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Estimation of daily intake of arsenolipids in Japan based on a market basket survey

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ABSTRACT

Arsenolipid concentrations were measured in 17 food composites prepared from 152 food items purchased in Shizuoka city, Japan, to (1) determine the food contributing to daily intake of arsenolipids, and (2) estimate the daily intake of arsenolipids. Analysis of arsenolipids was performed by high performance liquid chromatography-inductively coupled plasma mass spectrometry/electrospray ionization tandem mass spectrometry (HPLC-ICP-MS/ESI-MS-MS). Arsenic containing hydrocarbons (AsHCs), arsenic containing fatty acids (AsFAs), and arsenosugar phospholipids (AsSugPLs) were detected only in "algae" and "fish and shellfish" of the 17 food composites in a concentration range of 4.4–233 ng As/g fresh weight (fw). Two cytotoxic arsenolipids, AsHC332 and AsHC360, were detected in "algae" and "fish and shellfish" in the concentrations range of 33–40 ng As/g fw. The estimated average daily intake of AsHC332 and AsHC360 was ca 3000 and 360 ng As/person/day, or 50 and 6.0 ng As/kg bw/day, respectively. The present study indicated that arsenolipids from "algae" and "fish and shellfish" consumption contributed to the daily intake of toxic AsHCs, though the margin of exposure for the AsHC332 and AsHC360 does not appear to pose a health risk for the general Japanese population.

1. Introduction

The presence of inorganic arsenic (iAs) in drinking water is a major environmental health problem in many parts of the world. Since long, it has been known that food contains arsenic and, in the case of some marine foods, the levels can be high (190 μg As/g, dry weight) (Mania et al., 2015). Health authorities, however, have not been concerned by these high concentrations because arsenic in marine foods was generally considered to be present mainly as harmless organic compounds such as arsenobetaine and arsenosugars (Francesconi, 2005) except for the arsenate in some seaweed (FSA, 2010; CFS, 2011; FSANZ, 2013). Another group of organoarsenic species was known to be present in marine organisms since the 1920s (Sadolin, 1928); these compounds, known as arsenolipids, have properties very different from arsenobetaine and other organic arsenic compounds in marine foods, all of which are water-soluble. The first identification and characterization of the structure of arsenolipids was studied in a brown alga, *Undaria pinnatifida* (wakame), by Morita and Shibata (1988). In the two decades following this discovery, only a few attempts were made to identify lipid soluble arsenic species probably due to their chemical complexity and relatively low concentrations. However, during the last few years, identification and characterization of arsenolipids have been carried out on a wide range of marine foods such as fish oils (Rumpler et al., 2008; Sele et al., 2012, 2014; Lischka et al., 2013), fish liver (Arroyo-Abad et al., 2010), sashimi tuna (Taleshi et al., 2010), other fish (Amayo et al., 2014), and algae (García-Salgado et al., 2012; Raab et al., 2013).

Taleshi et al. (2014) chemically synthesized a range of naturally occurring organic arsenic compounds, including seven arsenolipids, to be used in toxicity tests. These tests revealed that one group of arsenolipids, the arsenic containing hydrocarbons (AsHCs), showed cytotoxicity to human bladder and liver cells and that the magnitude of cytotoxicity was comparable to that of arsenite (Meyer et al., 2014). This toxicity result has raised concern over the human health risk of arsenolipids particularly among populations that consume large amounts of marine foods.

There is pressing public concern in Japan about arsenolipids because marine foods, in both raw and cooked forms, represent a

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significant part of Japanese diet. The European Food Safety Authority has also requested scientific data on arsenolipids in foods ahead of a re-assessment of safe limits for arsenic in foods (EFSA, 2009). Therefore, the health risk of toxic arsenolipids intake via the consumption of marine foods has to be assessed.

Towards this objective, exposure assessment of arsenolipids was carried out in this study through a market basket survey in Japan where all categories of food that people usually have in their diet, and were available in the marketplace, were included for the arsenolipids analysis. Arsenolipid concentrations were determined using HPLC/ICP-MS in combination with HPLC/ESI-MS in different types of Japanese foods (1) to determine the foods contributing to the daily intake of arsenolipids, and (2) to estimate daily intake of arsenolipids. Possible health risk of arsenolipids intake was also assessed.

2. Materials and methods

2.1. Sample collection by market basket survey

A market basket survey was performed for this study in which 152 food items of 17 food categories were purchased in one day in December 2015 from supermarkets in Shizuoka city, Shizuoka Prefecture, Japan. This city is located in the central part of Japan, in the Tokai District, being intermediate between two mega cities, Tokyo and Osaka, with cultural characteristics that differ in many aspects. The average amount of food consumption of 17 food categories in Tokai district is similar to that of Japan as a whole. The 17 food categories, including cereals, potatoes, sugars and sweeteners, pulses, nuts and seeds, vegetables, fruits, mushrooms, algae, fish and shellfish, meats, eggs, milks, oils and fats, confectioneries, beverages, and seasonings and spices, were based on the Appendix Food Categories of the 2013 National Health and Nutrition Survey (NHNS) by the Ministry of Health, Labor and Welfare (MHLW) of Japan (MHLW, 2015). Food items in each category were chosen according to the Detailed Environmental Survey (DES) in 2006 (Ministry of the Environment of Japan; 2007). Purchased food samples were transported to our laboratory under refrigerated condition (4 °C) overnight, and kept in a refrigerator (vegetables, fruits, eggs, and some confectioneries), in a freezer at −18 °C (raw meat, fish and shellfish, etc.), or at room temperature (canned foods, dried foods, oils, nuts and seeds etc.) until sample preparation which was done within two days after purchase.

