Environmental Research 164 (2018) 18–23

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Arsenic methylation capacity in relation to nutrient intake and genetic polymorphisms in one-carbon metabolism

Brenda Gamboa-Loira[a], César Hernández-Alcaraz[a], A. Jay Gandolfi[b], Mariano E. Cebrián[c], Ana Burguete-García[a], Angélica García-Martínez[a,1], Lizbeth López-Carrillo[a,*]

[a] Instituto Nacional de Salud Pública, Av. Universidad 655, Col. Santa María Ahuacatitlán, C.P. 62100, Cuernavaca, Morelos, México
[b] Department of Pharmacology and Toxicology, College of Pharmacy, University of Arizona, Tucson, AZ 85721, USA
[c] Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del IPN, Av. Instituto Politécnico Nacional 2508, Col. San Pedro Zacatenco, Del. Gustavo A. Madero, C.P. 07360, Ciudad de México, México

ARTICLE INFO

Keywords:
Inorganic arsenic
Arsenic metabolism
Micronutrients
Polymorphisms
Northern Mexico

ABSTRACT

Background: Nutrients and genetic polymorphisms participating in one-carbon metabolism may explain inter-individual differences in inorganic arsenic (iAs) methylation capacity, which in turn may account for variations in susceptibility to iAs-induced diseases.

Objectives: 1) To evaluate the association between polymorphisms in five one-carbon metabolism genes (*FOLH1* c.223 T > C, *MTHFD1* c.1958 G > A, *MTHFR* c.665 C > T, *MTR* c.2756 A > G, and *MTRR* c.66 A > G) and iAs methylation capacity; 2) To assess if previously reported associations between nutrient intake and iAs methylation capacity are modified by those polymorphisms.

Methods: Women (n = 1027) exposed to iAs in Northern Mexico were interviewed. Blood and urine samples were collected. Nutrient dietary intake was estimated using a validated food frequency questionnaire. iAs methylation capacity was calculated from urinary iAs species (iAs, monomethylarsonic acid [MMA] and dimethylarsinic acid [DMA]) measured by high performance liquid chromatography (HPLC-ICP-MS). One polymorphism in each of the five genes evaluated was genotyped by allelic discrimination. Multivariable linear regression models were used to evaluate if genetic polymorphisms modified the associations between iAs methylation capacity parameters and nutrient intake.

Results: The median (min-max) concentration of total arsenic (TAs) was 20.2 (1.3–2776.0) μg/g creatinine in the study population. Significant interactions for iAs metabolism were only found with *FOLH1* c.223 T > C polymorphism and vitamin B12 intake, so that CT and CC genotype carriers had significantly lower %iAs, and higher DMA/iAs with an increased vitamin B12 intake, as compared to carriers of wild-type TT.

Conclusion: Differences in dietary nutrient intake and genetic variants in one-carbon metabolism may jointly influence iAs methylation capacity. Confirmation of these interactions in other populations is warranted.

1. Introduction

Several studies have suggested one-carbon metabolism related nutrient intake (*e.g.* folate, choline, methionine, betaine, *etc.)* is associated with inorganic arsenic (iAs) elimination efficiency through the generation of S-Adenosylmethionine (SAM) which serves as a methyl donor for iAs metabolism.

Ingested iAs is eliminated through urine as dimethylated metabolites (dimethylarsonuous acid [DMA^{+3}] and dimethylarsinic acid [DMA^{+5}]) in greater proportion than the monomethylated (monomethylarsinous acid [MMA^{+3}] and monomethylarsonic acid [MMA^{+5}]) products (60–70% *vs.* 10–20%), and the fraction that remains as iAs (10–30%) (Shen et al., 2016). The intermediates MMA^{+3} and DMA^{+3} are highly toxic and may be partially responsible for arsenic toxicity (Moe et al., 2016; Petrick et al., 2000; Vahter, 2002). Higher %MMA has been associated with increased risk of cancer and cardiovascular disease (Kuo et al., 2017). Our research group previously found that women with higher capacity to methylate iAs to MMA and/or a lower capacity to further methylate MMA to DMA were at higher risk for breast cancer (López-Carrillo et al., 2014). Individual

