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Effects of dietary folate intake and folate binding protein-2 (Folbp2) on urinary speciation of sodium arsenate in mice

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Abstract

Folate binding protein-2 (Folbp2$^{-/-}$) knockout mice have been previously shown to be highly susceptible to the teratogenic effects of arsenic. Arsenic biotransformation is achieved primarily by biomethylation. Given the potential close relationship between folate biochemistry and arsenic biotransformation, the aims of our study were to: (1) test whether Folbp2$^{-/-}$ mice have altered arsenic biotransformation which would suggest a potential mechanism for their enhanced susceptibility; (2) examine whether dietary folate deficiency alters arsenic biotransformation. Folbp2$^{-/-}$ mice were found to have slightly lower plasma folate levels than wildtype mice. No genotype-specific effects were observed in arsenic speciation thereby negating altered biotransformation of arsenic as the mechanism of the enhanced teratogenicity seen in Folbp2$^{-/-}$ mice. Reduction in excretion of organic arsenicals was observed during folate deficiency, suggesting an important role for folic acid homeostasis in arsenic biotransformation.

Keywords: Folic acid; Arsenic; Excretion; Urinary speciation

1. Introduction

Inorganic arsenic has long been suspected of being teratogenic in humans; however, the existing published literature is limited to a few case reports and epidemiological studies. As a result, the association between in utero human fetal exposure to arsenic and adverse pregnancy outcome remains controversial (Shalat et al., 1996; DeSesso, 2001). In a number of laboratory animal species, arsenic has consistently been shown to be teratogenic, primarily inducing exencephaly, the major form of teratogen-induced neural tube defects (NTDs; Shalat et al., 1996; Golub et al., 1998). However, arsenic-induced teratogenicity in animals has been achieved almost exclusively by acute injections of high doses, which does not represent the typical conditions of human arsenic exposure. Thus, extrapolating from animal studies to humans is not straightforward, and warrants additional scientifically valuable human epidemiological studies.

Folate deficiency has been shown to be associated with the occurrence of birth defects, and a considerable number of studies have shown the protective role of folic acid supplementation in preventing neural tube, conotruncal heart, and craniofacial malformations in humans. For example, daily supplementation with folic acid in the range of 0.4–5 mg has been shown to reduce the incidence of NTDs by up to 70%

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(Mulinare et al., 1988; Czeizel and Dudas, 1992). However, despite the accumulating evidence regarding the embryoprotective effect of folic acid, the mechanism by which this beneficial effect is achieved remains unknown.

Folates enter cells by way of the folate receptors (FRs), also known as folate binding proteins (Folbps) in mice, and the reduced folate carrier. While the reduced folate carrier is a ubiquitously expressed membrane transporter, most FRs are externally bound to the plasma membrane by glycosylphosphatidylinositol anchors, and have tissue-specific and cell-specific expression patterns (Antony, 1992, 1996; Ross et al., 1994). The human FR-β is variably expressed in most human tissues and binds various folate forms with relatively high affinity (Ratnam et al., 1989; Ross et al., 1994). Using homologous recombination, we have previously inactivated (knocked out) the Folbp2 gene, the mouse homologue of FR-β, and generated nullizygous Folbp2 (Folbp2$^{-/-}$) mice (Piedrahita et al., 1999). Folbp2$^{-/-}$ mice develop normally and have no apparent pathologies or abnormalities associated with the mutation. However, when pregnant Folbp2$^{-/-}$ dams were treated with sodium arsenate during gestational days 7.5 and 8.5, the surviving fetuses had an elevated incidence of NTDs compared to control mice (Wlodarczyk et al., 2001). Folate deficiency further exacerbated this phenomenon in the mutant Folbp2 mice but not in controls, and thus it was concluded that Folbp2$^{-/-}$ mice have an increased susceptibility to in utero arsenic exposure.

