

論文の内容の要旨

Daily exposure to arsenolipids and its associated health risk in the Japanese

(日本人におけるヒ素脂質の一日ばく露量とその健康リスク)

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Introduction

The presence of arsenic in food has long been known, however, in the last few years, new types of arsenic compounds have been identified in various marine foods. These compounds, known as arsenolipids, are lipid-soluble arsenicals and hence they have properties very different from arsenobetaine and the other arsenic compounds in marine foods, all of which are water soluble and well characterized to date. There are four major categories of arsenolipids identified so far in marine samples; the arsenic-containing hydrocarbons (AsHCs), the arsenic-containing fatty acids (AsFAs), the arsenosugar phospholipids (AsSugPLs), and the tri-methylated arsenic-containing fatty alcohols (TMAAsFOHs). Arsenolipids have been found, often as the major form of arsenic, in various types of fish, fish oils, algae, and other marine foods [1],[2]. People who consume large amount of marine food in their daily life also consume arsenolipids in accordance. Japan is one of those countries where people consume large amount of marine foods in their daily diet. The toxicity investigation of arsenolipids revealed that, one group of arsenolipids, the AsHCs showed toxicity to human bladder and liver cells that was comparable to the strength of toxicity of arsenite species [3]. This raised a serious public concern regarding the safety of AsHCs in foods. 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 [4]. Therefore, the health risk of toxic arsenolipids intake via the consumption of marine foods has to be assessed. With these concerns in mind, in the present study, health risk of arsenolipids in marine foods was focused.

The aim of this study is to estimate the possible health risk of ingesting arsenolipids via marine foods in the Japanese. For achieving the aim, information need to be obtained includes

- (1) the arsenolipids exposure of the Japanese,
- (2) the variation of arsenolipids exposure, and
- (3) the bioaccessibility of arsenolipids in marine foods.

Analytical methods

Arsenolipids were analyzed in various types of food samples. Extraction of arsenolipids from food samples was performed by an extraction method [5] using a mixture of dichloromethane (DCM) and methanol (MeOH). After purification, arsenolipids were determined by using high performance liquid chromatography (HPLC)-inductively coupled plasma- mass spectrometry (ICP-MS)/electrospray ionization tandem mass spectrometry (ESI-MS-MS) in the laboratory at Graz, Austria.

(1) Exposure assessment of arsenolipids of Japanese people

By performing a market basket survey, a total of 17 food composites, i.e., cereals, potatoes, sugars & sweeteners, pulses, nuts & seeds, vegetables, fruits, mushrooms, algae, fish & shellfish, meats, eggs, milks, oils & fats, confectioneries, beverages, and seasonings & spices, were prepared from 152 Japanese food items which were purchased in Shizuoka city, Japan in December 2015. A portion of food composites was freeze-dried individually and used for the arsenolipids analysis.

Arsenolipids were detected only in “algae”, and “fish & shellfish” of the 17 food composites at a concentration of 0.8-37.5 ng As/g fresh weight (fw). In the case of other food categories, arsenolipids concentration was below the detection limit. The daily intake of arsenolipids (**Table 1**) was calculated by multiplying the measured concentrations of arsenolipids in food composites and the average daily consumption weight of the corresponding food category of the Japanese [6].

Table 1 Estimated daily intake of arsenolipids by food categories

Food category	Arsenolipids (ng As/person/day)											
	AsHCs		AsFAs			AsSugPLs						
	AsHC 332	AsHC 360	AsFA 362	AsFA 388	AsFA 390	AsSugPL 720	AsSugPL 930	AsSugPL 958	AsSugPL 986	AsSugPL 1014	AsSugPL 1042	AsSugPL 1070
Algae	40.4	112	“0”	“0”	“0”	409	48	321	64.4	55.6	7.64	24.0
Fish and shellfish	1525	305	511	53	709	“0”	“0”	“0”	“0”	“0”	“0”	“0”
Total	1565	417	511	53	709	409	48	321	64.4	55.6	7.64	24

Intakes from composites with undetectable arsenolipids species were estimated by assuming their concentration as “0” (zero).

The daily intake of toxic AsHCs (AsHC332 and AsHC360) for the Japanese was estimated to be 1.6 and 0.4 μg As/person/day. In the present study, health risk of the toxic AsHC332 and AsHC360 was estimated by using margin of exposure (MOE), which was defined in this study as the ratio of IC_{50} values (given as concentration in medium solution) to tissue concentration estimated from the intake. IC_{50} values of 9.2 and 4.8 μM for AsHC332 and AsHC360, respectively [3], were used. Additionally also two assumptions were necessary for MOE calculation: AsHCs in the food are 100% bioaccessible and its concentration in the blood (approximately 5 L) is same as its body fluid concentration. Based on these assumptions and estimated daily intake, the body fluid concentrations of AsHC332 and AsHC360 were expected to be approximately 0.004 and 0.001 μM and hence, MOE was calculated to be approximately 2300 (9.2/0.004) and 4800 (4.8/0.001), respectively.

(2) Variation of arsenolipids concentration in various fish and their stability on cooking

Nine different fish species available in Japanese supermarket including, salmon, mackerel, yellow tail, tuna, sardine, sea bream, skipjack, pacific saury, and whitebait were purchased. The edible portion of the fish species were collected and homogenized individually. A portion of three of the homogenized fish samples (salmon, yellowtail and mackerel) were cooked by grilling on a frypan separately. An aliquot of all homogenized fish samples was individually freeze dried. Freeze dried fish samples were used for extraction and analysis of arsenolipids.

