δ -Aminolevulinic acid dehydratase genotype modifies four hour urinary lead excretion after oral administration of dimercaptosuccinic acid

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Abstract

Objectives—Previous research suggests that binding of lead by δ -aminolevulinic acid dehydratase (ALAD) may vary by ALAD genotype. This hypothesis was tested by examining whether ALAD genotype modifies urinary lead excretion (DMSA chelatable lead) after oral administration of dimercaptosuccinic acid (DMSA).

Methods—57 South Korean lead battery manufacturing workers were given 5 mg/kg oral DMSA and urine was collected for four hours. Male workers were randomly selected from two ALAD genotype strata (ALAD1-1, ALAD1-2) from among all current workers in the two plants (n = 290). Subjects with ALAD1-1 (n = 38) were frequency matched with subjects with ALAD1-2 (n = 19) on duration of employment in the lead industry. Blood lead, zinc protoporphyrin, and plasma aminolevulinic acid concentrations, as well as ALAD genotype, duration of exposure, current tobacco use, and weight were examined as predictors or effect modifiers of levels of DMSA chelatable lead.

Results—Blood lead concentrations ranged from 11 to 53 μ g/dl, with a mean (SD) of 25.4 (10.2) μ g/dl. After 5 mg/kg DMSA orally, the workers excreted a mean (SD) 85.4 (45.0) μ g lead during a four hour urine collection (range 16.5–184.1 μ g). After controlling for blood lead concentrations, duration of exposure, current tobacco use, and body weight, subjects with ALAD1-2 excreted, on average, 24 μ g less lead during the four hour urine collection than did subjects with ALAD1-1 (P = 0.05). ALAD genotype seemed to modify the relation between plasma δ -aminolevulinic acid (ALA) and DMSA chelatable lead. Workers with ALAD1-2 excreted more lead, after being given DMSA, with increasing plasma ALA than did workers with ALAD1-1 (P value for interaction = 0.01).

Conclusions—DMSA chelatable lead may partly reflect the stores of bioavailable lead, and the current data indicate that subjects with ALAD1-2 have lower stores than those with ALAD1-1. These data provide further evidence that the ALAD genotype modifies the toxicokinetics of lead—for example, by differential binding of current lead stores or by differences in

long term retention and deposition of lead.

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Keywords: δ -aminolevulinic acid dehydratase genotype; chelating agents; dimercaptosuccinic acid; lead

The factors that mediate the absorption, distribution, and excretion of lead are complex and poorly understood. None the less, binding of lead by specific proteins in different tissues probably has a major influence on the toxicokinetics, and perhaps the toxicity, of lead.^{1–6} Known polymorphisms in genes coding for several of these proteins may account for significant variation in intravascular and soft tissue binding and in the long term deposition of lead. One such possible polymorphism has been described for the δ -aminolevulinic acid dehydratase (ALAD) gene, which codes for one of three isozymic proteins (termed ALAD1-1, ALAD1-2, and ALAD2-2).^{7–14}

ALAD genotype seems to modify the toxicokinetics of lead. Investigators first found differences in blood lead concentrations by genotype, with higher concentrations in subjects with ALAD1-2.^{7,8,14} We previously reported that the prevalence of ALAD1-2 was increased in a battery manufacturing plant with high exposure to lead compared with plants with lower exposures.¹⁴ Prevalence of ALAD1-2 was also higher among workers with longer duration of exposures.¹⁴ Smith *et al* have reported that concentrations of lead in trabecular bone were higher and those in cortical bone were lower in carpenters with ALAD1-2, although these differences did not reach significance.¹⁵ Fewer studies have evaluated whether ALAD genotype influences health effects related to lead. Smith *et al* reported higher blood urea nitrogen and uric acid concentrations in workers with ALAD1-2,¹⁵ and Bellinger *et al* reported better neuropsychological test performance in five adolescents with ALAD1-2 exposed to lead compared with 67 adolescents with ALAD1-1 exposed to lead.¹³

