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Role of glutathione in reduction of arsenate and of γ-glutamyltranspeptidase in disposition of arsenite in rats

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Abstract

Arsenate (AsV), the environmentally prevalent form of arsenic, is converted sequentially in the body to arsenite (AsIII), monomethylarsonic acid (MMAsV), monomethylarsonous acid (MMAsIII), and dimethylarsinic acid (DMAsV) and some trimethylated metabolites. Although the biliary excretion of arsenic in rats is known to be glutathione (GSH)-dependent, involving transport of arsenic-GSH conjugates, the role of GSH in the reduction of AsV to the more toxic AsIII in vivo has not been defined. Therefore, we studied how the fate of AsV is influenced by buthionine sulfoximine (BSO), which depletes GSH in tissues. Control and BSO-treated rats were given AsV (50 μmol/kg, i.v.) and arsenic metabolites in bile, urine, blood and tissues were analysed by HPLC-HG-AFS. BSO increased retention of AsV in blood and tissues and decreased appearance of AsIII in blood, bile (by 96%) and urine (by 63%). The biliary excretion of MMAsIII was also nearly abolished, the appearance of MMAsIII and MMAsV in the blood was delayed and the renal concentrations of these monomethylated arsenicals were decreased by BSO. Interestingly, appearance of DMAsV in blood and urine remained unchanged and the concentrations of this metabolite in the kidneys and muscle were even increased in response to BSO. To test the role of γ-glutamyltranspeptidase (GGT) in arsenic disposition, the effect of the of the GGT inhibitor acivicin was investigated in rats injected with AsIII (50 μmol/kg, i.v.). Acivicin lowered the hepatic and renal GGT activities and increased the biliary as well as urinary excretion of GSH, but failed to alter the disposition (i.e. blood and tissue concentrations, biliary and urinary excretion) of AsIII and its metabolites. In conclusion, shortage of GSH decreases not only the hepatobiliary transport of arsenic, but also reduction of AsV and the formation of monomethylated arsenic, while not hindering the production of dimethylated arsenic. While GSH plays an important role in the disposition and toxicity of arsenic, GGT, which hydrolyses GSH and GSH conjugates, apparently does not influence the fate of the GSH-reactive trivalent arsenicals in rats.

Keywords: Arsenic; Biliary excretion; Urinary excretion; Glutathione; γ-Glutamyltranspeptidase; Buthionine sulfoximine; Acivicin

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1. Introduction

Arsenate (AsV) and the acutely more toxic arsenite (AsIII) are found in the environment, though AsV is typically the predominant form there (Tamaki and Frankerberger, 1992). Chronic arsenic exposure through consumption of contaminated drinking water is responsible for peripheral vascular disease, skin lesions and cancers (Hughes, 2002; Rossman, 2003). Arsenic trioxide, which is readily hydrated in vivo to form AsIII, once was a homicidal poison; however, it is now used as a chemotherapeutic drug in the treatment of acute promyelocytic leukaemia (Evens et al., 2004).

Inorganic arsenicals undergo extensive biotransformation in the body. Arsenate is eliminated by excretion into urine and by reduction to the more toxic AsIII. The latter in turn is converted into methylated metabolites, namely monomethylarsonic acid (MMAsV), monomethylarsonous acid (MMAsIII), and dimethylarsinic acid (DMAsV). Finally, dimethylarsinous acid and trimethylated metabolites may be formed and become detectable, especially after chronic arsenic exposure (Le et al., 2000; Hughes et al., 2003). In AsV- or AsIII-injected rats (Gregus et al., 2000) and other species (Csanaky and Gregus, 2002), AsIII is disposed into both bile and urine, MMAsIII is excreted into bile, whereas AsV, MMAsV and DMAsV are excreted into urine.

Glutathione (GSH) has multiple roles in the disposition of arsenic in the body. Among others, it is thought to participate in the reduction of AsV to AsIII, because depletion of GSH inhibited AsV reduction in isolated cells, such as rat erythrocytes (Winski and Carter, 1995) and mouse embryo cells (Bertolero et al., 1987), as well as in liver mitochondria (Németi and Gregus, 2002). Nevertheless, the role of this thiol compound in reduction of AsV in vivo has not been equivocally demonstrated. GSH is also important in the hepatobiliary transport of arsenic because the GSH depletor diethyl maleate virtually abolished the biliary excretion of arsenic in both AsIII- and AsV-injected rats (Gyurasics et al., 1991a). GSH-dependence of the hepatobiliary transport of arsenic is attributed to the fact that the trivalent arsenicals (AsIII and MMAsIII) are translocated from the liver cells into the bile canaliculi as labile GSH conjugates, namely As-triglutathione [$As(GSH)_3$] and MMAsIII-diglutathione [$MMAs(GSH)_2$], via mrp-2, a primary active transporter in the bile canalicular membrane (Kala et al., 2000). Furthermore, the presence of $As(GSH)_3$ and $MMAs(GSH)_2$ has been demonstrated recently in the urine of AsIII-injected γ-glutamyltranspeptidase-deficient mice that exhibit profound glutathionuria (Kala et al., 2004).

γ-Glutamyltranspeptidase (GGT) is abundant in the brush border membrane of renal tubular cells and is also located, albeit at much lower quantities, in the bile canalicular membrane of hepatocytes (Albert et al., 1964; Inoue et al., 1983). With its active site facing the lumen, GGT catalyses the first step in the catabolism of GSH and GSH conjugates via removal of the γ-glutamyl moiety from the tripeptide to produce cysteinylglycine (Cys-Gly) or Cys-Gly conjugates, respectively, which in turn may be broken down to cysteine or cysteine conjugates by Cys-Gly hydrolase, another membrane-bound extracellular enzyme. The breakdown products may then be reabsorbed into the renal tubular cells or the hepatocytes (Meister et al., 1981).

The extent of GGT-initiated degradation may markedly influence the excretion of xenobiotic-GSH conjugates and renal or intrabiliary reabsorption of their breakdown products. For example, GGT-deficient mice excreted into urine three- to five-fold more methymercury (that is known to form a GSH conjugate) than their wild-type counterparts (Ballatori et al., 1998), and pre-treatment with acivicin, a potent inhibitor of GGT (Allen et al., 1980; Griffith and Meister, 1980), enhanced the urinary excretion of methylmercury more than 30-fold in rats, while only slightly increasing the biliary excretion of this organometal compound (Gregus et al., 1987). It is unknown, however, whether the renal and hepatic GGT activities also influence the excretion of the AsIII and its trivalent metabolites, the GSH conjugates of which are candidate substrates of GGT.

The present studies have been designed to determine the importance of GSH in the in vivo reduction of AsV and the role of GGT in the disposition of AsIII in rats. For this purpose, we investigated first the effect of buthionine sulfoximine (BSO), an inhibitor of GSH synthesis and depletor of tissue GSH content (Griffith, 1982; Drew and Miners, 1984), on the elimination of AsV and formation of its metabolites in rats injected with AsV. Secondly, we studied the effect of the GGT-inhibitor acivicin on the disposition of AsIII and its metabolites in rats injected with AsIII.