In most mammalian species, the major route of arsenic detoxification occurs via biomethylation reactions (Fig. 1). For the first biomethylation reaction to occur, arsenate (As(V)) must be activated by reduction to its trivalent form, arsenite (As(III)). Arsenite serves as a substrate for a methyltransferase that generates the organic arsenical monomethylarsonate (MMA(V)) (Vahter and Concha, 2001; Lin et al., 2002). MMA(V) is then reduced to monomethylarsonous acid (MMA(III)) which is biomethylated to dimethylarsonic acid (DMA(V)), the major arsenic metabolite excreted in the urine (Vahter and Marafante, 1988). Both biomethylation reactions are catalyzed by methyltransferases that require *S*-adenosylmethionine (SAM) as the methyl donor (Vahter and Enval, 1983; Aposhian, 1997; Lin et al., 2002). The metabolic process that generates SAM occurs primarily via the transmethylation pathway (Fig. 1), where the conversion of homocysteine (Hcy) to methionine requires the transfer of a methyl group from 5-methyl-tetrahydrofolate (5M-THF), the primary bioactive folate form. Methionine is then converted to SAM by methionine adenosyltransferase and ATP. SAM, after donating its methyl group to acceptor molecules, is converted to *S*-adenosylhomocysteine (SAH), which is hydrolyzed to Hcy and adenosine.

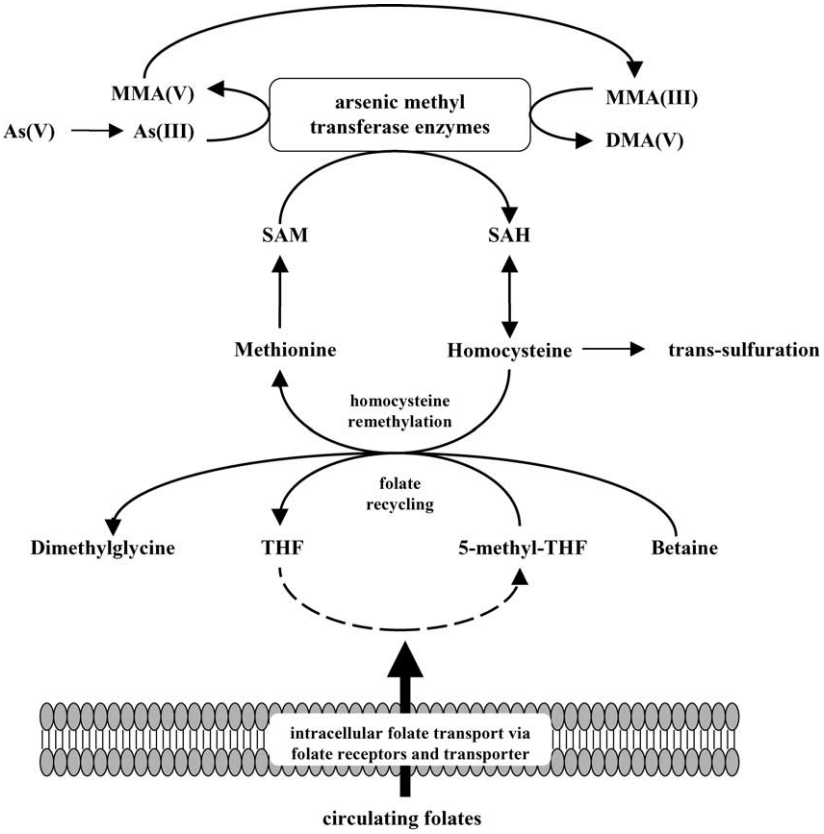

Fig. 1. Schematic representation of arsenic biotransformation pathway, the homocysteine transmethylation and folate metabolic recycling. As(III): arsenite; As(V): arsenate; DMA(V): dimethylarsonic acid; Hcy: homocysteine; MMA(III): monomethylarsinic acid; MMA(V): monomethylarsonic acid; 5M-THF: 5-methyl tetrahydrofolate; THF: tetrahydrofolate; SAH: *S*-adenosylhomocysteine; SAM: *S*-adenosylmethionine.