The hypotheses about altered lead binding by ALAD raise the question whether ALAD may mediate bioavailable lead stores. Dimercaptosuccinic acid (DMSA) is a lead chelating agent given orally. Urinary lead excretion after a dose of DMSA (DMSA chelatable lead) may be a biomarker of bioavailable lead burden.¹⁶ Data suggest that the predictors of urinary lead excretion after

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10 mg/kg DMSA orally and 1 g intravenous ethylene diamine tetra-acetic acid (EDTA) are different, and that earlier EDTA increases excretion of urinary lead when DMSA is given later.¹⁶ Blood lead is a strong predictor of EDTA chelatable lead,¹⁶⁻²⁰ whereas urinary δ -aminolevulinic acid (ALA) was reported to be the strongest predictor of DMSA chelatable lead.¹⁶ The DMSA may preferentially remove lead from soft tissue storage sites, perhaps only after conjugation with cysteine, whereas EDTA primarily removes lead from bone sites.²¹⁻²⁷ As ALAD may be an important site of lead binding in many soft tissue storage pools, we thought it would be of interest to compare DMSA chelatable lead by ALAD genotype. We report on differences in DMSA chelatable lead by genotype and associations of this measure with other biological markers of lead exposure.

Materials and methods

STUDY POPULATION

Current male employees from two lead storage battery factories in the Republic of Korea were studied to evaluate DMSA chelatable lead, neuropsychological function, and blood pressure by ALAD genotype. These employees were part of a larger study of 308 current workers in three factories who were genotyped for ALAD.¹⁴ The current study was limited to workers at two of the three factories, both owned by the same company. One factory manufactures batteries for cars and lorries (plant A) and the other manufactures large industrial batteries (plant B). Before the construction of these plants in the late 1980s, the company had a single plant with higher lead exposures. After completion of the new plants, the long term workers employed at the older plant were transferred to plant B. Plant A hired new workers.

Of the 30 subjects in the two plants who were ALAD1-2, 19 who were randomly selected from three exposure strata (< 2 years, 2-5 years, > 5 years), agreed to participate in the current study, and provided informed consent. A total of 38 subjects who were randomly selected from among the 259 subjects with ALAD1-1 in the two plants, agreed to participate, provided informed consent, and were frequency matched to subjects with ALAD1-2 by duration of exposure. The 57 subjects who completed testing had a mean (SD, range) age of 32.3 (5.9, 21-50) years and had been employed in the lead industry for 7.2 (3.3, 1.5-13.9) years. The study protocol was approved by the institutional review boards of the Johns Hopkins School of Hygiene and Public Health and Soonchunhyang University.

STUDY VARIABLES

Data collection, from 1 December 1994 to 30 April 1995, included a questionnaire administered by an examiner to obtain information on job history, medical history, current symptoms, and history of tobacco and alcohol consumption; neurobehavioral testing with the World Health Organisation neurobehavioural

core test battery and the trails A and B tests, blood pressure measurement, blood and urine collection for blood lead, zinc protoporphyrin (ZPP), plasma ALA, urinary ALA, and estimation of DMSA chelatable lead. Subjects were given 5 mg/kg dimercaptosuccinic acid (DMSA) orally and urine was collected for four hours. Total lead excretion in four hours was used as a measure of DMSA chelatable lead.

ALAD GENOTYPING

A modified protocol based on the polymerase chain reaction (PCR) was used for ALAD genotyping and has been previously described.^{6,14} Briefly, reactions were performed sequentially and in duplicate on 0.5 μ l of whole blood with nested primers. Reactions were completed with 1 unit of Taq polymerase (Perkin Elmer Cetus) in a buffer containing 300 ng of each primer, 200 μ M of each dNTP, 0.1% Triton X100, 100 mM Tris-HCl pH 8.9, 10 μ M KCl, and 2.5 μ M MgCl₂. Blood samples were initially preincubated for three cycles of three minutes at 94°C and then three minutes at 55°C. One unit of Taq polymerase was then added. Samples were heated for two minutes at 94°C and incubations were continued for 35 cycles of 10 s at 55°C, 30 s at 71°C, and 30 s at 94°C. The initial amplification, with 3' and 5' oligonucleotide primers (5'-AGACAGACAT-TAGCTCAGTA-3') and (5'-GGCAAAGAA-CACGTCCATTC-3'), generates a 916 base pair fragment. A second round of amplification with a pair of nested primers (kindly provided by J Wetmur), sequences (5'-CAGAGCT-GTTCCAAACAGTGGA-3') and (5'-CCAG-CACAATGTGGGAGTGA-3'), generates an 887 base pair fragment. The amplified fragment was cleaved at the diagnostic MSP1 site, electrophoresed through a 2% agarose gel, stained with ethidium bromide, and visualised by fluorescence.

