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Metabonomic analysis of serum of workers occupationally exposed to arsenic, cadmium and lead for biomarker research: A preliminary study

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ABSTRACT

Environmental metabonomics is the application of metabonomics to characterize the interactions of organisms with their environment. Metabolic profiling is an exciting addition to the armory of the epidemiologist for the discovery of new disease risk biomarkers and diagnostics. This work is a continuation of research searching for preclinical serum markers in a group of 389 healthy smelter workers exposed to lead, cadmium and arsenic. Changes in the metabolic profiles were studied using Proton Nuclear Magnetic Resonance Spectroscopy on pooled serum samples from both the metal exposed and control groups. These multivariate metabonomic datasets were analyzed with Principal Component Analysis and Partial Least Squares Discriminant Analysis. Analysis of metabolic profiles of people exposed to heavy metals suggests energy metabolism disturbance induced by heavy metals. Changes in lipid fraction (very-low-density lipoprotein — VLDL, low-density lipoprotein — LDL), unsaturated lipids and in the level of amino acids suggest perturbation of the metabolism of lipids and amino acids. This study illustrated the high reliability of NMR-based metabonomic profiling on the study of the biochemical effects induced by the mixture of heavy metals. This approach is capable of identifying intermediate biomarkers of response to toxicants at environmental/occupational concentrations, paving the way to its use in a monitoring of smelter workers exposed to low doses of lead, cadmium and arsenic.

1. Introduction

Humans are often exposed to contamination by metals in environmental and occupational settings. This situation is most commonly encountered by the general population of industrialized countries (de Burbure et al., 2006; Zhai et al., 2005). Much is known about the toxicity of individual agents, but little is known about their potential interactions (de Burbure et al., 2006; Hu et al., 2007). More and more researchers are interested in possible effects of chronic low environmental/occupational exposure to mixtures of heavy metals, particularly with regard to their possible interaction (de Burbure et al., 2006; Garçon et al., 2007; Hochadel and Waalkes, 1997; Hong et al., 2004; Kossowska et al., 2013; Liu et al., 2000; Navas-Acien et al., 2004; Vineis et al., 2009; Wang and Fowler, 2008). However, direct assessment of the effects of environmental toxicants in human populations is very difficult as the exposure is often at low concentration and responses are often subtle and early events (Ellis et al., 2011).

Modern analytical techniques currently used in classical epidemiological studies provide the possibility to develop single biomarkers that signal subtle, early changes in people exposed even to low doses of toxic substances (Muñoz and Albores, 2010; Silins and Högberg, 2011). However, it is very likely that a combination of several markers will be necessary to effectively detect and diagnose low-dose exposure and the early effects of heavy metals on human health. This is due to the multivarious mechanisms of metal toxicology. Multiple markers that function as the signature patterns/profiles are necessary to achieve higher sensitivity and to enable the early discovery of acute toxic onset or the molecular signatures of long-term toxicant exposure and disease (Harezlak et al., 2008; Kossowska et al., 2013; Luque-Garcia et al., 2011; Zhai et al., 2005).

To search for new markers with high sensitivity and specificity, in studies of human exposure to toxic substances, it is appropriate to incorporate "-Omics" techniques, like proteomics, genomics and metabonomics. These new methods could potentially contribute significantly to the identification of environmental causes of disease and

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could help to make advances towards an integrated view of the interaction between the environment and human health (Muñoz and Albores, 2010; Silins and Högberg, 2011; Vineis et al., 2009; Vlaanderen et al., 2010).

Metabonomics is commonly used for typing biomarkers associated with prognosis or diagnosis of disease, evaluation of the toxicity/efficacy and improved understanding of the pathophysiological substrate of the disease (Mamas et al., 2011). Cell response to toxic substances or other stress factors results in an adjustment of its intra-and/or extracellular environment in order to maintain the constancy of the internal environment (homeostasis). One way to maintain homeostasis in response to disease processes, medications and toxic substances is modulation of biofluids. This regulation is expressed as a metabolic pattern of biochemical changes that are specific to the disease process (Lindon et al., 2004). Comprehensive approaches to gain insights into metabolic variation and diseases, such as metabolite phenotyping, have become increasingly popular over recent years. These developments have been driven by mass spectrometry (MS) and proton nuclear magnetic resonance (^{1}H NMR) spectroscopy as the two key experimental technologies (Ala-Korpela et al., 2012).

Environmental metabonomics is the application of metabonomics to characterize the interactions of organisms with their environment. Metabolic profiling is an exciting addition to the armory of the epidemiologist in the discovery of new disease risk biomarkers and diagnostics. Global metabonomic analysis in studies of exposure to metals in laboratory animals has just begun (Feng et al., 2002; Griffin et al., 2000, 2001a, 2001b; Kim et al., 2010; Lei et al., 2008; Liao et al., 2007; Tripathi et al., 2008; Vineis et al., 2009; Wei et al., 2008, 2009).

Metabolic analysis is to a small extent, so far, used in molecular epidemiology to define the metabolic signatures of exposure to heavy metals in the human population. Ellis et al. (2012) studied the changes in metabolism of people living near a closed zinc foundry. This region is characterized by a higher level of environmental cadmium. Urine metabolic profiles of 178 volunteers were analyzed with the use of ^{1}H NMR techniques and analyzed with univariate and multivariate analyses. Exposure to cadmium, as determined by the concentration of cadmium in urine, correlated with six metabolites in the urine: citrate, 3-hydroxyisovalerate, 4-deoxy-erythronic acid (related to mitochondrial metabolism), dimethylglycine, creatinine and creatine. The authors emphasized the importance of continuing research on other biological fluids, in particular blood plasma or serum.

