The Protective Effect of δ-Aminolevulinic Acid Dehydratase 1-2 and 2-2 Isozymes against Blood Lead with Higher Hematologic Parameters

Hee-Seon Kim, Sung-Soo Lee, Gap-Soo Lee, Young Hwangbo, Kyu-Dong Ahn, and Byung-Kook Lee

1Department of Food Science and Nutrition and 2Institute of Industrial Medicine, Soonchunhyang University, Asan, Choongnam, Republic of Korea

Previous studies have suggested that δ-aminolevulinic acid dehydratase (ALAD) types 1-2 or 2-2 are protective against the toxicity of blood lead (PbB) when zinc protoporphyrin (ZPP) levels are low because of differential binding of lead in erythrocytes. The hypothesis is that subjects with the ALAD 1-1 genotype are more susceptible to lead exposure with impaired hematologic synthesis and therefore that iron nutrition is more important in those with the ALAD 1-1 genotype. The purpose of this study was to prove the protective effect of ALAD 1-2/2-2 against PbB with higher hematologic parameters. Data on 1,219 male workers from eight lead-using factories in the Republic of Korea were examined in this cross-sectional study. Blood samples were evaluated for PbB, ZPP, hemoglobin (Hb), and serum iron (SFe) concentrations and ALAD genotypes. The overall prevalence of the ALAD 1-2/2-2 genotype was 9.3%, which was associated with lower log ZPP (p < 0.001) and higher Hb (p = 0.014) levels. For the subjects with normal iron status (SFe levels > 60 µg/dL), those with the ALAD 1-1 genotype were more likely to be anemic (adjusted odds ratio of 5.2; 95% confidence interval, 1.2–22.6) than those with ALAD 1-2-2. The study confirms the protective effects of ALAD 1-2/2-2 polymorphisms against PbB on hematologic pathways. In order to promote health and to minimize the toxicity of lead exposure more effectively, the nutritional management of iron in Korean workers should take both their ALAD genotypes and occupational lead exposures into account. Key words: δ-aminolevulinic acid dehydratase polymorphism, hemoglobin, lead, zinc protoporphyrin.

DOI: 10.1289/ehp.6464 available via [Online 9 December 2003]

Materials and Methods

Study population. Participation in the study was voluntary, and all participants provided written, informed consent. The study protocol was approved by the Institutional Review Board of the College of Medicine, Soonchunhyang University.

Employees from eight lead-using factories [five storage-battery factories, two secondary-smelting factories, and one litharge (PbO) making factory] were studied. Storage-battery factories manufacture batteries for cars and trucks, large industrial batteries, and/or smaller batteries for consumer products. All of the workers included in the study were male, and they were either lead workers who were exposed to high environmental lead levels in modern society. The control of lead exposure by legislation and modern technology is undoubtedly responsible for the reduction in acute lead poisoning over the past few decades in Korea (Lee 1992). Despite these declines, there is still considerable concern regarding the toxicologic implications of lead exposure and the subtle health effects at blood lead (PbB) levels around 30 µg/dL in Korean lead workers with a significantly higher prevalence of iron deficiency (Kim et al. 2003).

There is a considerable interindividual variation in the toxicokinetics of lead (Fleming et al. 1998; Goering and Fowler 1987; Schwartz et al. 1995; Smith et al. 1995; Wettmur et al. 1991b). Lead inhibits three enzymes—aminolevulinic acid dehydratase (ALAD), coproporphyrinogen oxidase, and ferrochelatase—in the heme-synthesis pathway, with its effects on ALAD being most profound (Onalaja and Claudio 2000). The ALAD polymorphism codes for one of three isozymes (termed ALAD 1-1, ALAD 1-2, and ALAD 2-2) and has been reported to account for significant variation in intravascular and soft-tissue binding and in the long-term deposition of lead (Kelada et al. 2001). The ALAD 2 allele has been shown to modify the toxicokinetics of lead by coding for a more electronegative enzyme that may bind positively charged lead ions more tightly than does the ALAD-1 protein (Wettmur 1994; Wettmur et al. 1991a). PbB concentrations are reportedly higher in subjects carrying the ALAD-2 allele than in subjects with the ALAD-1 isozyme (Fleming et al. 1998; Wettmur 1994; Wettmur et al. 1991a, 1991b; Ziemsen et al. 1986). However, several other studies (Bergdahl et al. 1997; Hu et al. 2001; Schwartz et al. 1995; Smith et al. 1995) have reported no association between ALAD genotypes and PbB levels. The results of the studies of Smith et al. (1995) and Hu et al. (2001) suggest that ALAD modifies PbB and/or the toxicokinetics of lead only in those with a high body lead burden. Recent studies into occupational lead exposures support the hypothesis that the protective effect of the variant allele is due to the binding of lead and maintaining it in the intravascular space in a less bioavailable form with lowered zinc protoporphyrin (ZPP) levels, a parameter of the hematologic toxic consequences of lead (Fleming et al. 1998; Schwartz et al. 1997, 2000; Sithisarankul et al. 1997).

