TOXICOLOGICAL HIGHLIGHT

Measuring Arsenic Exposure, Metabolism, and Biological Effects: The Role of Urine Proteomics

Ana Navas-Acien*†1 and Eliseo Guallar†‡§

*Department of Environmental Health Sciences and †Department of Epidemiology and the Welch Center for Prevention, Epidemiology and Clinical Research, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland 21205; ‡Department of Medicine, Johns Hopkins Medical Institutions, Baltimore, Maryland 21205; and §Department of Cardiovascular Epidemiology and Population Genetics, Centro Nacional de Investigaciones Cardiovasculares, Madrid 28029, Spain

Received August 12, 2008; accepted August 13, 2008

Key Words: arsenic; biomarkers; defensins; proteomics; urine.

Millions of people worldwide are exposed to inorganic arsenic mainly through drinking water contaminated from natural mineral deposits (Smedley and Kinniburgh, 2002). Humans metabolize inorganic arsenic (arsenate and arsenite) to methylated compounds (predominantly methylarsonate and dimethylarsinate) that are largely cleared through the urine together with unchanged inorganic arsenic (Aposhian and Aposhian, 2006). Exposure to inorganic arsenic is an established cause of cancers of the bladder, lung, and skin (International Agency for Research on Cancer, 2002), and increasing evidence indicates that inorganic arsenic may also cause cancers of the kidney, prostate and liver, cardiovascular disease, diabetes, and developmental and reproductive effects (Benbrahim-Tallaa and Kuehnelt, 2004). In the 1970’s, new techniques to identify methylated species in urine (Braman and Foreback, 1973) opened up the investigation of human arsenic metabolism and the potential role of metabolism in arsenic toxicity. In addition to evaluating arsenic metabolism, urine arsenic speciation made also possible to differentiate inorganic from organic arsenic exposure (Francesconi and Kuehnelt, 2004), an important distinction because organic arsenicals have little or no toxicity compared to inorganic arsenic. While other biomarkers such as toenail, hair, saliva, or blood are also useful to evaluate arsenic exposure (Chen et al., 2005; Hughes, 2006), arsenic speciation in those specimens is technically challenging, and urine arsenic remains the preferred fluid to assess both exposure and metabolism in human population studies. Although some markers of arsenic effect have been evaluated (Chen et al., 2005; Hughes, 2006), no specific marker has been established to monitor subclinical outcomes, prognosis, or response to treatment among populations exposed to inorganic arsenic.

Hegedus et al. (2008) conducted urine proteomic analyses in 14 men and 16 women from Nevada, USA, and in 31 men and 26 women from Northern Chile. In both study populations, they identified a statistically significant decrease in a 4.37-kDa protein in men exposed to high arsenic levels (>500 μg/l) in drinking water compared to men exposed to low levels (<15 μg/l). No statistically significant difference was found in women, although protein peak intensities were not reported in this subgroup. In Nevada, the 4.37 protein was inversely
related with micronuclei data in exfoliated bladder cells, another marker of arsenic biological effect. The 4.37-kDa protein contained three disulfide bonds and was identified as human β-defensin-1. In a further step, Hegedus et al. (2008) confirmed the potential for inorganic arsenic to decrease human β-defensin 1 gene expression in two human cell lines (HeLa and 293T cells) treated with arsenite 3 µM (225 µg/l) or methylarsonite 1 µM (106 µg/l) for 48 h.

