Effect of Phosphate Transporter and Methylation Inhibitor Drugs on the Disposition of Arsenate and Arsenite in Rats

Iván Csanaky and Zoltán Gregus

Department of Pharmacology and Pharmacotherapy, University of Pécs, Medical School, Pécs, Hungary

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Arsenic (AsV) is biotransformed into the more toxic arsenite (AsIII) and monomethylarsonous acid (MMAsIII), but it is unknown how to decrease production of these harmful metabolites. We investigated the effects of fosfocarnet and fosfomycin, drugs interacting with the phosphate transporter, on biotransformation of AsV, an analog of inorganic phosphate. The effects of entacapone, an inhibitor of catechol-O-methyl transferase (COMT), and nitrous oxide, an inactivator of methylcobalamin, were also tested on the formation of MMAsIII from AsIII in order to clarify the role of COMT and methylcobalamin in biomethylation of AsIII. Arsenic in bile and urine of control and treated rats receiving AsV or AsIII was speciated by HPLC-HG-AFS. In AsV-injected rats, fosfomycin, but not fosfocarnet, increased the urinary excretion of AsV and decreased the biliary and urinary excretion of AsIII as well as biliary excretion of MMAsIII. In AsIII-injected rats, however, fosfocarnet failed to influence the excretion of AsIII and its metabolites, suggesting that this drug inhibits the hepatic uptake and renal reabsorption of AsV, thereby decreasing formation of AsIII and MMAsIII from AsV. Entacapone or nitrous oxide pretreatment slightly or not at all influenced the biliary excretion of MMAsIII and urinary excretion of dimethylarsinic acid (DMAsV) in AsIII-injected rats. In contrast, periodate-oxidized adenosine, an inhibitor of S-adenosylmethionine-dependent methyltransferases, nearly abolished appearance of methylated arsenic metabolites in bile and urine. Thus, fosfocarnet facilitates urinary clearance of AsV and decreases formation of toxic AsIII and MMAsIII, indicating that this drug may be used to promote elimination and counter toxification of AsV. Because entacapone and nitrous oxide influenced the excretion of MMAsIII and DMAsV negligibly, neither COMT nor methylcobalamin appears to be involved in arsenic methylation in rats.

Key Words: arsenic; monomethylarsonous acid; fosfocarnet (fosfomycin); nitrous oxide (N₂O); methylation; catechol-O-methyltransferase (COMT); methylcobalamin; biliary excretion; urinary excretion.

Arsenic is well known for its significant acute toxicity and carcinogenicity in humans. In several areas of the world, arsenic-rich minerals contaminate the drinking water, mainly with arsinite (AsV), whereas burning of coal and melting of metals pollute the air with the more toxic arsenic trioxide (the dehydrated form of arsenite, AsIII). The toxicity of arsenic is exploited therapeutically against harmful microorganisms and neoplastic cells. Currently, organic arsenicals are used in the treatment of African trypanosomiasis, whereas arsenic trioxide is employed to induce complete remission of acute promyelocytic leukemia (Chen et al., 1996).

Inorganic arsenicals are extensively biotransformed in the body and are excreted both in unchanged form and as metabolites. According to the most accepted scheme, the biotransformation involves alternating steps of reduction and oxidative methylation. Thus, the putative metabolic pathway of AsV is AsV → AsIII → MMAsV → MMAsIII → DMAsV, where MMAsIII is the trivalent monomethylarsonous acid, and MMAsV and DMAsV are the pentavalent monomethylarsenic acid and dimethylarsinic acid, respectively. Our recent study on bile-duct-cannulated rats injected with AsIII or AsV indicates that the pentavalent metabolites (AsV, MMAsV, DMAsV) are excreted exclusively into urine, MMAsIII appears only in bile, whereas AsIII is cleared into both bile and urine (Gregus et al., 2000). MMAsIII has been found in the urine of some arsenic-exposed humans, but it was undetectable in the urine of the majority of such individuals (Aposhian et al., 2000a), unless they were given an arsenic chelator (Aposhian et al., 2000b; Le et al., 2000).

Chemical reactivity and toxicity of arsenicals are markedly influenced by their oxidation state. The pentavalent AsV does not react covalently with thiols, whereas the trivalent AsIII forms stable compounds with dithiols, but relatively unstable complexes with monothiols, and monomethylarsenite (MMAsIII) binds to both mono- and dithiols (Knowles and Benson, 1983). Biotransformation of AsV contributes to its toxic effects, because it yields the more toxic AsIII and the even more toxic MMAsIII (Cullen, 1989; Petrick et al., 2000; Styblo et al., 2000). Thus, it is important to know how production of these toxic arsenic metabolites can be influenced.

In the body, AsV behaves, in part, similarly to inorganic phosphate, and it competes with phosphate in enzymatic and transport processes (Dixon, 1997). AsV undergoes renal tubu-
lar reabsorption, which is inhibited at high plasma-phosphate concentration and is purportedly mediated by the Na" dependen
phosphate transporter (Berner et al., 1976; Ginsburg and Lotspeich, 1963; Swaan and Tukker, 1995; Szczepanska-
Konkel et al., 1986). This transporter can be inhibited by alkylphosphonate compounds (Szczepanska-Konkel et al., 1986), including the antiviral drug foscarnet (phosphonoformic acid) and the antibiotic fosfomycin (Fig. 1).