Hypotheses concerning lower ZPP levels even in the presence of higher concentrations of PbB and the ALAD 1-2 or 2-2 isozyme question whether ALAD mediates the levels of hematologic parameters. The prevalence of the ALAD-2 allele varies with race and ethnicity. For example, it has been reported that only 9.9% of Koreans carry the allele (Lee et al. 2001), whereas approximately 20% of Caucasians have the allele (Onalaja and Claudio 2000; Wettmur et al. 1991b; Ziemsen et al. 1986). With increased attention being paid to nutrition as a secondary means of mitigating the effects of occupational and environmental exposure to lead (Cheng et al. 1998; Mahaffey 1990), improved iron nutrition has been found to be important to the reduction of the toxicity of PbB (Mahaffey 1995). One of the most prevalent nutritional concerns among Korean lead workers has been a higher prevalence of iron deficiency compared with the general population, although the prevalence of anemia in the overall Korean male population is no longer of serious concern. The recent study of Kim et al. (2003) showed that 42% of lead workers have low iron store status compared with only 21% of control subjects. For both populations, the prevalence of anemia determined by a hemoglobin (Hb) level < 13.5 g/dL was very low (13% lead workers vs. 0% control), and the mean Hb level was 14.7 g/dL. This study also indicated that the dietary iron intake was inversely associated with ZPP. If the ALAD genotype modifies hematologic parameters to reduce ZPP even in the presence of higher PbB, nutritional management strategies for secondary preventive intervention against lead toxicity should vary with ALAD genotype. Here we report a cross-sectional evaluation of differences of hematologic parameters by ALAD genotype in 1,219 male lead workers from Korea.

538 | VOLUME 112 | NUMBER 5 | APRIL 2004 • Environmental Health Perspectives

Address correspondence to B.-K. Lee, Institute of Industrial Medicine, Soonchunhyang University, 646 Eunpae-ri, Shinchang-myun, Asan, Choongnam, 336-745, Republic of Korea. Telephone: 82-41-530-1760. Fax: 82-41-530-1778. E-mail: seelkk@sch.ac.kr

The authors declare they have no competing financial interests. Received 15 May 2003; accepted 9 December 2003.
directly engaged in lead-using operations or nonlead workers who were engaged in administrative or clerical jobs at the same eight factories. Both lead and nonlead workers were screened at a mandatory semi-annual health surveillance program that included environmental and biologic monitoring (e.g., levels of PbB, ZPP, and Hb) and liver and renal function examinations by the Institute of Industrial Medicine, Soonchunhyang University. Workers without any detectable medical problems were considered eligible subjects for the present study. Out of a total of 1,287 male workers, the eligible study participants consisted of 1,074 lead workers (88%) and 145 nonlead workers (12%), with a mean age of 35.8 years (range, 19–72 years). The mean employment duration of lead workers was 6.7 years (range, 0–27 years), and that of nonlead workers was 5.9 years (range, 0–20 years).