AsHC332 and AsHC360 were detected in all of the fish species in a concentration range of 0.78 to 71.5 and 1.55 to 56.9 $\mu\text{g As/kg fw}$, respectively. Lipid content of the fish species could be a source of the concentration variation since arsenolipids are lipid soluble. Another reason of the variation in arsenolipids concentration was the part of fish for analysis in the present study: edible muscle tissue was used for the arsenolipids analysis for all the fish species except whitebait. Whole body was used for whitebait because it is eaten by people as whole. Additionally fish concentration of AsHCs reported in the literature [7] was adopted for supplementing the measured data for estimating concentration range of AsHCs in various fish species. The estimated concentration range of toxic AsHC332 and AsHC360 in fish species were 0.78 to 180 and 1.55 to 80 $\mu\text{g As/kg fw}$, respectively. AsHCs did not substantially change before and after cooking in all of the three fish species, which suggested that AsHCs were not decomposed by cooking. Considering the concentration variation of AsHC332 and AsHC360, the daily intake range would be 0.05-11.9 and 0.10-5.30 $\mu\text{g As/person/day}$ on the assumption that 66.3 g of fish is consumed daily: hence MOE would be approximately 92000-290 and 24000-340, respectively.

(3) Bioaccessibility arsenolipids in seafood

The food samples used for bioaccessibility test were “fish and shellfish” composite and hijiki seaweed (NMIJ CRM 7405-a, Hijiki). The *in vitro* gastrointestinal digestion model based on human physiology for the bioaccessibility test was performed in this study [8] where digestive juices were prepared artificially. The arsenolipids fraction that is mobilized from the food into the digestive juice represents the bioaccessible fraction and analyzed for arsenolipids. Bioaccessibility was calculated by the following equation:

$$\text{Bioaccessibility (\%)} = \frac{\text{Arsenolipids in chyme or supernatant (bioaccessible fraction)(ng)}}{\text{Arsenolipids present in non - treated sample (ng)}} \times 100\%$$

From the bioaccessibility test, bioaccessible arsenolipids concentrations were found to be lower than the arsenolipids concentration measured in “fish and shellfish” composite and hijiki not treated with digestive juices. This result indicated that arsenolipids in food are not completely bioaccessible for gastrointestinal absorption after the ingestion. The present experimental design for bioaccessibility test did not allow identifying whether the low bioaccessibility was due to decomposition of arsenolipids by digestive juices or to lower leachability of arsenolipids from food matrix. The bioaccessibility of AsHC332 and AsHC360 was calculated about 22% and 37% in “fish and shellfish” composite and about 68% and 100% in hijiki. In the case of bioaccessible toxic AsHC332, AsHC360 concentration, there were no great differences found between duodenal phase (16.3 and 5.42 ng As/g) and in gastric phase (22 and 5.64 ng As/g) in “fish and shellfish” composite. Moreover, no loss of AsHCs was found in duodenal phase (924 and 85.2 ng As/g) compared to the gastric phase (521 and 63.8 ng As/g) in hijiki which suggested that bioaccessible AsHCs in gastric juice did not decompose in intestinal conditions. Some arsenolipids were also found at higher concentrations in duodenal phase compared to the gastric phase in hijiki. Difference in pH between gastric and duodenal juices can to some extent contribute to the leachability for the bioaccessibility of arsenolipids. Some arsenolipids can be extracted in a specific pH range while would not be extracted if the pH changed; this is why probably some arsenolipids were extracted more in duodenal phase than

in gastric phase. By considering bioaccessibility data, daily intake of AsHC332 and AsHC360 from “fish and shellfish” composite and hijiki would be 0.36 and 0.22 μg As/person/day, respectively.

Overall evaluation of health risk

Based on the consumption data of “algae” (10.9 ± 19.5 g/person/day) and “fish and shellfish” (66.3 ± 71.5 g/person/day) [3] and by assuming the normal distribution, the 95 percentile of the people will consume “algae” and “fish and shellfish” approximately 50 and 209 g/person/day, respectively. Therefore, considering both concentration variation of arsenolipids and food consumption variation, the daily intake range of AsHC332 and AsHC360 would be 0.0007-37.80 and 0.0015-17.23 μg As/person/day, respectively. Then, by considering the bioaccessibility of AsHC332 (22-68%) and AsHC360 (37-100%), the body fluid concentration range of AsHC332 and AsHC360 is expected to be approximately 4.0×10^{-7} to 6.9×10^{-2} and 1.5×10^{-6} to 4.6×10^{-2} μM . Therefore, the MOE to the IC_{50} values (9.2 and 4.8 μM) for toxic AsHC332 and AsHC360 would be approximately 2.3×10^7 to 130 and 3.2×10^6 to 110, respectively.

Generally health risk is assessed by whether the intake exceeds the tolerable daily intake (TDI) or not. TDI is the product of NOAEL divided by Uncertainty Factors (UFs). Usually UF is set at 100, by considering inter-species variation ($\times 10$, difference between animal and humans) and intra-species variation ($\times 10$) into account. If $\text{MOE} < 100$, then, it implies the presence of risk. The minimum MOE in the present study was about 110, which is very close to 100, although there are additional sources of uncertainty which were not considered in the present study those could make MOE more variable, such as the fact that the concentration variation range of AsHCs was not determined in “algae”, due to the unavailability of NOAEL, and bioavailability data, the IC_{50} value, and the bioaccessibility data were used in MOE estimation. If the information of these uncertainties becomes available in future, all of those will make MOE to smaller direction except the bioavailability data of arsenolipids. However, despite of some uncertainties, the combined MOE estimated in the present study provided a notion about the possible health risk of toxic arsenolipids for Japanese people. It will also trigger to the necessity of further research on arsenolipids particularly on their toxicity, degradation pathway, and the estimation of their health risk for people in other countries.

References

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