Alterations in urinary metabolomic profiles due to lead exposure from a lead—acid battery recycling site☆

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ABSTRACT

Lead poisoning is considered a public health threat, particularly in developing countries. Health problems from Pb exposure occur in many parts of the world, especially near Pb mines, Pb smelters, and used lead—acid battery (ULAB) recycling plants. In this study, we analyzed the urine metabolome of residents in a village located near a ULAB recycling facility to investigate the biological effects of Pb exposure (ULAB: $n = 44$, Reference: $n = 51$). Lasso linear regression models were moderately predictive of blood Pb levels, as evaluated by a training set ($R^2 = 0.813$) and against an external test set ($R^2_{EXT} = 0.647$). In lasso logistic regression models, areas under receiver operating characteristic curves, as measured by 5-fold cross-validation ($AUC_{CV} = 0.871$) and against an external test set ($AUC_{EXT} = 0.917$), indicated accurate classification of urine samples from the affected village and from a reference site. Ten candidate biomarkers identified at false discovery rates of <0.05 were associated with ATP-binding cassette (ABC) transporters, possibly related to the disruption of small-molecule transport in the kidney; amino acid, porphyrin, and chlorophyll metabolism; and the heme biosynthetic pathway. Collectively, the results suggest that lead Pb is related to the health effects in individuals residing in ULAB site by alteration of these biological pathways.

1. Introduction

Lead poisoning is considered a public health threat, particularly in developing countries (WHO, 2010). Health problems from Pb exposure have been reported in many parts of the world, especially near Pb mines, Pb smelters, and used lead—acid battery (ULAB) recycling plants (Fujimori et al., 2016), where the diffusion of Pb to the surrounding environment may endanger both workers and residents. In a previous study, we surveyed Pb levels in the surface soil in Hung Yen, a ULAB recycling village in northern Vietnam, in 2011, 2013, and 2014 (Fujimori et al., 2016). We noted high Pb levels

along battery transportation routes in the smelter and at the battery collection site (Fujimori et al., 2016). Blood Pb levels in the residents of the village (median, 34 μg/dL) were also significantly higher than those in residents of a control site (median, 3.3 μg/dL) in 2011 (Noguchi et al., 2014). Importantly, levels in the former exceed the recommended levels for adults (<25 μg/dL) as set by the U.S. Department of Health and Human Services (Roscoe et al., 2002), indicating that health effects from Pb exposure are a concern to these residents, even though toxicological problems, if any, have not been documented.

It is well-known that Pb exposure induces porphyria and anemia by disrupting the heme biosynthetic pathway (Ahamed and Siddiqui, 2007). δ-aminolevulinic acid level is an initial biomarker in the heme synthesis pathway, and its concentration in urine can be used as a signature of Pb-related anemia (Morgan and Burch, 1975). In vivo study has shown alterations in the intercellular

apical region of proximal tubules in the kidney caused by chronic exposure of rats to Pb (Navarro-Moreno et al., 2009). The ensuing tubular damage manifests in children as Fanconi syndrome, which is characterized by the triad of glycosuria, hypophosphatemia, and aminoaciduria (Hammond, 1977), of which aminoaciduria has also been noted in workers at Pb smelters (Clarkson and Kench, 1956). However, the cellular mechanisms underlying renal toxicity due to chronic Pb exposure have not been fully elucidated (Reyes et al., 2013). Pb may also affect the central nervous system (Sanders et al., 2009). In children, Pb reportedly causes delayed growth, decreased intelligence, short-term memory and hearing loss, and even brain damage and death at high levels (Cleveland et al., 2008; Flora et al., 2012). Essentially, Pb behaves like other cations, such as Na^+, Ca^{2+}, and Fe^{2+}, high concentrations of which disrupt various fundamental biological and metabolic processes that depend on such ions (Lidsky and Schneider, 2003), including intra- and intercellular signaling, cell adhesion, protein folding and maturation, apoptosis, ion transport, enzyme regulation, and neurotransmitter release (Garza et al., 2006). However, toxicological pathways resulting from environmental exposure to Pb have not been well characterized in epidemiological studies.

Metabolome analysis can provide important knowledge related to the responses of metabolomic profiles to environmental changes in cells, tissues, and biofluids (Ramirez et al., 2013; Robertson et al., 2011). Alterations in metabolomic profiles by heavy metal exposure have been described in animals (Lafaye et al., 2003, 2004), but rarely in humans. However, the effects of high cadmium exposure on carbohydrate and amino acid metabolism in humans have been shown in the urinary metabolome; furthermore, intestinal flora metabolism and the tricarboxylic acid cycle were found to be affected by Cd exposure (Xu et al., 2016). In Taiwan, metabolomic urine profiles of residents living near oil refineries and coal-fired power plants showed that alterations in oxidative stress-related amino acids were linked to polycyclic aromatic hydrocarbon and heavy metal exposure. However, Pb was not discussed by that study (Chen et al., 2017). A study of workers in a copper foundry examined the relationship between lipid and amino acid profiles in serum and occupational exposure to Pb, Cd, and arsenic, but detailed mechanisms remain unclear (Dudka et al., 2014). In this study, we hypothesized that Pb exposure from ULAB recycling might change urine metabolomic profiles, and that these alterations might be linked to biological features. Thus, to investigate the biological effects of Pb exposure, we analyzed the profiles of urinary metabolomes in residents from a village located at a ULAB recycling site and from a control site.