The Department and Clinic of Internal Medicine, Occupational and Hypertension, Wroclaw Medical University has for many years carried out research, using a cohort of people employed in the copper smelters in Głogów and Legnica with exposure to low concentrations of lead, cadmium and arsenic. Their work, with application of conventional epidemiological methods, has shown a negative effect of heavy metals on the health of smelter workers, especially on the function of the cardiovascular system (Poręba et al., 2010, 2011a, 2011b).

In the present study we carried out a risk assessment of the health effects due to the heavy metal environment with the application of ^{1}H NMR-based metabonomics adopting the same study groups as in our previous proteomic study (Kossowska et al., 2010). The results obtained indicate very early preclinical changes in the metabolic profile of the metal exposed group. This is despite all individual clinical parameters within the standards for people occupationally exposed.

2. Material and methods

2.1. Study population and sampling

The study population has been described in detail in a previous study (Kossowska et al., 2010). Briefly, the study was conducted on two groups: a metal-exposed group of 389 men working in the copper foundry and a control group of 45 age-matched, non-exposed healthy men, who had no previous history of occupational exposure to metals. Criteria for inclusion into the study involved work with exposure to lead, cadmium and arsenic. The size and quality of the environmental exposure were similar for all of the men included in the study. The method of individual measurement (dosimetry) was used in order to establish the noxious factors at the work place. The results of the measurements showed that the air concentrations of lead (Pb) were in the range between 0.05 and 3.97 of the maximum acceptable concentration (MAC) for lead in the environment in the work place, for arsenic (As) between 0.0 and 4.64 of MAC, and for cadmium (Cd) it ranged between 0.01 and 0.82 of MAC. Maximum acceptable air concentration norms in the country of study (Poland) for lead, cadmium and arsenic amount to 0.05 mg/m^3, 0.01 mg/m^3 and 0.01 mg/m^3, respectively. The characteristics of the study subjects in control and metal-exposed groups, including measurements of 37 clinical parameters could be found in the paper of Kossowska et al. (2010).

Blood and urine samples were collected within the framework of routine occupational health surveillance of people chronically exposed to heavy metal mixtures. The evaluation of toxicological status and cancer risk assessment was an important part of medical care for copper foundry workers. Investigations were carried out by physicians from the Department of Internal and Occupational Medicine, Wroclaw Medical University. Each participant underwent physical examination according to a standard survey. Information on sociodemographic characteristics, medical history, alcohol consumption, smoking cigarettes and occupational history were obtained by a structured questionnaire. All participants gave written, informed consent to the study. The study protocol was approved by the Bioethic Committee of the Wroclaw Medical University (no. KB 487/2008; grant of Wroclaw Medical University no. 1907).

For all subjects, blood samples were collected in the morning before work and urine samples after a day shift. Blood and urine samples of smelter workers were collected in Głogów and Legnica and shipped at +4 °C to Wroclaw Medical University on the same day. Blood and urine samples from people from a control group were collected in Wroclaw Medical University. Blood samples for metabonomic and proteomic analyses were collected by venipuncture into tubes using an aspiration vacuum set with serum clotting activator (SARSTEDT) and spun at 2500 rpm at +4 °C for 10 min and stored at −80 °C until analysis. All serum samples from the control and metal exposed groups were stored at the same time at +4 °C, and that time did not exceed 6 h. Barton et al. (2008) have shown previously that storage for up to 24 h at +4 °C does not detectably affect the metabolic profile. Metabonomic and proteomic research were carried out in parallel and were performed three months after the collection of samples. A study by Pinto et al. (2014) proved that the impact of long-term −80 °C storage (up to 2.5 years) of human plasma was almost negligible on NMR metabolomics results. No systematic influence of time-in-storage was observed in blood samples stored over a period of 13–17 years on metabolomics results with application of ultra performance liquid chromatography–time-of-flight mass spectrometry (UPLC–ToFMS) in research by Hebels et al. (2013).

Blood samples for morphology, measurements of Pb, Cd, and FEP were collected by venipuncture into tubes using an aspiration-vacuum set with EDTA-K2 (SARSTEDT), frozen at −20 °C, and stored at −80 °C until analysis. Blood samples for measurements of Mg, Ca, Cu, Zn, Fe, glucose, creatinine, total iron binding capacity, unsaturated iron binding capacity, transferrin saturation, transferrin, ferritine, total prostate-specific antigen, free prostate-specific antigen in serum were collected by venipuncture into tubes using an aspiration vacuum set with serum clotting activator (SARSTEDT) and spun at 2500 rpm at +4 °C for 10 min, frozen at −20 °C, and stored at −80 °C until analysis (Kossowska et al., 2010).

Mathematical analysis performed in our previous study (Kossowska et al., 2010) has been applied to the metabonomic study. Only data of persons with complete clinical tests were analyzed. The outliers in the control group were eliminated by the Q-Dixon test. Finally, the mathematical analysis and then metabolomic analysis were performed for