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Cytotoxic effects of *S*-(dimethylarsino)-glutathione: A putative intermediate metabolite of inorganic arsenicals

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Abstract

Glutathione (GSH) plays an important role in the metabolism of arsenite and arsenate by generating arsenic-glutathione complexes. Although dimethylarsinic acid (DMA^V) is the major metabolite of inorganic arsenicals (iAs) in urine, it is not clear how DMA^V is produced from iAs. In the present study we report that *S*-(dimethylarsino)-glutathione (DMA^{III}(SG)), a putative precursor of dimethylarsinic acid DMA^V, was unstable in the culture medium without excess GSH and generated volatile substances which were highly cytotoxic for both rat heart microvascular endothelial cells and HL60, a human leukemia cell line. Cytotoxicity of DMA^{III}(SG) was higher than that of iAs and its LC_{50} value was calculated to be 7.8 μM in the endothelial cells. To our surprise DMA^{III}(SG) effectively killed cells in the neighbor wells of the same multi-well dish, indicating that volatile toxic compounds generated from DMA^{III}(SG) in the culture medium. High performance lipid chromatography–inductively coupled plasma mass spectrometry (HPLC–ICPMS) analyses suggested that the freshly generated volatile compounds dissolved into aqueous solution and formed an unstable arsenic compound and the unstable compound was further converted to DMA^V. These results suggested that DMA^{III}(SG) exerts its cytotoxicity by generating volatile arsenicals and is implicated in the metabolic conversion of inorganic arsenicals into DMA^V, a major final metabolite of inorganic arsenicals in most mammals.

Keywords: Arsenic; Glutathione; Cytotoxicity; Metabolism; *S*-(Dimethylarsino)-glutathione; HPLC–ICPMS

1. Introduction

Globally, millions of people are at risk for the adverse effects of inorganic arsenicals (iAs) (Mead, 2005) and suffer from chronic arsenicosis such as skin lesions (Shraim et al., 2003), cancers (Hu et al., 2003; Lee and Bebb, 2005), diabetes mellitus (Tseng, 2004), and vascular and heart diseases (Engel and Smith, 1994; Lewis et al., 1999; Tseng et al., 2005). Excess intake of iAs is also associated with spontaneous abortion, still birth, and preterm birth (Ahmad et al., 2001). In the urine of people in arsenism endemic areas, dimethylarsinic acid (DMA^V) and monomethylarsonic acid (MMA^V) have been found to be two major metabolites of iAs (Shraim et al., 2003). Their trivalent forms of arsenics, dimethylarsinous acid (DMA^{III}) and monomethylarsonous acid (MMA^{III}) are possibly present in the fresh urine of arsenic-poisoned patients in West Bengal (Mandal et al., 2001), Romania (Aposhian

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et al., 2000), and in Mexico (Valenzuela et al., 2005) and As-chelator-treated patients in inner Mongolia (Le et al., 2000). However, the reported DMA^{III} in urine might have been pentavalent dimethylthioarsenicals (Hansen et al., 2004; Suzuki et al., 2004).

Recently, S-adenosyl-L-methionine (SAM)-dependent arsenic methyltransferase was purified from rat liver cytosol and the rat arsenic methyltransferase was an analogue to human Cyt19 (Lin et al., 2002). It is generally accepted that iAs are metabolized to MMA^V and DMA^V or possibly to trimethylarsine oxide (TMAO) by repetitive reduction and oxidative methylation (Waters et al., 2004). The methylation was once recognized as a detoxification process for harmful iAs, since the toxicities of MMA^V and DMA^V are much lower than those of iAs (Hirano et al., 2004). However, the methylation of arsenicals is now considered to be a bioactivation rather than a detoxification process, because the toxicity of intermediate trivalent metabolites such as MMA^{III} and DMA^{III} was found to be much higher than that of iAs^{III} (Dopp et al., 2004; Petrick et al., 2001; Styblo et al., 2000).