**Study variables.** ALAD genotype, PbB, and Hb levels were determined in EDTA-treated whole-blood specimens obtained during medical surveillance in autumn 2000 at Soonchunhyang University. A modified polymerase chain reaction (PCR)-based protocol was used for ALAD genotyping (Wetmur et al. 1991a, 1991b), performed sequentially and in duplicate on 0.5 µL whole blood using nested primers. Detailed descriptions of the ALAD genotyping methods are available elsewhere (Lee et al. 2001). PbB was measured in duplicate with a Zeeman background-corrected atomic absorption spectrophotometer (model Z-8100; Hitachi, Tokyo, Japan) using the standard addition method of the National Institute of Occupational Safety and Health (Kneip and Crable 1988) at the Institute of Industrial Medicine, Soonchunhyang University, which is a certified reference laboratory for lead measurement in Korea. ZPP levels were measured by a portable hematofluorometer (Aviv-206; Aviv, Lakewood, NJ, USA) at the medical surveillance sites (Blumberg et al. 1977). Hb was assayed by the cyanmethemoglobin method (model Ac-T; Beckman Coulter, Fullerton, CA, USA). Serum iron (SFe) levels were measured by spectrophotometry (TBA-40FR biochemical analyzer; Hitachi, Tokyo, Japan).

**Data analysis.** The primary goals of the analysis were to examine relations of the ALAD genotypes with hematologic parameters (blood ZPP and Hb), PbB, and SFe while controlling for covariates, and to evaluate whether the ALAD genotype modified relations with PbB or SFe on above relations. Statistical analyses were performed using SAS, version 8.12 (SAS Institute, Cary, NC, USA). Detailed frequency distributions and summary statistics were examined for all study variables. Relations between variables were assessed by correlations and multiple linear regressions for continuous outcome data, and multiple logistic regression for dichotomous outcomes. These analyses were conducted on data from all workers in both direct lead-using and nonlead-using environments in the same factories because the PbB levels of nonlead workers in the study were significantly higher than the mean PbB level of the general Korean population (0.24 µmol/L, 5 µg/dL) (Kim et al. 2001).

Covariates examined in linear regression and logistic regression models comprised age, employment duration, body mass index (BMI), and alcohol and tobacco consumption. Covariates were retained in the final regression and logistic models if they were significant predictors of hematologic parameters. We transformed ZPP values to a logarithmic scale to account for the skewness of the ZPP distribution. Multiple linear regression was used to examine the association between ALAD and log ZPP or Hb concentration after controlling for PbB, SFe, and other covariates. To evaluate the effect of modification by ALAD, we added cross-product terms of ALAD × PbB and ALAD × SFe to the models of log ZPP and Hb (one cross-product at a time).

### Table 1. Characteristics of study subjects.

| Variables | Lead workers (n = 1,074) | Nonlead workers (n = 145) |
|-----------|------------------------|--------------------------|
| Age (years) | 35.4 ± 8.1 (19.0–70.0) | 36.1 ± 7.6 (20.0–72.0) |
| Employment duration (years) | 6.7 ± 4.6 (0.0–27.0) | 5.9 ± 4.5 (0.0–20.0) |
| BMI | 22.7 ± 1.7 (16.5–34.4) | 23.6 ± 2.7 (17.4–32.7) |
| PbB | 1.25 ± 0.66 (0.19–4.06) | 26.0 ± 13.7 (4.0–16.0) |
| µg/dL | 0.45 ± 0.14** (0.17–0.80) | 9.4 ± 3.0** (3.5–16.6) |
| ZPP | 53.3 ± 10.0* (22.2–79.8) |
| µmol/mol heme | 50.8 ± 36.5 (23.0–440.0) |
| µg/dL | 38.0 ± 7.1* (23.0–57.0) |
| Hb (g/dL) | 14.7 ± 1.9 (10.1–17.7) |
| SFe (µmol/L) | 20.2 ± 7.7 (4.1–85.4) |
| Smoking (n[%]) | 20.0 ± 8.6 (8.6–64.1) |
| Current | 106 (49.8) | 96 (66.2) |
| Never smoked | 200 (91.5) | 48 (33.8) |
| Alcohol consumption (n[%]) | 114 (78.6) |
| Current | 250 (23.2) | 31 (21.4) |
| Never or no longer | 971 (90.4) | 135 (93.1) |
| ALAD genotype (n[%]) | 103 (9.6) |
| 1-1 | 103 (9.6) |
| 1-2 or 2-2 | 103 (9.6) |

Values shown are mean ± SD (range).