LABORATORY ANALYSES

Plasma and urinary ALA were measured by high performance liquid chromatography (HPLC)²⁸ with a modification of the method of Tomokuni *et al.*²⁹⁻³¹ Blood and urinary lead were measured in duplicate by flameless atomic absorption spectrophotometry (Hitachi-Zeeman 8100, Japan) by standard addition methods.^{32,33} These methods for measurement of lead in blood and urine have reported blood lead precisions of 5%-8%, or estimated urinary lead precision of 15%.^{32,33} Also, the laboratory participates in Korean and Japanese government quality control programmes that require regular analysis of reference samples provided by the programmes. During January and February 1995, the average accuracy for five reference samples was $\pm$ 3.1%. The ZPP was measured by haematofluorimetry (ZP Hematofluorometer—Model 206, Aviv Biomedical Inc, Lakewood, NJ, USA).

DATA ANALYSIS

Statistical analyses were performed with the BMDP statistical software program (BMDP

Table 1 Summary statistics of selected study variables by *δ*-aminolevulinic acid dehydratase genotype in 57 Korean lead workers

Characteristic	ALAD genotype		P value*
	ALAD1-1 (n = 38)	ALAD1-2 (n = 19)	
Age (y) (mean (SD))	32.1 (5.9)	32.8 (6.1)	0.68
Duration of exposure (y) (mean (SD))	7.3 (3.3)	7.0 (3.3)	0.76
Weight (kg) (mean (SD))	62.8 (11.5)	65.7 (7.0)	0.32
Employed in plant A (%)	71.1	78.9	0.52
Current cigarette smokers (%)	71.1	78.9	0.52
Past cigarette smokers (%)	10.5	10.5	1.0
Current alcohol users (%)	76.3	78.9	0.82
Blood lead in early 1995 (µg/dl) (mean (SD))	26.1 (9.8)	24.0 (11.3)	0.48
Blood lead in late 1994 (µg/dl) (mean (SD))	38.5 (10.7)	34.5 (13.6)	0.23
ZPP in early 1995 (µg/dl) (mean (SD))	57.1 (26.8)	48.3 (22.2)	0.23
ZPP in late 1994 (µg/dl) (mean (SD))	68.9 (31.9)	55.6 (28.8)	0.13
Urinary ALA in late 1994 (ng/ml) (mean (SD))	1395 (984)	1022 (679)	0.15
Plasma ALA in late 1994 (ng/ml) (mean (SD))	17.3 (9.2)	11.8 (3.3)	0.03
Four hour urinary lead excretion after 5 mg/kg oral DMSA (µg) (mean (SD))	92.9 (45.1)	70.3 (42.1)	0.07

P values are from *t* tests comparing the means in the two ALAD genotype groups.

Statistical Software, Los Angeles, CA, USA). Detailed frequency distributions and summary statistics were examined for all study variables. Relations between variables were assessed by correlations, analysis of variance, and multiple linear regression.

Linear regression was used to determine predictors of four hour urinary excretion after 5 mg/kg DMSA orally. The ALAD genotype was evaluated as both a main effect on DMSA chelatable lead levels, and as an effect modifier of the relation between blood lead, ZPP, plasma ALA, urinary ALA, and duration of

exposure and DMSA chelatable lead. Only interactions between blood lead, ZPP, plasma ALA, urinary ALA, and duration of exposure and ALAD genotype were examined; these interactions were identified originally as being of interest because of previous research and hypotheses on binding of lead by ALAD within erythrocytes.⁷⁻¹⁶ Non-linear relations between the various lead biomarkers—for example, blood lead, ZPP, plasma ALA—and DMSA chelatable lead were evaluated by inclusion of quadratic terms for the biomarkers in the linear regression models. All linear regression models were assessed for departures from normality, multicollinearity, and heteroscedasticity.

