Chemosphere 81 (2010) 206–213

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Distribution and speciation of arsenic after intravenous administration of monomethylmonothioarsonic acid in rats

Hua Naranmandura [a,*], Na Bu [a], Kazuo T. Suzuki [b], Yijia Lou [a], Yasumitsu Ogra [c]

[a] Institute of Pharmacology & Toxicology and Biochemical Pharmaceutics, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou 310058, China
[b] Graduate School of Pharmaceutical Sciences, Chiba University, Chuo, Chiba 260-8675, Japan
[c] Laboratory of Chemical Toxicology and Environmental Health, Showa Pharmaceutical University, Machida, Tokyo 194-8543, Japan

ARTICLE INFO

Article history:
Received 16 February 2010
Received in revised form 30 May 2010
Accepted 9 June 2010
Available online 1 July 2010

This article is dedicated to late Professor Kazuo T. Suzuki suddenly passed away during the manuscript preparation.

Keywords:
Arsenic
Thioarsenical
Speciation
Arsenic metabolism
Monomethylmonothioarsonic acid

ABSTRACT

Quite a few new thioarsenicals have recently been found in urine of arsenic-exposed humans and animals, and some of them have been shown to be highly toxic to cells. However, little is known about their toxic effects and metabolism in the body. In order to elucidate the toxic mechanism of thioarsenicals, we further focused on the distribution and metabolism of monomethylmonothioarsonic acid ($MMMTA^V$) in rats. $MMMTA^V$ was synthesized chemically and injected intravenously into rats at the dose of 0.5 mg As/kg, followed by speciation analysis of selected organs and body fluids at 10 min and 12 h after the injection. $MMMTA^V$ was excreted into urine in its intact form, and approximately 35% of the dose was recovered in urine at 12 h after the injection, suggesting that $MMMTA^V$ was taken up more effectively by organs/tissues than non-thiolated, monomethylarsonous acid (MMA^V) previously studied. On the other hand, the liver and kidneys contained arsenic that was in a protein-binding form with free forms of DMA^V or $DMDTA^V$ at 10 min, and disappeared at 12 h after the injection. Moreover, these bound arsenic species in kidneys were converted back to MMA^V after oxidation with H_2O_2, suggesting that the arsenic bound to proteins had been reduced within the body and was in a trivalent oxidation state. In red blood cells (RBCs), most of the arsenic was in the form of DMA^{III} bound to hemoglobin (Hb), and approximately 40% of the dose was recovered in RBCs at 12 h after injection. These results indicate that arsenic accumulated preferentially in RBCs after being transformed to DMA^{III}. In addition, we have also discussed the effect of $MMMTA^V$ on viability of human bladder cancer T24 cells in comparison with MMA^V. Consequently, $MMMTA^V$ was assumed to be a more toxic arsenic metabolite than non-thiolated MMA^V.

1. Introduction

Arsenic is a well documented human carcinogen, which is associated with increased risk of developing cancer of the skin, lungs, liver, kidney and bladder (NRC, 1999, 2001). In the environment, naturally occurring arsenic in water is present as the two major inorganic forms of arsenic, which are arsenite (As^{III}) and arsenate (As^V) (Oremland and Stolz, 2003; Le et al., 2004; Miyashita et al., 2009). Worldwide, millions of people are chronically exposed to arsenic by arsenic-contaminated drinking water, and suffer from the related diseases (NRC, 1999, 2001; Ng et al., 2003). In contrast to the toxic effects, arsenic (arsenic trioxide) is also successfully used clinically to treat patients with acute promyelocytic leukemia (APL) (Shen et al., 1997; Bobé et al., 2006). However, little is known about the mechanisms underlying arsenic-induced carcinogenicity in the target organs, and the mechanisms underlying the antileukemic activity of arsenic.