First described in 1985, defensins are a family of plant and animal proteins with antimicrobial, inflammatory, and chemotactic properties (Ganz, 2003). Although their functions in vivo are not completely understood, potentially important roles in cell differentiation, angiogenesis, and apoptosis have been proposed (Kougias et al., 2005; Pazgier et al., 2006). Defensins are small proteins (3–5 kDa) characterized by six cysteine residues forming three disulfide bonds. They are subdivided into two main families, α and β, depending on the location of the disulfide bonds. While human α-defensins are mainly expressed in leukocytes, human β-defensins are typically expressed in epithelial tissues, including the urogenital tract, the respiratory tract, and the skin (Ganz, 2003). Human β-defensin-1, the first β-defensin identified in humans, is encoded on chromosome 8 band p23 and is constitutively secreted in several epithelia including the urogenital tract. Mature human β-defensin-1 consists of 36 amino acids, although multiple forms with the characteristic three disulfide bonds but a different number of amino acids have been identified in human urine.

The expression of human β-defensins is not only increased by bacteria and fungi but also by inflammatory cytokines such as interferon-γ and interleukins and may be regulated through the nuclear factor-κB (Ganz, 2003). Human β-defensin-1 is downregulated in kidney and prostate cancer tissues, suggesting a connection between the loss of this protein and carcinogenesis (Donald et al., 2003; Young et al., 2003). In a bladder cancer cell line (TSU-Pr1), purified β-defensin-1 at 50 µg/ml delayed cell proliferation compared to cells receiving a random sequence peptide, indicating that β-defensin-1 could act as a tumor suppressor for urological cancers (Sun et al., 2006). On the other hand, urine proteomic analyses of patients with transitional cell carcinoma of the bladder found increased peaks for 3.3- and 3.4-kDa peptides that were identified as α-defensins (Vlahou et al., 2001), and expression of α-defensins has been associated with bladder cancer invasiveness (Holterman et al., 2006).

Few other proteomic studies have compared the protein profile of individuals exposed to different arsenic levels (Harezlak et al., 2008; Tan et al., 2008; Zhai et al., 2005). Comparing the serum proteomic profile of 46 male smelter workers (mean urine arsenic 220 µg/l) to 45 male office employees (mean urine arsenic 70 µg/l), Zhai et al. (2005) identified 12 statistically increased and 9 statistically decreased protein peaks. In a study conducted in Bangladesh comparing 67 men and 49 women exposed to high arsenic levels (median toenail arsenic 7.31 µg/g) and 55 men and 43 women exposed to low arsenic levels (median toenail arsenic 0.59 µg/g), Harezlak et al. (2008) identified 14 statistically increased and 10 statistically decreased protein peaks in plasma after adjusting for age, sex, body mass index, and tobacco accounting for multiple comparisons. Interestingly, both studies noted decreased peaks for several proteins in the 3- to 5-kDa range, including a 4.3-kDa protein, in arsenic-exposed individuals compared to controls, but the specific proteins were not identified. Finally, a small qualitative urine proteomic study in Southwestern Taiwan identified 33 unique proteins in eight patients with black foot disease (a vascular condition typical of the high arsenic areas of Southwestern Taiwan) and bladder cancer that were absent among patients with bladder cancer or other urological conditions but no black foot disease (Tan et al., 2008). Using SEQUEST software, one of the 33 proteins was identified as human α-defensin 1.

The potential role of defensins in kidney, prostate, and bladder cancers and the carcinogenic effect of arsenic in these organs add to the interest of the findings of urine β-defensin-1 as a target of arsenic toxicity by Hegedus et al. (2008). The validation of the findings in two independent populations exposed to arsenic, the efforts to purify and identify the protein, the consistent relationship of the identified protein with micronuclei data in exfoliated bladder cells, and the supportive evidence from in vitro data are the major strengths of the study. Several steps, however, are needed before urine β-defensin-1 can be established as a useful biomarker of arsenic effects in population studies. First, the study must be replicated in larger samples exposed to a wider range of arsenic levels, including low and moderate levels of exposure. While Hegedus et al. (2008) found a correlation between arsenic levels and human β-defensin-1 peak intensity, further studies should evaluate the dose-response relationship over a wide range of arsenic exposure levels. It is also important to evaluate the temporal relationship between changes in arsenic exposure and changes in β-defensin-1 levels, including the short- or long-term nature of the relationship. Because human β-defensin-1 is expressed in epithelial cells of the lungs, skin, kidney, and bladder, all of them tissues that are affected by arsenic exposure, the relationship of arsenic with human β-defensin-1 could be evaluated in other fluids, including respiratory secretions.

