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Title of research project	Cancer Risk assessment of food-derived dimethylmonothioarsinic acid
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【Abstract】

Dimethylmonothioarsinic acid (DMMTA) is a highly toxic organic arsenical metabolite of arsenic including various food-derived arsenical compounds. The clear evidence of carcinogenic potential of inorganic arsenic in humans and the ample evidence for the carcinogenicity of organic arsenicals in experimental animals have raised the concerns about toxicity and carcinogenicity of DMMTA. However, the detailed metabolic activation pathway, *in vivo* mutagenicity and carcinogenicity remain unknown. The purposes of the present study were to: 1) determine the *in vivo* mutagenicity of DMMTA; 2) determine the correlation between the urinary concentration of DMMTA and the urinary bladder cancer susceptibility in rats and mice; 3) examine the changes in AsSug structure in a mimic gastrointestinal system; 4) evaluate the permeability of bladder epithelial cells to arsenic compound; and 5) elucidate the metabolic processing of DMMTA.

We firstly established a novel intravesical treatment model in which DMMTA was stable in urinary bladder at least for 3 hours. In the mutagenicity study, urinary bladders of female *gpt* delta F344 rats were exposed to 70 ppm DMMTA by intraurethral catheter twice a week for 4 weeks using above model. Urinary bladder urothelium DNA were examined for point mutations by *gpt* assay and deletion mutations by Spi-assay. To compare the urinary concentration of DMMTA in rats and mice treated with dimethylarsinic acid (DMA^V), mice and rats were treated with 100 ppm DMA^V in the drinking water for 4 weeks. Speciation analyses for arsenic compounds were performed using high performance liquid chromatography with inductively coupled plasma mass spectrometry (HPLC-ICP-MS), HPLC with time of flight mass spectrometer, HPLC with a photodiode array detector, gas chromatography-mass spectrometry (GC-MS), and GC with a flame photometric detector. The bladder cell permeability to arsenic was assessed using rat bladder epithelial cells cultured in a Transwell® system.

There were no significant differences in the mutation frequencies in *gpt* assay and Spi-assay in bladder urothelium between the control and DMMTA treatment group, while DMMTA increase the cell proliferation in the bladder urothelium. Similar concentrations of DMMTA were detected in urine of mice and rats treated with DMA^V in drinking water, regardless the fact that DMA^V is a bladder carcinogen in rats but not in mice.

AsSug328 was decomposed to AsSug254 by the mimic gastric juice. The bile extract and pancreatin did not change the chemical structure of AsSug328. The human intestinal bacteria changed AsSug328 to AsSug254, thio-AsSug328, and a few unidentified forms of arsenic. An *in vitro* reaction of DMA^V with GSH generated DMA^{III} or DMA^{III}-SG. We also confirmed that the reaction of $DMMTA^V$ with GSH directly produced the stable complex of DMMTA-SG, and then produced hydrogen sulfide (H_2S) and dimethylmercaptoarsine (DMA^{III}-SH), DMA^{III} and DMA^{III}-SG in the decomposition process of $DMMTA^V$-SG. The apparent

permeability coefficient of the rat bladder epithelial cells for sodium arsenite and dimethylarsinic acid ranged between 5×10^{-7} to 1×10^{-6} cm/s.

DMMTA is not mutagenic in rat urinary urothelium. Rather than urinary concentration of DMMTA, the genetic differences in expression of arsenic transporter and cancer-related genes might contribute to the difference in urinary bladder cancer susceptibility between rats and mice. Furthermore, toxicity of DMMTA depends not only on the formation of DMA^{III} but also on at least those of H_2S and DMA^{III}-SH.