSA-chelatable lead could be useful in both clinical and epidemiological settings.

The data suggest that DMSA and EDTA lead excretion correlated; that lead excretion after EDTA was generally higher than after DMSA; that peak urinary lead concentrations and return to baseline are attained more rapidly after DMSA than after EDTA; and that lead excretion after DMSA is rapid, such that cumulative lead excretion at 2, 4, 6, 8, and 24 hours were all highly correlated. Blood lead was an important predictor of lead excretion after EDTA whereas ALAU was an important predictor of lead excretion after DMSA. Age, work duration, subject weight, and baseline urinary lead concentration were not consistently associated with lead excretion after either DMSA or EDTA in adjusted analyses. Interestingly, cumulative lead excretion after DMSA was higher in workers who received EDTA two weeks before DMSA. Also, EDTA given before DMSA was an important modifier of the relation between ALAU and eight hour lead excretion after DMSA.

The DMSA only group and the DMSA *v* EDTA group were recruited from different work sites. The two groups had similar ages, blood lead concentrations (although a statistically significant difference was found, we do not think that the difference in blood lead concentrations was biologically important), baseline urinary lead concentrations, and ALAU, but the DMSA *v* EDTA group had higher mean ZPP concentrations and work durations. We do not think, however, that uncontrolled confounding by work site is likely to have influenced the study results. When we controlled for these important measured confounding variables—that is, ones that we thought from the start were most likely to influence the relation between chelatable lead concentrations and the main independent variables such as blood lead and ALAU—there was no important change in the associations found. We thus think that it is unlikely that unmeasured confounders had a meaningful influence on these relations.

Although not entirely resolved,[25 26] DMSA

*Table 4 Linear regression results of models of eight hour cumulative lead excretion after 1 g intravenous EDTA in 17 Korean lead workers**

Independent variable†	*β Coefficient*	*SEM β*	*P value*	*Total r²*
Model 1—with PbB:				0·56
PbB	97·680	39·944	0·03	
Weight	75·656	38·042	0·07	
DMSA first	−624·245	582·121	0·30	
Model 2—with ZPP:				0·36
ZPP	0·430	9·370	0·96	
Weight	72·818	53·858	0·20	
DMSA first	−967·452	1137·707	0·41	
Model 3—with ALAU				0·39
ALAU	82·589	99·232	0·42	
Weight	74·111	44·852	0·12	
DMSA first	−786·072	712·270	0·29	

*Regressions were performed separately with PbB, ZPP, and ALAU. All workers also received 10 mg/kg of oral DMSA two weeks before or after EDTA.
†DMSA first = 1 if DMSA was given before EDTA.

seems to mobilise lead primarily from soft tissue and does not seem to have a significant effect on lead in bone.[27] As such, redistribution of lead from bone to soft tissue target organs such as the brain and kidneys is not thought to occur. This not only makes DMSA theoretically safer in terms of long term organ function with chelation treatment, but perhaps also more relevant for use in provocative chelation. The soft tissue compartment of lead storage may be most relevant to the function of the target organ so to assess DMSA-chelatable lead could be an easy and convenient measure of this type of lead storage site. Studies in animals have shown that lead concentrations decreased dramatically in brain, liver, and kidney after DMSA was given.[25 27] This supports the notion that DMSA-chelatable lead could be used to estimate the lead burden in target organs for use in epidemiological studies. In one study in a small number of animals with restricted ranges of lead tissue burdens low correlations were found between DMSA-chelatable lead and lead concentrations in organs (kidney, liver, brain).[27] Nevertheless, the ultimate test of the validity of the measure is whether it predicts health effects in humans. This has not been evaluated to date.

Several of the findings in our study are consistent with the hypothesis that DMSA-chelatable lead is a measure of bioavailable lead stores, primarily of soft tissue origin. Lead excretion after DMSA is enhanced if EDTA is given before the DMSA. This is consistent with research in animals that suggested that EDTA results in a redistribution of lead from bone to soft tissue. It is thus possible that EDTA increased concentrations of soft tissue lead and DMSA then mobilised lead from these sites. In contrast with EDTA-chelatable lead, which was most strongly associated with blood lead concentrations (consistent with previous research[9 28–31]) DMSA-chelatable lead was most strongly associated with ALAU, but notably not with blood lead. The lack of correlation with blood lead concentrations may be due to the important influence of bone lead on blood lead concentrations; DMSA does not seem to chelate lead from bone.