Results

Blood lead and ZPP concentrations suggested that lead exposures in these plants were moderate (table 1). Blood lead concentrations ranged from 11 to 53 µg/dl, with a mean (SD) of 25.4 (10.2) µg/dl. After 5 mg/kg DMSA orally, the 57 workers excreted a mean (SD, range) of 85.4 (45.0, 16.5–184.1) µg of lead during a four hour urine collection.

There were no differences by ALAD genotype in age, duration of exposure, proportion of workers employed in plant A, cigarette or alcohol use, blood lead concentrations, or ZPP concentrations (table 1). There were differences in plasma ALA (*P* = 0.01) and concentrations of DMSA chelatable lead (*P* = 0.07) by ALAD genotype (figure). The DMSA chelatable lead was correlated with blood lead

Blood lead, DMSA chelatable lead, and plasma ALA concentrations by ALAD genotype in 57 Korean male lead workers. P values are for differences in the three biomarkers by ALAD genotype. The adjusted P value for DMSA chelatable lead is after control for concentrations of blood lead, duration of exposure, weight, and current cigarette use.

Table 2 Pearson's correlations for selected study variables in 57 Korean lead workers

Variable**	Pearson's r correlation coefficient*					
	Chellead	Blood lead	ZPP	Duration of exposure	Plasma ALA	Urinary ALA
Chellead (μg)†	—					
Blood lead in early 1995 ($\mu\text{g/dl}$)	0.40	—				
ZPP in early 1995 ($\mu\text{g/dl}$)	0.10	0.55	—			
Duration of exposure (y)	-0.18	0.10	0.14	—		
Plasma ALA in late 1994 (ng/dl)‡	0.35	0.35	0.21	0.51	—	
Urinary ALA in late 1994 (ng/dl)	-0.12	0.18	0.04	0.21	0.06	—

*Pearson's r values ≥ 0.35 are associated with P values ≤ 0.01 . No other r values have associated P values ≤ 0.05 .

†Chellead = four hour urinary lead excretion after 5 mg/kg DMSA orally.

‡All correlations with plasma ALA are after elimination of three extreme values (outliers) for this variable.

($r = 0.40$) and plasma ALA ($r = 0.35$) concentrations (table 2). As expected, blood lead concentrations were correlated with ZPP ($r = 0.55$) and plasma ALA ($r = 0.35$) concentrations (table 2).

ALAD genotype was an independent predictor of DMSA chelatable lead, after control for blood lead concentrations, duration of exposure, current tobacco use, and weight (table 3, model 1). Subjects with ALAD1-2 excreted a mean of 24 μg less lead during the four hour urine collection than did subjects with ALAD1-1 ($P = 0.05$). Similar relations were found when ZPP and plasma ALA were substituted for blood lead in this model (table 3, models 2a and 3). The ALAD genotype did not modify the relations between blood lead or ZPP concentrations and DMSA chelatable lead. In a separate model, genotype did seem, however, to modify the relation between plasma ALA and DMSA chelatable lead. Workers with ALAD1-2 excreted more lead, after DMSA, with increasing plasma ALA (a larger regression coefficient for the relation between plasma ALA and DMSA chelatable lead, table 3, model 2b) than did workers with ALAD1-1 (P value for interaction < 0.01). Some caution should be expressed as three

people with ALAD1-1 and high plasma ALA concentrations had a strong influence on this relation.

Although there were some differences in DMSA chelatable lead ($P = 0.07$) and duration of exposure ($P = 0.01$) by month of testing, there were no such differences in blood lead, ZPP, urinary ALA, or plasma ALA concentrations. Month of testing was not associated with DMSA chelatable lead in linear regression models after adjustment for blood lead, duration of exposure, ALAD genotype, weight, and current smoking status.