*p < 0.05; **p < 0.01.

### Table 2. Lead biomarker variables and hematologic indices by ALAD gene status.

| Variables | ALAD 1-1 (n = 1,106) | ALAD 1-2/2-2 (n = 113) |
|-----------|---------------------|-----------------------|
| Age (years) | 35.5 ± 8.0 (20.0–70.0) | 35.6 ± 8.0 (19.0–63.0) |
| Employment duration (years) | 6.8 ± 4.6 (0.0–27.0) | 6.8 ± 4.6 (0.0–20.0) |
| BMI | 22.9 ± 2.8 (16.3–34.4) | 22.7 ± 2.4 (16.5–28.0) |
| PbB | 1.15 ± 0.67 (0.17–4.66) | 1.23 ± 0.66 (0.19–3.18) |
| µg/dL | 23.9 ± 14.0 (3.5–84.6) | 25.6 ± 13.7 (3.9–66.3) |
| ZPP µmol/mol heme | 69.9 ± 50.2 (32.2–620.2) |
| µg/dL | 49.9 ± 35.8 (23.0–443.0) |
| Hb (g/dL) | 14.7 ± 0.9 (10.1–17.8) |
| SFe (µmol/L) | 20.2 ± 7.9 (4.12–85.38) |

Values shown are mean ± SD (range).
alcohol. Ninety percent of both types of workers were homozygous for the ALAD 1-1 genotype (Table 1). Age, duration of job, BMI, and Hb and SFe levels did not vary with ALAD genotype (Table 2). There were also no significant differences in PbB and SFe levels by ALAD genotype.

Correlation of variables and associations with ALAD genotype. Table 3 lists the unadjusted correlations of study variables. As expected, PbB concentrations were strongly positively correlated with log ZPP (r = 0.35***) and negatively correlated with Hb (r = -0.313) and showed a very weak negative correlation with SFe (r = -0.059, p < 0.05).

In multiple linear regression models, ALAD genotype was associated with log ZPP (Table 4, model 1). The results of Hb (model 2) with ALAD genotype showed that subjects with ALAD 1-2 or 2-2 isozymes had significantly (p < 0.05) higher Hb levels when

**Table 3. Correlation analysis of study variables.**

| Age      | Employment duration | BMI     | PbB    | Log ZPP | Hb     |
|----------|---------------------|---------|--------|---------|--------|
| 0.50***  | 0.10**              | 0.35**  | 0.34***| -0.22***| -0.01  |
| 0.12***  | 0.03                | 0.18*** | 0.03   | 0.22*** | 0.01   |
|         |                     | -0.05   | -0.03  | -0.20***| 0.00   |
|         |                     |         | 0.69***| -0.31***| -0.06* |
|         |                     |         |        |         | 0.16***|

*Unadjusted Pearson’s correlation for all subjects. *p < 0.05. **p < 0.01. ***p < 0.001.

**Table 4. Multiple linear regression models of log ZPP (model 1) and Hb (model 2) with SFe, PbB, and ALAD genotype.**

| Estimate | SE | t-Statistic | No. | R²  |
|----------|----|-------------|-----|-----|
| Model 1: log ZPP with ALAD Intercept | 3.63 | 0.08 | 45.23*** | 1,219 | 0.50 |
| SFe (µmol/L) | -0.003 | 0.001 | -2.40* | -0.05 |
| PbB (µmol/L) | 0.39 | 0.01 | 30.10*** | 0.03 |
| ALAD 1-2/2-2 | -0.11 | 0.03 | -4.03* | -0.03 |
| Model 2: Hb with ALAD Intercept | 123.13 | 2.55 | 52.19*** | 1,219 | 0.16 |
| SFe (µmol/L) | 0.19 | 0.03 | 5.78*** | 0.41 |
| PbB (µmol/L) | -1.67 | 0.07 | -4.11*** | 0.88 |
| ALAD 1-2/2-2 | 2.16 | 0.88 | 2.46* | 0.88 |

*In addition to variables listed under each model, models also controlled for age, employment duration, alcohol consumption, and cigarette smoking for all subjects. *p < 0.05. **p < 0.01. ***p < 0.001.

