Available online at www.sciencedirect.com
ScienceDirect
Toxicology and Applied Pharmacology 216 (2006) 446–457
Toxicology and Applied Pharmacology
www.elsevier.com/locate/ytaap

Glutathione-*S*-transferase-omega [MMA(V) reductase] knockout mice: Enzyme and arsenic species concentrations in tissues after arsenate administration☆

Uttam K. Chowdhury [a], Robert A. Zakharyan [a], Alba Hernandez [c], Mihaela D. Avram [a], Michael J. Kopplin [b], H. Vasken Aposhian [a,b,*]

[a] *Department of Molecular and Cellular Biology, The University of Arizona, Tucson, Arizona, 85721-0106, USA*
[b] *College of Pharmacy, The University of Arizona, Tucson, Arizona, 85721-0106, USA*
[c] *Grup de Mutagènesi, Department of Genètica i de Microbiologia, Facultat de Ciències, Universitat Autónoma de Barcelona, Campus de Bellaterra, 08193 Cerdanyola del Vallés, Spain*

Received 2 May 2006; revised 26 June 2006; accepted 29 June 2006
Available online 7 July 2006

Abstract

Inorganic arsenic is a human carcinogen to which millions of people are exposed via their naturally contaminated drinking water. Its molecular mechanisms of carcinogenicity have remained an enigma, perhaps because arsenate is biochemically transformed to at least five other arsenic-containing metabolites. In the biotransformation of inorganic arsenic, GSTO1 catalyzes the reduction of arsenate, MMA(V), and DMA(V) to the more toxic +3 arsenic species. MMA(V) reductase and human (hGSTO1-1) are identical proteins. The hypothesis that GST-Omega knockout mice biotransformed inorganic arsenic differently than wild-type mice has been tested.

The livers of male knockout (KO) mice, in which 222 bp of Exon 3 of the GSTO1 gene were eliminated, were analyzed by PCR for mRNA. The level of transcripts of the GSTO1 gene in KO mice was 3.3-fold less than in DBA/1lacJ wild-type (WT) mice. The GSTO2 transcripts were about two-fold less in the KO mouse. When KO and WT mice were injected intramuscularly with Na arsenate (4.16 mg As/kg body weight); tissues removed at 0.5, 1, 2, 4, 8, and 12 h after arsenate injection; and the arsenic species measured by HPLC-ICP-MS, the results indicated that the highest concentration of the recently discovered and very toxic MMA(III), a key biotransformant, was in the kidneys of both KO and WT mice. The highest concentration of DMA(III) was in the urinary bladder tissue for both the KO and WT mice. The MMA(V) reducing activity of the liver cytosol of KO mice was only 20% of that found in wild-type mice. There appears to be another enzyme(s) other than GST-O able to reduce arsenic(V) species but to a lesser extent. This and other studies suggest that each step of the biotransformation of inorganic arsenic has an alternative enzyme to biotransform the arsenic substrate.

Keywords: Arsenic; Knockout; GST-Omega; MMA(III); DMA(III)

Abbreviations: MMA(V), monomethylarsonate; MMA(III), methylarsonous acid; DMA(V), dimethylarsinic acid; DMA(III), dimethylarsinous acid; GST, glutathione-*S*-transferase; As(V), arsenate; As(III), arsenite; KO, knockout; WT, wild-type; HPLC, high-performance liquid chromatography; ICP-MS, inductively coupled argon plasma mass spectrometry; GSH, glutathione.

☆ This paper is dedicated to recently retired William R. Cullen, Professor of Chemistry at The University of British Columbia. His generous gifts of arsenic compounds synthesized in his laboratory and made available to all who requested them for research has aided the advancement of our knowledge about one of the oldest group of toxic compounds that humans have used for good (cancer chemotherapy) and bad (homicides). In addition, his subtle, gentle humor has enlivened a deadly subject. His research productivity and presence will be missed by all of us investigating the biochemistry and molecular biology properties of arsenic compounds.

* Corresponding author. Department of Molecular and Cellular Biology, The University of Arizona, Life Sciences South, Room 444, Tucson, Arizona, 85721-0106, USA.
E-mail address: aposhian@u.arizona.edu (H.V. Aposhian).

0041-008X/$ - see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.taap.2006.06.014

Introduction

The mechanisms of the toxicity and carcinogenicity of inorganic arsenic at the molecular level in humans remain an enigma (Abernathy et al., 1999; Aposhian and Aposhian, 2006; Goering et al., 1999; Liu et al., 2002; Kitchin, 2001; Rossman et al., 2004; NRC, 2001; IARC, 1987) even though there are millions of people in the world drinking water containing carcinogenic concentrations of inorganic arsenic. For example, more than 25 million people in Bangladesh and 6 million people in West Bengal, India, are drinking water containing arsenic concentrations above 50 μg/L (Chakraborti et al., 2002) even though the WHO recommends that arsenic in drinking water not exceed 10 μg/L. An estimated 36 million people in the Bengal Delta also are at risk for arsenic-caused cancer (Nordstrom, 2002). Chronic exposure to inorganic arsenic has led to cancer of the skin, lungs, urinary bladder tissue, kidneys, and liver (Hopenhayn-Rich et al., 1996; Chiou et al., 1995; Chen et al., 1992; Smith et al., 1992).

