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A novel metabolic activation associated with glutathione in dimethylmonothioarsinic acid ($DMMTA^V$)-induced toxicity obtained from in vitro reaction of $DMMTA^V$ with glutathione

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ABSTRACT

The purpose of the present study was to elucidate the metabolic processing of dimethylmonothioarsinic acid ($DMMTA^V$), which is a metabolite of inorganic arsenic and has received a great deal of attention recently due to its high toxicity. The metabolites produced from an in vitro reaction with GSH were analyzed by high performance liquid chromatography-time of flight mass spectrometer (HPLC-TOFMS), HPLC with a photodiode array detector (PDA), and also gas chromatography-mass spectrometry (GC-MS) and GC with a flame photometric detector (FPD). The reaction of dimethylarsinic acid (DMA^V) with GSH did not generate DMA^V-SG but did generate dimethylarsinous acid (DMA^{III}) or DMA^{III}-SG. On the contrary, we confirmed that the reaction of $DMMTA^V$ with GSH directly produced the stable complex of $DMMTA^V$-SG without reduction through a trivalent dimethylated arsenic such as DMA^{III} and DMA^{III}-SG. Furthermore, the present study suggests the production of hydrogen sulfide (H_2S) and dimethylmercaptoarsine (DMA^{III}-SH), a trivalent dimethylated arsenic, as well as DMA^{III} and DMA^{III}-SG in the decomposition process of $DMMTA^V$-SG. These results indicate that the toxicity of $DMMTA^V$ depends not only on the formation of DMA^{III} but also on at least those of H_2S and DMA^{III}-SH.

1. Introduction

In humans and animals, consumption of inorganic arsenic brings about the excretion of dimethylarsinic acid [$(CH_3)_2AsO(OH)$, DMA^V] as a main metabolite of not only inorganic arsenic [1,2] but also, according to recent reports, arsenosugars and arsenolipids derived from seafood [3,4]. Accordingly, DMA^V is not available as an accurate biomarker for exposure of inorganic arsenic unless the subject has a diet history that excludes seafood consumption [5]. On the other hand, the metabolic methylation pathway from inorganic arsenic to DMA^V has long been considered as a detoxification process for inorganic arsenic, at least for its acute toxicity. However, because recent reports have indicated that metabolic intermediates of DMA^V, e.g., probably its trivalent form, are highly toxic and that DMA^V has a carcinogenic action in rodents [6,7], the dimethylated arsenic produced in the metabolic methylation as well as inorganic arsenic are now presumed to possibly play an important role in the toxic mechanism of arsenic [8–12]. Therefore, the International Agency for Research on Cancer (IARC) has classified DMA^V into group 2B, a possible carcinogen in humans [13].

The most common view for this is that DMA^V is further reduced metabolically to trivalent dimethylated arsenics such as dimethylarsinous acid [$(CH_3)_2AsOH$, DMA^{III}] and dimethylarsenic-glutathione conjugate [$(CH_3)_2As$-SG, DMA^{III}-SG] and that these trivalent dimethylated arsenics are considered to be more toxic than DMA^V and to be the ultimate substances responsible for arsenic carcinogenesis [14]. In addition, dimethylated arsenics containing a sulfur atom such as dimethylmonothioarsinic acid [$(CH_3)_2AsS(OH)$, $DMMTA^V$] and dimethyldithioarsinic acid [$(CH_3)_2AsS(SH)$, $DMDTA^V$] have been predicted as metabolites from

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DMA^V [15,16] and DMA^{III} [17–19]. In humans, $DMMTA^V$ has been identified as a trace arsenical excreted in urine after ingestion of arsenosugars [20], in the urine of male Japanese [21], and in the urine of women who reside in an area of arsenic contamination in Bangladesh [22]. In animal studies, $DMMTA^V$ was detected not only in the urine but also in the plasma of arsenite-treated F344 rats [23,24].

$DMMTA^V$ has both genotoxicity and cytotoxicity that have quite similar levels to those of DMA^{III}, while the same is not true for $DMDTA^V$ and trimethylarsine sulfide [$(CH_3)_3AsS$] [25]. The reason why the toxic potentials of pentavalent $DMMTA^V$ resemble those of trivalent dimethylated arsenic is that the cellular uptake system of $DMMTA^V$ resembles that of DMA^{III}, in fact, both are promptly taken up by cells [17]. Our previous study proposed a novel metabolic interrelation among $DMMTA^V$, DMA^V and DMA^{III} [26]. $DMMTA^V$ would be converted into DMA^V by receiving enzymatic oxidative desulfuration, possibly by cytochrome P450 (CYP), and then DMA^V formed here is further converted into DMA^{III} with GSH. Ultimately, the toxicity of $DMMTA^V$ has been thought to be caused by metabolic formation of DMA^{III} [27]. The toxic mechanisms of $DMMTA^V$, however, are not yet fully understood and elucidation of the details is needed. We further revealed that rhodanese, the enzyme that converts CN^- to SCN^-, catalyzed the sulfur atom-addition reaction from DMA^{III} to $DMMTA^V$ [26].