Discussion

In male lead workers with similar blood lead concentrations, duration of exposures, and ages, the data suggested that ALAD genotype influenced urinary lead excretion after a 5 mg/kg oral dose of DMSA. Subjects with ALAD1-2 excreted significantly less lead than did subjects with ALAD1-1. If urinary lead excretion after DMSA is conceptualised as a surrogate for bioavailable lead stores, then the data imply that subjects with ALAD1-2 have lower current bioavailable lead stores. These data provide further evidence that ALAD genotype modifies the toxicokinetics of lead, although the nature of the interaction between the different variants of this protein and lead seems to be complex. For example, ALAD1-2 could influence DMSA chelatable lead by differential current binding of lead in the intravascular space, by modifying the long term retention and deposition of lead, or by acting as a selection factor in the workplace (subjects with ALAD1-1 end employment earlier, subjects with ALAD1-2 select for more highly exposed areas) by an as yet unrecognised mechanism independent of these two.

There is supporting evidence for all of these potential mechanisms. ALAD genotype influences plasma ALA concentrations, which sup-

Table 3 Linear regression results modelling four hour urinary lead excretion after 5 mg/kg DMSA orally (four hour DMSA chelatable lead)

Variable	Units of β	β coefficient (95% CI)	P value	Model r^2
Model 1 (with blood lead):				
Blood lead, $\mu\text{g/dl}$	$\mu\text{g}/\mu\text{g/dl}$	1.510 (0.442 to 2.578)	0.01	0.30
ALAD genotype (1-2 v 1-1)	μg	-23.761 (-46.67 to -0.854)	0.05	
Duration of exposure (y)	$\mu\text{g}/\text{y}$	-2.105 (-5.433 to 1.223)	0.22	
Smoker (current v not-current)	μg	19.270 (-6.026 to 44.57)	0.14	
Weight (kg)	$\mu\text{g}/\text{kg}$	0.735 (-0.302 to 1.772)	0.17	
Model 2a (with plasma ALA):				
Plasma ALA (ng/ml)	$\mu\text{g}/\text{ng/ml}$	-0.188 (-1.719 to 1.343)	0.81	0.19
ALAD genotype (1-2 v 1-1)	μg	-29.048 (-54.50 to -3.592)	0.03	
Duration of exposure (y)	$\mu\text{g}/\text{y}$	-1.321 (-4.890 to 2.248)	0.47	
Smoker (current v not-current)	μg	27.485 (1.045 to 53.93)	0.05	
Weight (kg)	$\mu\text{g}/\text{kg}$	0.864 (-0.281 to 2.009)	0.15	
Model 2b (effect modification by ALAD with plasma ALA):				
Plasma ALA (ng/ml)	$\mu\text{g}/\text{ng/ml}$	-0.653 (-2.094 to 0.788)	0.38	0.32
ALAD genotype (1-2 v 1-1)	μg	-133.58 (-205.4 to -61.74)	<0.01	
Duration of exposure (y)	$\mu\text{g}/\text{y}$	-1.597 (-4.882 to 1.688)	0.35	
Smoker (current v not-current)	μg	25.529 (1.842 to 49.22)	0.04	
Weight (kg)	$\mu\text{g}/\text{kg}$	0.972 (-0.082 to 2.026)	0.08	
Cross product term (ALAD genotype and plasma ALA, ng/ml)	$\mu\text{g}/\text{ng/ml}$	8.649 (3.030 to 14.27)	<0.01	
Model 3 (with ZPP):				
ZPP ($\mu\text{g/dl}$)	$\mu\text{g}/\mu\text{g/dl}$	0.324 (-1.133 to 0.781)	0.17	0.22
ALAD genotype (1-2 v 1-1)	μg	-26.171 (-50.25 to -2.090)	0.04	
Duration of exposure (y)	$\mu\text{g}/\text{y}$	-1.500 (-4.969 to 1.969)	0.40	
Smoker (current v not-current)	μg	32.494 (5.593 to 59.40)	0.02	
Weight (kg)	$\mu\text{g}/\text{kg}$	0.965 (-0.125 to 2.055)	0.09	

Three separate main models were fitted, each with a different primary biomarker of lead exposure (blood lead, ZPP, and plasma ALA), because these biomarkers lie along the same casual pathway of lead exposure and the haeme synthetic system.

