RESEARCH REPORT - No. 1102 FY 2011–2013

Title of research project	Risk assessment of the ingestion of foods containing arsenosugers and arsenolipids
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Name of principal research Investigator（PI）	Ginji Endo

【Abstract】

Objective

In order to assess the health risk of arsenic from ingested foods containing arsenosugars (AsSugs) and arsenolipids (AsLipid), we studied the following subjects;

1) Examination of effective methods for extracting AsSug and AsLipid from seafoods.
2) Chemical synthesis of AsSug and their intermediate metabolites.
3) Identification and quantification of AsSug in seafoods.
4) The relation between AsSug ingestion and urinary metabolites levels.
5) Safety assessment of AsSug and the intermediate metabolites using animals.
6) *In vitro* assay of intermediate metabolites from AsSug in cultured cells.
7) Reviewing the methods reducing carcinogenic risk by seafoods ingestion.

Methods

1) We used wakame seaweed for an extraction study, and found an effective enzymatic treatment using cellulase and arginate lyase. For extraction of fat-soluble arsenic compounds in seafoods, we used the Folch's method.
2) AsSug328, which is a major AsSug of seafoods, was synthesized. In first reaction step, tetraacetyl-β-D-ribofuranose was treated with (S)-2,3-dibenzyloxy-1-propanol in the presence of Lewis acid. Then, eight reaction steps were followed.
3) Identification of AsSugs in wakame seaweed was performed by LC-MS/MS and LC-TOFMS. Quantification of arsenic compounds in wakame seaweed, processed anchovy, and fresh tuna were performed by arsenic speciation analysis using HPLC-ICP-MS.
4) Five volunteers ingested wakame seaweed and subsequently, urinary excretion of arsenic compounds were determined. Identification and quantification of the metabolites were performed by LC-TOF/MS and HPLC-ICP-MS, respectively.
5) *In vivo* mutation assay was performed using *gpt* delta rat.
6) Cytotoxicity tests of arsenic metabolites were performed using MYP3 and 1T1 cells. To elucidate metabolic process of toxic metabolites from AsSug, cell-free in vitro system was attempted. The analysis of their metabolites was carried out by using HPLC-ICP-MS and HPLC-TOFMS.
7) We collected information about genotoxicity test, adverse effect of inorganic and organic arsenics based on

animal experiments, epidemiological findings, and the evaluation by the International Agency.

Results

1) We established the best extraction method of seaweed which has a strong cell wall using cellulase and arginate lyase.
2) We succeed in total synthesis of AsSug328 consisting of nine reaction steps and also the toxic intermediate metabolites of dimethylmonothioarsinic acid (DMMTA).
3) We detected 4 arsenic compounds and identified AsSug328 and AsSug482 in wakame seaweed. Soluble arsenic compounds of dimethylarsinic acid (DMA), monomethylarsonic acid, arsenobetaine, trimethylarsineoxide (TMAO) and a fat-soluble arsenic compound, phosphatidylarsenocholine (PAC), were detected in both processed anchovy and fresh tuna.
4) Five volunteers ingested 0.06 mg of arsenic and excreted 30% of the total arsenic into urine. The identified urinary metabolites were DMA, oxo-dimethylarsinoylethanol (oxo-DMAE), oxo-dimethylasenoacetate (oxo-DMAA), and thio-DMAE.
5) Both point and deletion mutation were not induced by the treatment with DMA or arsenite. DMMTA was taken in bladder epithelium cells from the urine of rats.
6) DMMTA is the most toxic arsenic metabolite and the LC_{50} of DMMTA was 4.6 μM in MYP3 cells, 5.4 μM in 1T1 cells. DMMTA was transformed into DMMTA-SG conjugate by the reaction with GSH, then changed into trivalent dimethylated arsenic containing sulfur atom and H_2S.
7) We assessed risk of arsenic from food ingestion, finally, an evaluation book was published from the Japan Food Safety Commission.

Conclusion

The major AsSug in Japanese wakame seaweed was AsSug 482.

The major urinary metabolite was DMA after wakame ingestion. The contents were significantly different among volunteers.

The most toxic arsenic metabolite was DMMTA. Trivalent arsenical containing sulfur atom and H_2S produced metabolically from DMMTA would be involved in its metabolic activation.