* Correspondence to: Instituto Nacional de Salud Pública. Av. Universidad 655, Sta. Ma. Ahuacatitlán, C.P. 62100, Cuernavaca, Morelos, México.
E-mail addresses: brenda.gamboa@espm.insp.mx (B. Gamboa-Loira), cesar.hernandez@insp.mx (C. Hernández-Alcaraz), gandolfi@pharmacy.arizona.edu (A.J. Gandolfi), mcebrian@cinvestav.mx (M.E. Cebrián), aburgete@insp.mx (A. Burguete-García), angelica.garcia67@gmail.com (A. García-Martínez), lizbeth@insp.mx (L. López-Carrillo).
[1] Un Kilo de Ayuda A.C. Av. Paseo de la Reforma 1110, Col. Lomas de Chapultepec, Del. Miguel Hidalgo, C.P. 11000, Ciudad de México, México.

https://doi.org/10.1016/j.envres.2018.01.050
Received 19 May 2017; Received in revised form 22 January 2018; Accepted 31 January 2018

methylation capacity depends not only on age, sex, body mass index (BMI), smoking, and exposure level but also on nutrient intake and polymorphisms in one-carbon metabolism (Engström et al., 2009; Hall et al., 2012; Tseng, 2009).

Observational studies have reported a negative association between urinary %MMA and dietary intake and/or plasma levels of nutrients involved in one-carbon metabolism. In several studies, an increase in % DMA was observed in relation to greater consumption of these nutrients (Basu et al., 2011; Gamble et al., 2005; Heck et al., 2007; López-Carrillo et al., 2016; Spratlen et al., 2017; Steinmaus et al., 2005a). Results from a randomized clinical trial showed that folic acid supplementation in folate-deficient adults was significantly associated with higher %DMA and lower %MMA in urine (Gamble et al., 2006), as well as with lower concentrations of iAs and MMA in blood (Gamble et al., 2007).

Additionally, one-carbon metabolism polymorphisms have been associated with iAs methylation capacity. Consistently, studies have shown that carriers of TT genotype in methylene tetrahydrofolate reductase (*MTHFR)* c.665 C > T polymorphism have impaired iAs methylation capacity, *i.e.* increased %iAs and %MMA, and decreased % DMA (Deng et al., 2007; Engström et al., 2007; Lindberg et al., 2007; Steinmaus et al., 2007). In contrast, scarce and inconsistent information is available regarding methionine synthase (*MTR*) c.2756 A > G and methionine synthase reductase (*MTRR*) c.66 A > G polymorphisms (Engström et al., 2009, 2007; Porter et al., 2010). The joint effect of nutrient intake and genetic variants on iAs metabolism has not been studied previously. In addition to the aforementioned polymorphisms, folate hydrolase 1 (*FOLH1* c.223 T > C) and methylenetetrahydrofolate dehydrogenase 1 (*MTHFD1* c.1958 G > A) have a key role in folate absorption and metabolism (Krajinovic, 2008; O'Keefe et al., 1998), although no information is available regarding their relationships with iAs metabolism.

Therefore, the aims of this study are 1) to evaluate the association between polymorphisms in one-carbon metabolism and iAs methylation capacity; 2) to assess if previously reported associations between nutrient intake (methionine, choline, vitamin B12, folate, riboflavin, vitamin B6, and betaine) and iAs methylation capacity (López-Carrillo et al., 2016) are modified by those polymorphisms, among Mexican women residing in Northern Mexico, where iAs concentrations in water have been reported to be in the range of 7–740 μg/l (Camacho et al., 2011).

2. Materials and methods

2.1. Study population

A cross-sectional study was undertaken among 1027 healthy Mexican women (only one pregnant) aged at least 20 years and residing for one or more years in any of the following northern states: Chihuahua, Coahuila, Durango, Nuevo Leon, and Sonora, during the period 2007–2011 (López-Carrillo et al., 2014). Women were identified with the Master Sampling System for National Health Surveys in Mexico, which provides a list of homes located in both urban and rural areas. Detailed information about sample procedures is published elsewhere (López-Carrillo et al., 2016). The response rate was 99.6% (1027/1031).

Pending informed consent, participants were interviewed face to face in one occasion, at their homes by trained interviewers about sociodemographic characteristics, diet, alcohol, and tobacco consumption. Anthropometric measurements for calculating the BMI were also obtained. Participants donated one blood sample and a first-morning void urine sample.

The project was approved by the Research, Biosecurity and Bioethics Committees at the National Institute of Public Health (Mexico).