Second, the association between arsenic and human β-defensin-1 in men but not in women must be better understood. Previous studies have found higher concentrations of β-defensin-1 in urine from women compared to men and even higher urine concentrations in pregnant women (Valore et al., 1998). Unfortunately, Hegedus et al. (2008) did neither report average peak intensities among women nor formal statistical tests of effect modification for arsenic exposure by sex on proteomic findings. Both samples studied by Hegedus et al. (2008) were relatively small, and the lack of association in women could be a false-negative finding. If the difference between men and women is confirmed, a lack of effect of
arsenic on β-defensin-1 in women could point to biological differences of arsenic effect by sex. While there is suggestion that men may be more susceptible to the toxic effects of arsenic (as the authors discussed), no clear patterns in bladder cancer rates by sex have been observed in arseniasis areas of Chile, Taiwan, or Argentina. Future adequately powered studies need to formally test for sex differences in arsenic proteomic effects.

Third, the operating characteristics of proteomic testing need to be well established. These aspects include establishing the validity, reproducibility, and comparability of the assays; the short-term and long-term within-person variability; and the sensitivity and specificity of β-defensin-1 levels to arsenic exposure and to other causes of bladder and kidney cancers. The investigation of β-defensin 1 expression in established in vitro and in vivo models of arsenic toxicity as well as the incorporation of arsenic to models of β-defensin-1 expression in bladder, kidney, and prostate cancers may help understand the biological mechanisms in arsenic carcinogenesis. Additional information including the association of β-defensin-1 levels with changing arsenic levels, with markers of mechanisms for arsenic toxicity (e.g., markers of oxidative stress, inflammation, or DNA repair), and with arsenic clinical endpoints will further clarify the potential role of β-defensin-1 level as a diagnostic, prognostic, or therapeutic marker of arsenic exposure.

Finally, β-defensin 1 is one of several protein urine markers that may be influenced by arsenic. Other proteins in urine or other biological fluids may also be affected. Hagedus et al. (2008) identified only one single urine protein consistently associated in men with arsenic exposure. Since arsenic affects many biological pathways and arsenic exposure levels in this study were high, multiple proteins as identified in other arsenic proteomic (Harezlak et al., 2008; Zhai et al., 2005) and gene expression studies (Andrew et al., 2008) could have been affected. Future studies will thus be needed to determine if urine β-defensin-1 captures a large proportion of the variability in early effects of arsenic or if this information needs to be combined with other protein peaks or with additional information derived from exposure biomarkers or genetic data.

Most of the human evidence available on the health effects and potential mechanisms of arsenic toxicity comes from populations exposed to high arsenic levels in drinking water (>100 μg/l) or in occupational settings. From a public health perspective, there is substantial interest in extending arsenic research to populations exposed to low and moderate levels. Research in these populations, however, is challenging because of the variability and relatively high limits of detection for urine arsenic species assays, the extra variability added by the use of spot urine samples in most population studies, the substantial cost of the arsenic speciation assays, the short biological half-life of urine arsenic, the long latency between arsenic exposure and effect for most cancers, and the need to study large sample sizes to identify small but public health relevant effects of arsenic. As with other environmental exposures, however, growing evidence supports that even at low exposure levels, arsenic may have important health consequences (Andrew et al., 2008; Meliker et al., 2007; Navas-Acien et al., 2008). The availability of markers of arsenic biological effects in addition to biomarkers of exposure and metabolism will add to our research tools and to our understanding of arsenic health consequences. In the mean time, standardization, common protocols, and high-quality experimental and prospective population studies are urgently needed to characterize the full impact of arsenic exposure in human populations.

REFERENCES