Dimethylmonothioarsenic-glutathione conjugate [$(CH_3)_2AsS$-SG, $DMMTA^V$-SG], a conjugate of $DMMTA^V$ with GSH, has been detected in cabbages after exposure to DMA^V [28]. Although it is assumed that pentavalent arsenicals do not directly bind to sulfhydryl groups, a recent report using a theoretical chemistry approach suggested that $DMMTA^V$-SG could be formed under weakly acidic conditions [29]. $DMMTA^V$-SG generation was more stable in an experiment using cultured cells [30]. Thus, since pentavalent $DMMTA^V$ is likely to form a complex with GSH, the characteristics of the thioarsenicals are different from those of the other pentavalent arsenicals. It would be important to elucidate the relevance of the interaction between $DMMTA^V$ and GSH in the early elucidation of the toxic mechanism of $DMMTA^V$. In particular, elucidation of the metabolic reduction processing of $DMMTA^V$-SG is extremely important. We believe that the toxicity of $DMMTA^V$ might be induced not only by its metabolic conversion to DMA^{III} but also to dimethylmercaptoarsine [$(CH_3)_2AsSH$, DMA^{III}-SH]. The generation of DMA^{III}-SH in theoretical chemistry [29] and in vitro reactions [31,32] has been suggested. On the other hand, a recent study has reported that biosynthesized persulfides (R-SSH) and hydrogen sulphide (H_2S) are highly reactive and have crucial regulatory actions in redox cell signaling [33]. Therefore, it would be important to clarify the toxicological significance of R-AsSH as well as R-SSH.

The toxicity of $DMMTA^V$ was the strongest among dimethylated thioarsenic compounds and further enhanced under the coexistence of GSH [34]. Therefore, to elucidate the reaction mechanism of $DMMTA^V$ and GSH in an in vitro system, we attempt to identify arsenic species formed by the reaction with $DMMTA^V$ with GSH using high performance liquid chromatography-time of flight mass spectrometry (HPLC-TOFMS) and gas chromatography-mass spectrometry (GC-MS) analysis, because identification of other intermediate metabolites was indispensable to elucidate a metabolic pathway.

2. Materials and methods

2.1. Caution

The arsenic compounds used in this study are highly toxic. These compounds should be handled with care.

2.2. Reagents and standards

All reagents were of analytical grade. DMA^V was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) and recrystallized with methanol twice. L-Glutathione reduced (GSH), carbon disulfide (CS_2), diethyl ether and other general chemicals were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ammonium acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA). $DMMTA^V$, $DMDTA^V$ and DMA^{III}-SG were synthesized in our laboratory according to the report methods described by Fricke et al. [35], by Suzuki et al. [36] and by Cullen et al. [37], respectively, and then kept at −30 °C under dryness. DMA^{III}-SH was synthesized according to the report by Mandal et al. [31]. In brief, a solution of 1 mol DMA^{III}-SG dissolved in 1.6 mol sulfuric acid was added slowly to a 1.6 mol sodium sulphide water solution under stirring. DMA^{III}-SH generated here was trapped in diethyl ether overlaid on the reaction mixture.

2.3. HPLC-TOFMS analysis

An HPLC system (Nexera LC-30A, Shimadzu, Kyoto, Japan) was used to separate thio-arsenic compounds using a reversed phase polymer column (SunFire C18, 150 mm × 2.1 mm i.d., Waters, Milford, MA, USA) under the following conditions. The mobile phase was a linear gradient between 96% 10 mM ammonium acetate (pH 5.0) with 4% methanol (solvent A) and methanol (solvent B), and programmed for solvent B as follows: 0–5 min, 0%; 5–20 min, 0 → 100% and held for 5 min. The flow rate, column temperature, and injection volume were 0.2 mL/min; 40 °C, and 5 μL, respectively. For detection a TOFMS (maXis 4G, Bruker Daltonics, Bremen, Germany) with ESI positive ion mode was used; ion source temperature, 200 °C; dry nitrogen gas temperature, 200 °C; dry gas flow, 8.0 L/min; capillary voltage, 4000 V; endplate offset, −500 V; and collision energy, 35 eV. The instrument was used to obtain high-resolution spectra in the MS/MS modes.

2.4. HPLC-photodiode array detector (PDA) analysis

An HPLC system (Prominence LC-20A, Shimadzu) equipped with a SunFire column and PDA detector at 190–350 nm (SPD-M20A, Shimadzu, Kyoto, Japan) was used to separate and determine thio-arsenicals and dimethylated arsenicals, under the same conditions as the HPLC system in TOFMS analysis mentioned above. Furthermore, each arsenical was measured by PDA. Briefly, we semi-quantified on the basis of a calibration curve method using 0.01–1 mM concentrations of each arsenic standard, $DMMTA^V$, $DMDTA^V$ and DMA^{III}-SG. However, since there is no standard $DMMTA^V$-SG, we calculated the concentration of $DMMTA^V$-SG produced in the reaction by subtracting the quantitative values of $DMMTA^V$, $DMDTA^V$ and DMA^{III}-SG, respectively, determined after reaction from the initial concentration of $DMMTA^V$ at the start of the reaction.

There were no arsenic species other than four arsenicals found in the TOFMS and PDA detectors. Also, since HPLC-PDA analysis using the standard DMA^{III}-SG could not detect any arsenic species (DMA^V and DMA^{III}-SG) other than the peak of DMA^{III}-SG, it was quantified by a calibration curve calculated from the peak area of DMA^{III}-SG.

2.5. GC-MS and GC-flame photometric detector (FPD) analysis

A GC-MS system (GCMS-QP2010Plus, Shimadzu) was used to separate and detect DMA^{III}-SH using a fused silica capillary column (HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm thickness, Agilent Technologies, Inc. Santa Clara, CA, USA) under the following conditions. The column temperature was programmed as follows; at 35 °C for 4 min, increased 15 °C/min to 180 °C and then held at