In mammals, the inorganic arsenicals are known to be primarily taken up by the liver, transformed to monomethylated (MMA) and dimethylated arsenicals (DMA) through consecutive reductive methylation, in forms bound to proteins (Naranmandura et al., 2006), and then finally excreted into urine as pentavalent methylated arsenic forms (Vahter and Marafante 1983; Mandal and Suzuki 2002; Suzuki et al. 2002). With respect to arsenic metabolites in urine, arsenite (iAs^{III}), arsenate (iAs^V) and monomethylarsonic (MMA^V) and dimethylarsinic (DMA^V) acids can be commonly detected in human and animals (Buchet et al., 1981; Suzuki et al., 2002). In addition, several papers have reported that trivalent monomethylarsonous acid (MMA^{III}) was also identified in

Abbreviations: iAs^V, arsenate; iAs^{III}, arsenite; AsB, arsenobetaine; DMA^V, dimethylarsinic acid; $DMMTA^V$, dimethylmonothioarsinic acid; $DMDTA^V$, dimethyldithioarsinic acid; MMA^{III}, monomethylarsonous acid; MMA^V, monomethylarsonic acid; $MMMTA^V$, monomethylmonothioarsonic acid; ICP MS, mass spectrometry with ionization by inductively coupled argon plasma.

* Corresponding author. Tel./fax: +86 571 8820 8402.
E-mail address: narenman@zju.edu.cn (H. Naranmandura).

0045-6535/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2010.06.043

human urine from the arsenic–affected areas and APL patients treated with arsenic trioxide (As_2O_3) (Aposhian et al., 2000; Mandal et al., 2001; Wang et al., 2004).

Recently, a few new thioarsenicals have been found in urine of arsenic-exposed humans and animals, and also in microflora of mouse cecum, which were identified as pentavalent monomethylmonothioarsonic acid ($MMMTA^V$), dimethylmonothioarsinic acid ($DMMTA^V$), and dimethyldithioarsinic acid ($DMDTA^V$) (Naranmandura et al., 2007b; Raml et al., 2007; Mandal et al., 2008; Kubachka et al., 2009; Suzuki et al., 2010). However, little is known about metabolism of these arsenicals, i.e., how these thioarsenicals are produced during the metabolism of inorganic arsenic in the body. Likewise little is known about how thioarsenicals are metabolized (Suzuki et al., 2007; Mandal et al., 2008).

Arsenic toxicity is mainly dependent on its chemical form (Hirano and Kobayashi, 2006). Of all of the arsenic compounds, trivalent arsenicals are more efficiently taken up by most mammalian cells compared to the corresponding non-thiolated pentavalent arsenicals (Drobná et al., 2005). Trivalent arsenicals are suggested to be more cytotoxic and genotoxic than the pentavalent arsenicals (Styblo et al., 2000; Mass et al., 2001; Kligerman et al., 2003; Hernandez-Zavala et al., 2005). In recent years, the thioarsenicals have received particular attention due to their toxicity (Naranmandura et al., 2007a; Suzuki et al., 2007; Ochi et al., 2008). Although the dimethylated pentavalent arsenic compound, DMA^V, is less toxic against most cell types than trivalent arsenicals, it has been shown that the sulfur-containing pentavalent arsenic metabolite, $DMMTA^V$, is more toxic and shows a similar toxicity to trivalent arsenicals in different human cell lines (Naranmandura et al., 2007a, 2009; Ochi et al., 2008). It is suggested that this high toxicity is caused by efficient production of ROS in the cells. Hence, thioarsenicals may be the arsenicals associated with an increased risk of urinary bladder cancer as toxic metabolic intermediates rather than an end product of the metabolism of inorganic arsenicals (Naranmandura et al., 2009). However, little information about the metabolism of $MMMTA^V$ in animals and the toxic effect of $MMMTA^V$ in cells is available.

Arsenic is a known human carcinogen, inducing tumors of the skin and urinary bladder (NRC, 1999, 2001). However, a good animal model has not yet been found. Cohen et al. (2006) has been reported that DMA^V was found to be a bladder carcinogen only in rats and only when administered in the diet or drinking water at high doses for long time. Although it is known that arsenic accumulates preferentially in rat RBCs as DMA, and it is different from that in humans (Lu et al., 2004), rats can be used as experimental animals to reveal arsenic toxicity because large amounts of experimental data for rats have been cumulated, in particular, arsenic metabolites in rats are well characterized. Thus, it may easy to compare the differences in metabolisms for each arsenic species in rats.