Human MMA(V) reductase (Zakharyan and Aposhian, 1999) and human GSTO1 (Board et al., 2000) are identical proteins (Zakharyan et al., 2001). Because the reductions of arsenate to arsenite, MMA(V) to MMA(III), and DMA(V) to DMA(III) are catalyzed in vitro by GSTO1, this enzyme is crucial in the pathway for the methylation of inorganic As since only arsenic species having an oxidation state of +3 can be methylated (Zakharyan and Aposhian, 1999; Cullen and Reimer, 1989) (Fig. 1). Thus, we hypothesized that a deletion in the GSTO1 gene might seriously impair arsenic metabolism.

GSTO1 is a member of the glutathione-*S*-transferase superfamily. There are seven major types of human cytosolic GSTs: alpha, mu, pi, sigma, theta, zeta, and omega. These enzymes detoxify xenobiotics usually by the catalysis of the nucleophilic attack by reduced glutathione on an electrophilic compound. GSTO1 is a dimer of identical subunits. It has the characteristic GST fold of an N-terminal GSH-binding domain and a C-terminal domain made up of α-helices (Board et al., 2000).

An insoluble hGSTO2 is also known. GSTO1 and GSTO2 are two functional class glutathione transferase (GST) genes in humans that are separated by 7.5 kb on chromosome 10q24.3 (Whitbread et al., 2003). Recently, Schmuck et al. (2005) solubilized hGSTO2 and found it had MMA(V) and DMA(V) reducing activity. In addition, there has been the suggestion that GSTO1 may have a role as a nuclear antioxidant system (Yin et al., 2001). An excellent review of these important enzymes has appeared recently (Hayes et al., 2005).

A new pathway for inorganic arsenic biotransformation has been suggested by Hayakawa et al. (2005). It proposes that arsenic triglutathione (ATG) and monomethylarsonic diglutathione [$MA(SG)_2$] are substrates of CYT 19. CYT 19 may be one possible methylating enzyme that forms MADG and DMAG in the presence of *S*-adenosyl-methionine (SAM). As yet, this has not been tested experimentally. The methylated glutathionalated compounds are then oxidized to MMA(V) and DMA(V). The most original part of this new proposal is that +3 arsenic species are formed before the +5 analogous species. The latter are proposed end products of arsenic metabolism. But CYT 19 has not been purified and isolated from human tissues. A critical review of these enzymes for inorganic arsenic biotransformation has appeared recently (Aposhian and Aposhian, 2006).

The chemical forms of arsenic determine the toxicity and bioavailability of arsenic compounds (NRC, 2001; Cullen and Reimer, 1989). Although the tissue distributions of arsenic species with an oxidation state of +5 have been investigated extensively, this is not the case for the more reactive MMA(III)

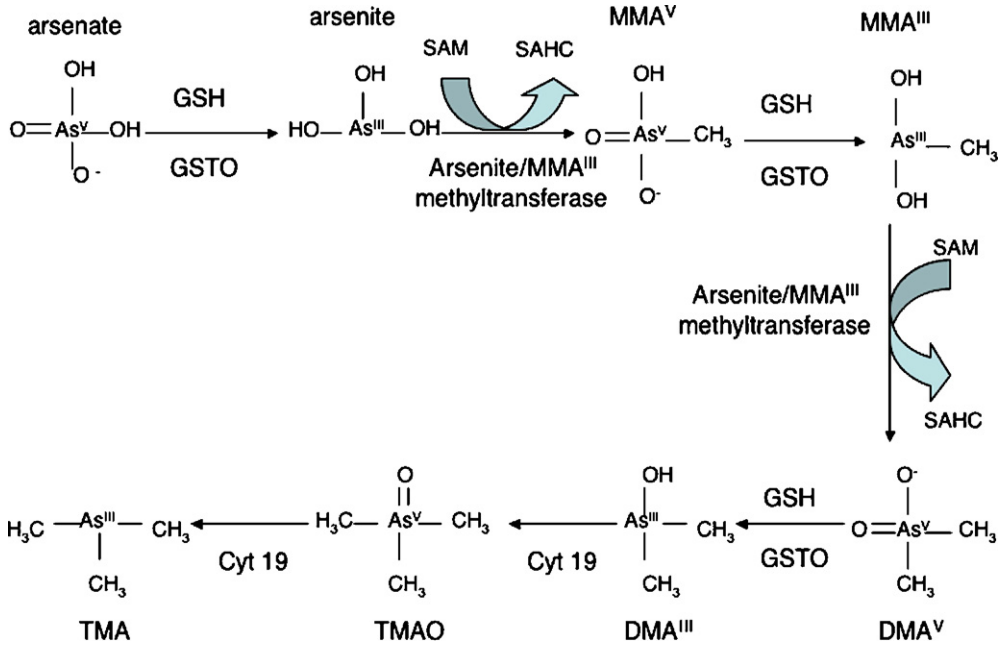

Fig. 1. Proposed pathway for inorganic arsenic biotransformation. Abbreviations: SAM, *S*-adenosyl-L-methionine; SAHC, *S*-adenosyl-L-homocysteine; GSTO, glutathione-*S*-transferase-omega; GSH, glutathione.