Glutathione plays a pivotal role in the metabolism of iAs. It has been shown that biliary metabolites of iAs^{III} were GSH-conjugated products such as arsenic triglutathione (ATG) and S-(monomethylarsino)-diglutathione ($MMA^{III}(SG)_2$) in the rat (Cui et al., 2004; Kala et al., 2000; Kobayashi et al., 2005). Mutant rats lacking MRP2 hardly excreted arsenic into bile and buthionine sulfoximine (BSO)-treated or hepatic GSH-depleted rats excreted a small amount of arsenic and GSH into bile and urine (Cui et al., 2004; Kala et al., 2004). It seems contradictory that ATG and $MMA^{III}(SG)_2$ are major metabolites of iAs^{III} in bile, while DMA^V is the major urinary metabolite in rats. However, it has been reported that approximately 60–70% of urinary arsenic was present as one of these GSH conjugates. They did not detect $DMA^{III}(SG)$, although DMA^V represented approximately 30% of urinary arsenic, using γ-glutamyl transpeptidase deficient mice that are unable to metabolize GSH (Kala et al., 2004).

It has been shown that ATG, $MMA^{III}(SG)_2$, and $DMA^{III}(SG)$ generated, when iAs, MMA^V and DMA^V were reacted with GSH (Scott et al., 1993). The authors suggested that those trivalent arsenic-GSH complexes were possible substrates for methylating enzymes. Recently we proposed that ATG and GSH-conjugated arsenicals were methylated by human arsenic (+3 oxidation state) methyltransferase (AS3MT, EC2.1.1.137, previously Cyt19) (Hayakawa et al., 2005). Taken together, it is reasonable to suppose that iAs^{III} is first conjugated with GSH producing ATG in the liver, ATG is then methylated or dimethylated by AS3MT, and finally trivalent arsenic-GSH complexes are secreted into bile or hepatic blood circulation.

However, an important question arises in the metabolic scheme for iAs. $DMA^{III}(SG)$ has not been identified either bile, urine, or tissues, although DMA^V is the prominent arsenic metabolite in urine (Suzuki et al., 2004). It is plausible that $DMA^{III}(SG)$ is unstable and quickly converted to DMA^V in blood stream after excretion from the liver. We report that $DMA^{III}(SG)$, a highly toxic metabolic intermediate of iAs, generates volatile arsenic compounds when GSH concentration is low and the volatile arsenical, possibly dimethylarsine, was finally converted to DMA^V, a less toxic major metabolite of iAs in urine.

2. Materials and methods

2.1. Chemicals

Sodium arsenite ($NaAsO_2$) and SAM were purchased from Sigma (St. Louis, MO). DMA^V and MMA^V, arsenobetaine (AsB) of analytical grade were purchased from TRI Chem. (Yamanashi, Japan). GSH, tetrabuthylammonium hydroxide (TBAH) solution, malonic acid, and other chemicals of analytical grade were purchased from Wako Pure Chem. (Osaka, Japan). $DMA^{III}(SG)$ was synthesized according to the method described in the literature (Kala et al., 2000; Scott et al., 1993). Briefly, 1 mol of DMA^V was reacted with 3 mol of GSH in 2-ml degassed water with continuous stirring and the solution was incubated overnight under a nitrogen atmosphere at room temperature. The products were collected as white precipitates, recrystalized in methanol:water = 1:1, and dried in a desiccator under reduced pressure.

2.2. Cells

Rat heart microvessel endothelial (RHMVE) cells were purchased from VEC Tech Inc. (population doublings < 6, Rensselaer, NY). They were grown in rat endothelial cell growth medium (cell applications, San Diego, CA) on gelatin-coated (attachment factor, cell applications) culture dishes in a humidified incubator under 95% air and 5% CO_2. The cells were detached by trypsinization, suspended in fresh growth medium at 2.5×10^5 cells/ml, and aliquoted into fibronectin-coated (attachment factor, cell applications, bovine plasma, 10 μg/ml) culture dishes. The cells were used for the experiments at the 6th to 10th passage after arrival. HL60 cells were obtained from RIKEN (Tsukuba, Japan) and subcultured in RPMI1640 medium containing 10% heat-inactivated fetal bovine serum.

2.3. Viability assay

As we describe later, $DMA^{III}(SG)$ is not stable in the culture medium and generates volatile toxic arsenicals. Thus, each test