**Table 5. Association between dichotomous outcome variables of hemoglobin with ALAD genotype and SFe status.**

| ALAD genotype | Iron status | Hb     | Crude OR (95% CI) | Adjusted OR (95% CI) |
|---------------|-------------|--------|-------------------|----------------------|
|               | Normal      | Anemic | Total             |                      |
| 1-2/2-2       | 110 (97%)   | 3 (3%) | 113               | 1.0                  |
| 1-1           | 1,013 (91%) | 93 (9%)| 1,106             | 3.4 (1.2–13.5)       |
| Total         | 1,123       | 96     | 1,219             |                      |
| 1-2/2-2       | Normal      | 105 (86%)| 2 (2%)   | 107                | 1.0                  |
| 1-1           | 982 (92%)   | 79 (8%)| 1,061           | 4.3 (1.3–26.4)       |
| Total         | 1,123       | 96     | 1,219             |                      |
| 1-2/2-2       | Deficient   | 5 (83%)| 1 (17%) | 6                  | 10.5 (0.4–130.1)    |
| 1-1           | 51 (78%)    | 14 (22%)| 65               | 14.4 (3.8–93.9)     |
| Total         | 1,123       | 96     | 1,219             |                      |

*Logistic regression models for all subjects. *Cutoff value for anemia: Hb level = 13.5 g/dL. *Cutoff value for iron-deficiency: SFe level = 10.74 µmol/L (60 µg/dL) (Gibson 1990).

**Discussion**

The decline in PbB levels among Korean lead workers over the past few decades reflects primary interventions including legislation on adequate industrial hygiene practices, improved work environments, and mandatory health surveillance. Nonetheless, considerable concern remains regarding lead levels, and proper nutrition has been recognized as an adjunct to the reduction of occupational lead exposure by altering susceptibility to lead toxicity. In addition, studies using genetic markers of susceptibility to environmental toxicants have suggested that certain genes can make individuals more vulnerable to environmental toxins such as lead. The present study focused on the relationship between different ALAD genotypes and differentiated lead-induced impairment of hematopoiesis, as an aid to seeking appropriate nutritional strategies to counteract the hematologic toxicity of lead exposure.

Data from male Korean lead workers with similar PbB and SFe concentrations, durations of employment in lead industry, BMIs, and ages suggest that the ALAD genotype influences levels of ZPP and Hb and, consequently, the prevalence of anemia. Subjects with the ALAD 1-2 or 2-2 genotype showed significantly lower log ZPP and higher Hb levels compared with ALAD 1-1 individuals (Table 4). This analysis was conducted to evaluate whether ALAD genotype modified the relation between lead exposure and Hb synthesis with differentiated ZPP levels (log ZPP).
ZPP levels, derived from the substrate (protoporphyrin IX) of the last enzyme in the heme-synthesis system (ferrochelatase), should only be increased by the presence of bioavailable lead; PbB that is strongly bound by an ALAD isozyme, for example, should not inhibit ferrochelatase. Therefore, lead binding by ALAD isozymes with different affinities differentially limits its bioavailability (Schwartz et al. 1995). We hypothesized that the different bioavailability of PbB determined by ALAD isotypes differentially affects ferrochelatase activity and therefore Hb synthesis. Our data involving different Hb concentrations of different ALAD isotypes confirmed this hypothesis.