ports the current binding hypothesis. Subjects with ALAD1-2 have lower ALAP concentrations after controlling for age and blood lead concentrations.²⁸ Earlier studies also suggest that subjects with ALAD1-2 have lower concentrations of lead in cortical bone (adjusted for age, $P = 0.17$), higher concentrations in trabecular bone (adjusted for age, $P = 0.22$), a larger trabecular cortical bone lead difference (adjusted for age, $P = 0.06$), higher uric acid concentrations (adjusted for blood lead, age, and alcohol consumption, $P = 0.09$), and higher blood urea nitrogen concentrations (adjusted for blood lead, age, and alcohol consumption, $P = 0.06$).¹⁵ These data suggest that ALAD genotype may influence lead excretion and hence long term deposition of lead in important in vivo storage sites.¹⁵ Finally, the prevalence of ALAD1-2 has been reported to be higher in lead plants with high exposures and among workers with longer durations of exposure, evidence that ALAD genotype may be a selection factor in workplaces that contain lead.¹⁴ It is possible, however, that differences in current binding or long term deposition of lead may explain these associations with plant and duration of exposure.

In an earlier study of DMSA chelatable lead, Lee *et al* reported that blood lead was not associated with eight hour urinary lead excretion after 10 mg/kg DMSA orally but that urinary ALA was correlated with this measure.¹⁶ In the current study, the dose of DMSA was less (5 mg/kg) and the urine collection time was shorter (four hours). We chose this dose and collection time to determine the predictors of a measure of low dose short duration DMSA chelatable lead, a protocol which is less expensive and time consuming. The low dose four hour measure was correlated with current blood lead and plasma ALA concentrations, but was not correlated with urinary ALA concentrations, by contrast with the higher dose eight-hour measure. It is possible that DMSA primarily accesses blood lead stores at low doses and blood lead and soft tissue stores at higher doses.

It is interesting to note that DMSA seems to preferentially remove lead from soft tissue sites that are rich in ALAD. Early research in several species of animals determined that ALAD activity is high in liver, kidney, and bone marrow, and lower but still present in blood.³⁴ Although there are few comparable data in humans, this organ and tissue distribution of ALAD is likely to be at least similar to that in people. It is not known whether ALAD binding sites of lead in soft tissues represent a primary or even majority source of lead that is subsequently bound by DMSA.

Associations of plasma ALA with ALAD genotype from this population have been previously reported.²⁸ In the current study, plasma ALA was correlated with DMSA chelatable lead and ALAD genotype seemed to modify that relation. Workers with ALAD1-2 excreted more lead after DMSA for each ng/ml plasma ALA than did workers with ALAD1-1. Although this association was

heavily influenced by three subjects with ALAD1-1 who had high plasma ALA concentrations, it was still present after elimination of these subjects but did not reach significance. This association is also consistent with our hypotheses about lead binding by ALAD. These data suggest that, in subjects with ALAD1-2, the lead storage compartments that influence ALAD activity (and thus plasma ALA concentrations) and from which lead is chelated by DMSA are more similar than in subjects with ALAD1-1.

The increasing number of publications on ALAD genotype now provides significant evidence that ALAD may be an important effect modifier of the toxicokinetics of lead. Subjects with ALAD1-2 have higher concentrations of blood lead, lower concentrations of DMSA chelatable lead, lower concentrations of plasma ALA, and possibly lower concentrations of lead in cortical bone and higher in trabecular bone. In the only published studies of differences in health effects by genotype, Bellinger *et al* reported that five subjects with ALAD1-2 had consistently better neuropsychological test performance than did 67 subjects with ALAD1-1,¹³ but Smith *et al* reported higher blood urea nitrogen concentrations in subjects with ALAD1-2.¹⁵ More definitive evidence about possible differential toxicity by genotype awaits further study.

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Examples of common forms of references are:

- 1 International Steering Committee of Medical Editors, Uniform requirements for manuscripts submitted to biomedical journals. *BMJ* 1979;1:532-5.
- 2 Soter NA, Wasserman SI, Austen KF. Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis during cold challenge. *N Engl J Med* 1976;294:687-90.
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