2.2. Food composite preparation

Collected food samples were processed (washing or soaking, boiling or baking or frying etc.) as Japanese people usually do in their households (Ministry of the Environment of Japan; 2007). The food sample preparation methods are briefly listed by food item in Table 1. A portion of each prepared food item in a food category was mixed to prepare a composite for that food category. The weight of each food item in a composite was determined based on food consuming statistics of Tokai District in the 2013 NHNS (MHLW, 2015).

Each of the food composites was homogenized in a food processor (Cuisinart, San-ei Co., Ltd, Tokyo, Japan) for 5 min. A portion (25 g) of the homogenized composite was individually freeze-dried in a 50 mL polypropylene tube. The weight of the freeze-dried composite was measured and the weight loss after freeze-drying was assigned as moisture content. Some composites (sugars and sweeteners, nuts and seeds, oils and fats, and beverages) were not freeze-dried. The concentrations of arsenolipids in these food composites are expressed as arsenic weight (not the compound-weight) on a fresh weight (fw) basis.

2.3. Chemicals and reagents

Water used for the experiments was obtained from a Milli-Q system (18.2 MΩ cm, Millipore GmbH, Vienna, Austria). Ethanol (≥99.9%, EtOH), methanol (≥99.9%, MeOH), methyl *tert*-butyl ether (≥99.5%, MTBE), formic acid (≥98%), and ammonia solution (25%) were obtained from Carl Roth GmbH (Karlsruhe, Germany). Ammonium acetate was obtained from Merck (Buchs, Switzerland).

The certified reference material (CRM) used was NMIJ CRM 7405-a (Trace Elements and Arsenic Compounds in Seaweed-Hijiki) obtained from the National Metrology Institute of Japan (Tsukuba, Japan). Standard compounds used for the determination of arsenolipids were the arsenic hydrocarbon AsHC332 (Fig. 1 C15-HC, $C_{17}H_{37}AsO$) and the arsenic fatty acid AsFA362 (Fig. 1 C15-FA, $C_{17}H_{35}AsO_3$), which were available in our laboratory in Austria from a previously reported synthesis (Taleshi et al., 2014).

2.4. Extraction procedure for arsenic speciation analysis

Freeze-dried food composite samples (ca 50 mg) were extracted with a mixture of MTBE (5 mL) and MeOH (1.5 mL), and rotated on the rotatory cross for 1 h at room temperature. Water (1.25 mL) was added to the sample, and the mixture was shaken on the rotatory cross for 10 min, and centrifuged (4032 g, 10 min), and the upper layer was removed. The lower layer was re-extracted with 2 mL of a mixture of MTBE/MeOH/H_2O (10:3:2.5, v/v), as described above, and the upper layer (MTBE/MeOH) obtained after centrifugation was combined with that from the first extraction. The combined solvent was evaporated overnight, and the residue was re-dissolved in 250 µL EtOH with ultrasonication (15 min) and vortexing (3 min) at room temperature. This solution was centrifuged (21380 g, 15 min) and the supernatant was analyzed for arsenolipids.

2.5. Identification and quantification of arsenic species (RP-HPLC-ICP-MS/ESI-MS-MS)

Determination of arsenolipids was performed by high-performance liquid chromatography (HPLC) - inductively coupled plasma mass spectrometry (ICP-MS)/electrospray ionization tandem mass spectrometry (ESI-MS-MS) using an Agilent 1260 Infinity HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a degasser (G 4225A), a binary pump (G 1312B), an isocratic pump (G1310A), a thermosstatted auto-sampler (G 7617A), and a thermosstatted column compartment (G 1316A). The ICP-MS (Agilent 7900ce ICP-MS, Agilent Technologies, Waldbronn, Germany) was equipped with an Ari Mist HP nebulizer (Burgerner, Mississauga, Canada) and an ESI PC^3 Peltier cooled cyclonic spray chamber (Elemental Scientific, Omaha, USA), and signals were recorded at m/z 75 (^{75}As and $^{40}Ar^{35}Cl$) and m/z 77 ($^{40}Ar^{37}Cl$, for possible chloride interferences) at dwell times of 300 ms, and for internal standards at m/z 74 (^{74}Ge), m/z 115 (^{115}In) and m/z 125 (^{125}Te) at dwell times of 100 ms. Measurement with ESI-MS-MS (Agilent 6460, Agilent Technologies, Waldbronn, Germany) was performed in the positive ion mode with a precursor ion scan. Product ions at m/z 123 and 105 from the precursor ions of m/z 100–1000 were measured at a fragmentor voltage of 135 V and collision energy of 30 V; product ions at m/z 237 and 409 from the precursor ions of m/z 400–1200 were measured at a fragmentor voltage of 220 V and collision energy of 50 V. Source conditions were: gas temperature: 100 °C, gas flow 12 L/min, nebulizer pressure: 45 psi, sheath gas temperature: 350 °C; sheath gas flow: 11 L/min, capillary voltage: 4500 V, nozzle voltage: 500 V.

Separation was performed by reversed-phase HPLC using an ACE Ultra Core super C18 (4.6 × 250 mm, 5 µm particle size). Eluent used was an aqueous solution containing 20 mM ammonium acetate at pH 9.2 and MeOH containing 20 mM ammonium acetate at pH 9.2 with the following gradient: 0–15 min, 20%–100% MeOH, 15–35 min, 100% MeOH, 35–35.1 min, 100%–50% MeOH, 35.1–40 min, 50% MeOH. The flow rate was 1 mL/min and the injection volume for ICP-MS and ESI-MS-MS detections were 20 µL. For our measurement, the HPLC effluent was split, whereby 10% was transferred to the ICP-MS unit and 90% to