Furthermore, the prevalence of anemia in subjects with ALAD 1-2/2-2 isotypes was significantly lower than in subjects homozygous for ALAD 1-1 (Table 5). Anemia is a late sign in industrial lead poisoning, and in the past it was severe among subjects exposed to lead (Mahaffey 1995). However, recent data indicate that anemia is less intense and less common in Korean lead workers than previously thought, although impaired iron status has been reported in this population (Kim et al. 2003). Lead intoxication impairs iron use and produces a hemolytic tendency, both of which complicate the mechanism of anemia (Albahary 1972). Above mentioned, the two target sites in the biosynthetic pathway of heme by PbB are the sites of activity of ALAD and ferrochelatase (Moore and Goldberg 1985). Lead is also known to interfere with the mitochondrial energy metabolism that is necessary to reduce ferric iron to ferrous iron before the insertion of iron into the porphyrin ring. Therefore, protoporphyrin accumulates when there is insufficient ferrous iron for its incorporation by ferrochelatase into heme (Mahaffey 1990). Ferrochelatase activity is sensitive to both lead and iron. Kapoor et al. (1984) reported that the enzyme kinetics of ferrochelatase in isolated human reticulocytes changes with both iron and lead concentrations. Ferrochelatase is more sensitive to lead effects when iron deficiency is present. Our data provide evidence that the ALAD genotype modifies both the toxicokinetics of lead in the hematologic pathway and the prevalence of anemia in lead exposure. In addition, our findings suggest that with impaired iron status and the presence of the ALAD 1-1 genotype, reduction of heme synthesis causes higher incidences of anemia when PbB levels are controlled (Table 5). These data imply that subjects with ALAD 1-2 or 2-2 are better able to use iron for Hb synthesis. Therefore, the impaired iron nutrition of individuals with the ALAD 1-1 isozyme will make them more susceptible to the toxic effects of lead in heme biosynthesis than subjects with other ALAD isozymes. Because the prevalence of anemia can be modified by iron status, it is important to reduce the more severe adverse effects of lead on the hematologic system of subjects with the ALAD 1-1 genotype, by improving their iron nutritional status.

Many investigators have reported that most Asians have the ALAD 1-1 genotype (Lee et al. 2001; Onalaja and Claudio 2000; Ziemsen et al. 1986). The Korean subjects comprising the population of the present study confirmed that tendency. The combined risks of marginal iron nutritional status found previously by Kim et al. (2003) and the higher prevalence of ALAD 1-1 isozyme among Koreans together result in Korean lead workers being at greater risk of lead toxicity in the hematologic pathway. Therefore, careful nutritional intervention as a secondary prevention strategy for reducing the risk of lead toxicity needs to be developed while considering the ALAD genotypes involved.

REFERENCES

Albahary C. 1972. Lead and hemopoiesis. The mechanism and consequences of the erythropoietic of occupational lead poisoning. Am J Med 52:367–378.
Bergdahl IA, Gerhardsson L, Schutz A, Desnick RJ, Wetmur JG, Skerfving S. 1977. Delta-aminolevulinate acid dehydratase polymorphism: Influence on lead levels and kidney function in humans. Arch Environ Health 52:91–96.
Blumberg WE, Eisinger J, Lamola AA, Zuckerman DM. 1977. Zinc protoporphyrin level in blood determined by a portable hematoftluorometer: a screening device for lead poisoning. J Lab Clin Med 89:712–723.
Cheng Y, Willet WC, Schwartz J, Sparrow D, Weiss S, Hu H. 1998. Relation of nutrition to bone lead and blood lead levels in middle-aged to elderly men. The Normative Aging Study. Am J Epidemiol 147:1162–1174.
Fleming DEB, Chettle DR, Wetmur JG, Desnick RJ, Robin JP, Boulby D, et al. 1998. Effect of the δ-aminolevulinate dehydratase polymorphism on the accumulation of lead in bone and blood in lead smelter workers. Environ Res 77:49–61.
Gibson RS. 1990. Assessment of iron status. In: Principles of Nutrition Assessment (Gibson RS, ed). New York:Oxford University Press, 349–376.
Goering PL, Fowler BA. 1987. Regulatory roles of high-affinity metal-binding proteins in mediating lead effects on δ-aminolevulinate acid dehydratase. Ann NY Acad Sci 514:235–247.
Hu H, Wu MT, Cheng Y, Sparrow D, Weiss S, Kelsey K. 2001. The δ-aminolevulinic acid dehydratase (ALAD) polymorphism and bone and blood lead levels in community-exposed men: the Normative Aging Study. Environ Health Perspect 109:827–832.
Kapoor S, Seaman C, Hurst D, Matsos S, Piomelli S. 1984. The biochemical basis of the clinical interaction of Fe deficiency and lead intoxication [Abstract]. Pediatr Res 18:242A.