The substrate, ALA, for the lead sensitive enzyme δ-aminolevulinic acid dehydratase (ALAD) is a measure of an early biological effect of lead in the haem synthetic system. It would seem that only bioavailable lead inhibits ALAD. The association of ALAU with DMSA-chelatable lead in our study is consistent with the interpretation of DMSA-chelatable lead as a measure of bioavailable lead stores. The dose-response relation between ALAU and DMSA-chelatable lead was much steeper when EDTA was given two weeks before DMSA. We speculate that EDTA redistributed large amounts of lead to soft tissue sites, which led to increased inhibition of ALAD, accumulation of ALA, increased lead excretion after DMSA two weeks later, and a stronger association between ALAU and DMSA-chelatable lead.

Cory-Slechta has proposed that one strategy in the chelation of lead workers might be to give DMSA before EDTA to prevent the redistribution of lead mobilised from bone and enhance depletion of soft tissue lead stores.[27] The present data indicate that such redistribution may, in fact, occur in humans, because DMSA mobilised more lead if EDTA was given first. If the goal is to remove as much lead as possible, however, this strategy would be less efficient, and perhaps EDTA should be given first followed shortly by DMSA. It should be noted that the ultimate value of any of these strategies in improving the health of lead workers has not been rigorously evaluated to date.

Other data suggest that the toxicokinetics of DMSA are favourable to its use in a provocative chelation test. After an oral dose of DMSA to normal human volunteers, urinary excretion of the unaltered (not metabolised) drug peaks at about two hours and is essentially complete by nine hours.[32] Urinary excretion of altered DMSA, which consists of several oxidised species including mixed DMSA-cysteine disulphides, peaks at about four hours and is not complete for 24–48 hours.[32 33] By 14 hours, about 21% of the DMSA given had appeared in the urine, with 88% as the altered form. Urinary lead excretion peaked at four hours and returned to baseline between six and eight hours. The DMSA is extensively bound to plasma proteins, mainly albumin, and does not seem to penetrate the erythrocytes.[33] Although the structure of the DMSA-lead chelate is not currently known, some investigators hypothesised that the mixed DMSA-cysteine disulphides may be the active chelating species.[34] It is interesting to note that published cumulative excretion curves of unaltered DMSA are similar to those of cumulative lead excretion after DMSA in these Korean lead workers.

The DMSA-cysteine mixed disulphide has not been detected in blood but is the primary form of altered DMSA in urine.[33] Plasma proteins may serve as a depot for DMSA in the blood. After transport to the kidney, exchange with cysteine may occur that results in excretion of the mixed disulphide. Although the biochemical basis for these transformations is not currently known, this suggests that DMSA may not be available in the blood for chelation of lead. Other data suggest that DMSA is primarily extracellular in its distribution.[35] Lead may be chelated by DMSA just before excretion from the kidney. If DMSA only forms complexes with lead in the kidney just before excretion, this may also explain why DMSA does not cause redistribution of lead to soft tissue. The DMSA-chelatable lead may thus be very relevant to the epidemiological study of renal function and perhaps blood pressure.

Two workers, one of whom received DMSA first and the other EDTA first, excreted large quantities of lead after EDTA but small quantities after DMSA. It can be speculated that there could be differences between people in the formation of these

mixed disulphides, which are likely to be enzymatically mediated, and perhaps this could account for the relatively low excretion of lead in these two subjects after DMSA.

As DMSA itself seems to be mainly distributed in plasma and does not mobilise lead in bone, an important question is the interpretation of the DMSA provocative chelation test. Clearly, bone lead measured by *x* ray fluorescence is a better measure of cumulative lead absorption and retained body burden, but much of this lead is biologically inactive and *x* ray fluorescence is not widely available. The plasma compartment is thought to be very important to the health effects of lead in that all lead that is deposited in target organs passes through this compartment.[2 3] Although whole blood lead concentrations were not a predictor of DMSA-chelatable lead, plasma lead concentrations may be, but measurement of plasma lead was beyond the scope of this study. The ultimate validation of DMSA-chelatable lead awaits the results of prospective epidemiological studies.

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