Rats are unique and the most tolerant animal species to arsenic among rodents, and the material balance of arsenic can be easily estimated in rats by determining the concentrations in RBCs and urine. In addition, immediate distribution can be detected from the concentrations of arsenic in organs/tissues up to 10 min after the injection, although the sum of arsenic distributed in the organs/tissues can be estimated from the concentration of arsenic in RBCs after a sufficient time period for metabolic transformation.

The present study was performed to gain an insight into the metabolism of thioarsenic species such as monomethylmonothioarsonic acid ($MMMTA^V$) in rats. Their distributions in major organs/tissues and body fluids were determined at two time points, i.e., 10 min and 12 h after an intravenous injection to investigate the initial distributions and the later distributions after its metabolism.

2. Materials and methods

2.1. Chemicals and reagents

All reagents were of analytical grade. Milli-Q water (Millipore) was used throughout the experiment. Arsenobetaine (AsB) and monomethylarsonic acid (MMA^V) were obtained from Tri Chemicals (Yamanashi, Japan). Hydrogen peroxide (H_2O_2), nitric acid, hydrogen chloride, ammonium acetate, acetic acid, 28% ammonia solution, glutathione, sodium arsenite, sodium arsenate, sodium sulfide (Na_2S), dimethylarsinic acid (DMA) were purchased from Wako Pure Chemicals (Osaka, Japan). An MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt) assay kit was purchased from Promega Corporation (Tokyo, Japan). Trizma® Base, and salt mixture of phosphate-buffered saline (PBS) were purchased from Sigma (St. Louis, MO). The arsenic standard solution (1000 $\mu g\ mL^{-1}$) for ICP–MS was purchased from SPEX CertiPrep, (Metuchen, NJ). Stock solutions of all arsenic compounds (10 mM) were prepared in purified water (Milli-Q), stored in the dark at 4 °C, and diluted daily prior to use.

2.2. HPLC–ICP–MS analysis

The HPLC system consisted of a liquid chromatograph solvent delivery PU-610 pump and a DG 660B-2 degasser (GL Sciences Co., Tokyo). A polymer-based gel filtration column (Shodex Asahipak GS-220 HQ, 300 mm × 7.6 mm i.d., Showa Denko, Tokyo) with an exclusion limit of 3000 Da was used to separate protein-unbound arsenic species from protein-bound arsenicals. A 20-μL aliquot of a sample solution was applied to the column, and then the column was eluted with 50 mM ammonium acetate buffer (pH 6.5 at 25 °C) at a flow rate of 0.6 mL min^{-1}. Arsenic in the eluate was monitored with an ICP MS (HP4500; Agilent Technologies, Hachiouji, Japan) at m/z 75. The signal at m/z 77 was also monitored to compensate for the molecular interference by $ArCl^+$.

2.2.1. Analytical procedure

Analyses were carried out using our developed HPLC–ICP MS technique (Mandal et al., 2001). The methods used to analyze for arsenic species, detection limit, quality control, precision, and sensitivity of these analytical measurements and validation of the procedure were described in details elsewhere (Mandal et al., 2001; Mandal et al., 2004). Briefly, the HPLC column (GS 220 HQ) used for this study of five arsenic species (i.e., MMA^V, DMA^V, AsB, $MMMTA^V$, and $DMDTA^V$) was a polymer-based column. The detection limits (LODs), defined as three times the standard deviation of the five blank readings, were calculated. It was found that the detection limits were 0.19–0.43 $\mu g\ L^{-1}$ for arsenic compounds. The precision was estimated five times with a solution containing approximately 10 times the LOD concentrations; the percentages of relative standard deviation (RSD%) were calculated, and it was 1.8–4.6.

Accuracy values were calculated by spiking standard compounds of all the species that were studied in all biological samples, and were also confirmed by analyzing the Standard Reference Material Bovine Liver (freeze-dried) SRM-1577b (NIST, Gaithersburg, MO). The recovery of the added compounds was 96–100%. This indicates that there is no significant interference.

2.3. Preparation of monomethylmonothioarsonic acid ($MMMTA^V$)

$MMMTA^V$ was prepared by stepwise addition of concentrated H_2SO_4 to an aqueous solution of MMA^V and Na_2S to give a final molar ratio of MMA^V:Na_2S:H_2SO_4 = 1:2:3, the reaction solution being