2. Materials and methods

2.1. Sample collection

This study was approved by the Biomedical Research Ethics Committee of the Graduate School of Medicine at Chiba University and the Ethical Committee of Ehime University. Informed consent was obtained from all participants.

In January 2010 and 2011, blood and urine were collected from 93 residents of Dong Mai, a ULAB recycling village, and from 71 residents of Duong Quang, a reference site. All participants agreed to donate samples (Noguchi et al., 2014). Donors were informed in advance about the purpose of the study at local government health stations where all volunteers registered their consent to participate in this study. Demographic, health, and diet information were collected through personal interviews. Samples were collected in the morning and placed on ice packs immediately after collection,

and then sent to the Center for Environmental Technology and Sustainable Development, Hanoi University of Science and frozen at $-20\,°C$. Subsequently, frozen samples were maintained on ice packs at $-80\,°C$ and transported by air to the Environmental Specimen Bank at the Center for Marine Environmental Studies, Ehime University, Japan, and stored at $-25\,°C$ until analysis (Tanabe, 2006). Participants below 18 years of age were removed from the original study (Noguchi et al., 2014). Moreover, due to limited analyte volume in some samples, only those volunteers from whom sufficient sample volumes were available were included in the analysis. Finally, 44 Dong Mai (ULAB) and 51 Duong Quang (reference) samples were used in the analysis (Table 1). In this study, no significant differences were found in the characteristics of participants analyzed and non-analyzed adult individuals.

2.2. Analysis of blood Pb and urinary δ-aminolevulinic acid

Blood Pb levels were obtained from a previous study (Noguchi et al., 2014) in which blood samples were digested for 3 h on a hot plate at $200\,°C$ in Teflon vials containing nitric acid. Pb was then measured on an inductively coupled plasma mass spectrometer (Agilent 7500cx; Agilent Technologies, Tokyo, Japan) using rhodium as an internal standard to correct for matrix effects and instrumental drift. The accuracy of the Pb values was assessed using A-13 bovine blood, a standard reference material provided by the International Atomic Energy Agency, Austria. Elemental recovery was 87–106% of the certified value (Noguchi et al., 2014). Urinary δ-aminolevulinic acid was measured by SRL Inc., Tokyo, Japan. Briefly, urine samples were mixed with acetyl-acetone, ethanol, and formaldehyde; boiled; and analyzed on a high-performance liquid chromatography system coupled to a fluorometer (Noguchi et al., 2014) to quantify fluorescent derivatives of δ-aminolevulinic acid.

2.3. Metabolome analysis by hydrophilic interaction Chromatography–Tandem mass spectrometry

Methanol, *N,N*-diethyl-2-phenylacetamide, D-camphor-10-sulfonic acid, and acetonitrile were purchased from Wako Pure Chemical Industries (Osaka, Japan). Ultrapure water was obtained using an RFD280NC system (Advantec, Dublin, CA, USA).

Urine levels of 263 ionic, water-soluble metabolites (which covered 10% of human urine compounds registered in the Human Metabolome Database [HMDB]) (Wishart et al., 2013) were quantified by hydrophilic interaction chromatography-tandem mass spectrometry according to published methods (Eguchi et al., 2016; Soga et al., 2009; Yuan et al., 2012) with slight modifications. The target metabolites were selected to cover major metabolic pathways, including glycolysis, the tricarboxylic acid cycle, the pentose phosphate pathway, and amino acid and nucleotide metabolism (Yuan et al., 2012). Samples were pretreated according to published methods (Eguchi et al., 2016; Soga et al., 2009; Yuan et al., 2012). In brief, samples were centrifuged at $17,960 \times g$ for 10 min, and 150 μL was transferred to Amicon® Ultra-0.5 3 kDa filter columns (Merck Millipore, Tokyo, Japan) and mixed with 250 μL methanol containing internal standards (1 nM lidocaine, *N,N*-diethyl-2-phenylacetamide, and D-camphor-10-sulfonic acid). The columns were then centrifuged at $17,960 \times g$ for 1 h, and filtrates were analyzed by selected reaction monitoring on a QTRAP 4500 system (AB SCIEX, Tokyo, Japan). Using positive/negative switching, some metabolites were analyzed in both positive and negative ion mode for 297 selected reaction monitoring (SRM) transitions (Yuan et al., 2012). Metabolome peaks were normalized to the creatinine level in the same sample to correct for urine concentration. Creatinine is