2.2. Urinary arsenic determination

Samples were collected in a sterile disposable polypropylene urine collection cup, stored in a fridge and maintained at least for two years at −70 °C until analysis. Concentrations (μg/L) of urinary species arsenite (As^{+3}), arsenate (As^{+5}), MMA^{+5}, DMA^{+5} and arsenobetaine (AsB) were determined by high performance liquid chromatography inductively coupled plasma mass spectrometry (HPLC-ICP-MS), according to methodology previously described (Gilbert-Diamond et al., 2011). Measurements below the limit of detection (LOD) (AsB: 24.25%; As^{+3}: 19.28%; As^{+5}: 56.28%; MMA^{+5}: 1.95%; DMA^{+5}: 0.49%) were given the corresponding LOD: AsB: 0.08; As^{+3}: 0.15; As^{+5}: 0.41; MMA^{+5}: 0.12 and DMA^{+5}: 0.12, divided by the square root of two (LOD/√2), as suggested by Barr et al. (2006). The urinary concentration of creatinine (mg/dl) was measured using an enzymatic method kit (Randox, Antrim County, UK). Coefficients of variation were: MMA^{+5} = 8%, DMA^{+5} = 9%, As^{+3} = 8%, AsB = 18% and creatinine = 2.76%. iAs concentration was the sum of As^{+3} and As^{+5}. Arsenic exposure was assessed as the sum of iAs, MMA^{+5}, and DMA^{+5} (total arsenic [TAs]), excluding AsB.

In order to evaluate iAs methylation capacity, we calculated the following parameters: 1) percentages of iAs (%iAs), MMA^{+5} (%MMA) and DMA^{+5} (%DMA) with respect to TAs and 2) methylation ratios: first = MMA^{+5}/iAs (MMA/iAs); second = DMA^{+5}/MMA^{+5} (DMA/MMA); and total = DMA^{+5}/iAs (DMA/iAs).

2.3. Nutrient intake evaluation

Daily consumption over the last year of 119 foods and 14 dishes was estimated using a validated semi-quantitative food frequency questionnaire (Galván-Portillo et al., 2011). Based on the frequency of food consumption reported by participants and tables of nutrient composition No. 20 of the United States Department of Agriculture (USDA), the daily intake of total energy was estimated, as well as that of riboflavin, vitamin B6, folate, vitamin B12, choline, methionine and betaine (López-Carrillo et al., 2016).

2.4. Genotyping of *FOLH1 c.223T > C, MTHFD1 c.1958 G > A, MTHFR c.665C > T, MTR c.2756A > G and MTRR c.66A > G*

The following single nucleotide polymorphisms involved in one-carbon metabolism were included in this study: *FOLH1* c.223T > C (rs202676); *MTHFD1* c.1958 G > A (rs2236225); *MTHFR* c.665C > T (rs1801133); *MTR* c.2756A > G (rs1805087) and *MTRR* c.66A > G (rs1801394). They were genotyped by allelic discrimination, using TaqMan® assays and an ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA), under the following conditions: 95 °C per 10 min, 40 cycles to 95 °C during 15 s and 60 °C per 1 min. Conditional upon DNA availability, samples were analyzed in duplicate (kappa coefficients were: *FOLH1* c.223T > C = 1.00; *MTHFD1* c.1958 G > A = 1.00; *MTHFR* c.665C > T = 1.00; *MTR* c.2756A > G = 0.31; *MTRR* c.66A > G = 0.17). Negative controls were included on each plate. Sample amplification failures were as follows: 18 for *FOLH1* c.223T > C; 23 for *MTHFD1* c.1958 G > A; 7 for *MTHFR* c.665C > T; 6 for *MTR* c.2756A > G and, 13 for *MTRR* c.66A > G, which rendered final sample sizes for subsequent analysis of: 1009 (*FOLH1*), 1004 (*MTHFD1*), 1020 (*MTHFR*), 1021 (*MTR*) and 1014 (*MTRR*).

2.5. Statistical analysis

Selected characteristics of the study population were described using measures of central tendency and dispersion.

The observed distributions of the study genotypes were assessed by the Hardy-Weinberg Equilibrium test. Linkage Disequilibrium between *MTHFR* c.665 C > T and *MTR* c.2756 A > G genetic variants was evaluated by the D' statistic.