Although AsIII and MMAIII methyltransferase enzymes have been partially purified from liver of some species (Wild-
fang et al., 1998; Zakharyan et al., 1995), they are still incompletely characterized. It has been suggested recently that ar-
senic methyltransferases belong to a class of enzyme with an active site related to that of catechol-O-methyl transferase
(COMT; De Kimpe et al., 1999). Furthermore, it has been shown that methylcobalamin (methylvitamin B₁₂) can directly
methylate AsIII in vitro (Buchet and Lauwerys, 1988; Zakharyan and Apostjian, 1999). However, the in vivo role of
COMT and methylcobalamin in arsenic methylation remains uncertain.

The objective of the present study was to test specific drugs to determine whether they inhibit the formation of toxic arsenic metabolites from AsV (i.e., AsIII and MMAIII) and from AsIII (i.e., MMAIII). The candidate drugs included foscarnet and fosfomycin, which interact with the phosphate transporter and thus might interfere with cellular uptake and intracellular toxification of AsV. We also tested the effects of entacapone, a potent and selective inhibitor of COMT (Nissinen et al., 1992), and the anesthetic gas nitrous oxide (N₂O) that rapidly and long lasting inactivates vitamin B₁₂ in rats, purportedly by oxidizing its Co(I) atom into Co(III) form, which in turns leads to inactivation of the methylcobalamin-requiring enzyme methionine synthetase (Deacon et al., 1980; Lumb et al., 1983). These drugs might inhibit formation of MMAIII, provided COMT or methylcobalamin, respectively, is involved in the biomethylation of AsIII. The effects of entacapone and nitrous oxide on arsenic methylation was compared with that of periodate-oxidized adenosine (PAD), which is an indirect in-
hibitor of all S-adenosylmethionine-dependent methyltrans-
fersases (Hoffman, 1980). We performed this investigation on rats, despite the controversy about the use of this species in arsenic metabolism studies, because rats, unlike other species, retain DMAAs in their red blood cells (Aposhian, 1997). We share the view of others (Lerman and Clarkson, 1983) that the fact that DMAAs, a late arsenic metabolite, binds to rat erythrocytes should not exclude the possibility of using rats for short-term mechanistic studies on earlier steps of arsenic disposition, such as transport of AsV and AsIII, reduction of AsV to AsIII, as well as formation and excretion of monomethylated arsenic metabolites. In addition, because rats produce and excrete large amounts of MMAIII into the bile (Gregus et al., 2000), this species appears especially suitable for studying the process of MMAIII formation in vivo and its responsiveness to drugs.

MATERIALS AND METHODS

Chemicals. Sodium arsenite (AsIII) was purchased from Carlo Erba (Mi-
lan, Italy); disodium hydrogen arsenate (AsV), high purity hydrochloric acid, potassium dihydrogen phosphate, adenosine and sodium periodate were from Reanal (Budapest, Hungary); disodium methylarsenate (MMAV) was ob-
tained from Chem Service (West Chester, PA); cacodylic acid sodium salt
(DMAAs) from Sigma, dipotassium hydrogen phosphate from Merck, and sodium borohydride from Aldrich. Fosfomycin was obtained from Zambon Group (Italy), whereas foscarnet (Foscavir™) was a product of Astra Läkemedel AB (Södertälje, Sweden). Entacapone was a generous gift from Orion Farmos–Pharmaceuticals, Orion Research Center (Espoo, Finland). Nit-
roglycerin containing 50% nitrous oxide and 50% oxygen as well as com-
pressed air were the products of AGA (Budapest, Hungary). Periodate-oxi-
dized adenosine (PAD) was prepared by incubation of adenosine with sodium periodate, according to the method of Hoffman (1980). Immediately before use, a saturated aqueous solution of PAD was prepared and its PAD concen-
tration was determined spectrophotometrically as described by Tandon et al. (1986).

Animal experiments. Male Wistar rats (LATI, Gödöllö, Hungary) weigh-
ing 240–310 g were used. The animals were kept at 22–25°C room tempera-
ture, at 55–65% relative air humidity, and on a 12 h light/dark cycle and provided with tap water and lab chow (Charles River VRF1, Budapest, Hungary) ad libitum.

The biliary and urinary excretion studies were performed largely as de-
scribed (Gregus et al., 1998). To induce urine production, the rats were hydrated by gavage of 30 ml/kg of saline containing 10 mM potassium chloride, anesthetized by ip injection of a mixture of fentanyl, midazolam, and droperidol (0.045, 4.5, and 5.5 mg/kg, respectively), and their body tempera-
ture was maintained at 37°C by means of heating radiators. Subsequently, the right carotid artery was cannulated with a PE-50 tubing, the urinary bladder was exteriorized through a low abdominal incision in the midline, and the common bile duct was cannulated with the shaft of a 23-gauge needle attached to PE-50 tubing (Clay Adams, Parsippany, NY) through a high abdominal incision. The rats, thus prepared, were administered 6 ml/kg 10% mannitol in saline, via the carotid cannula, to promote urine flow and were subsequently injected with AsIII or AsV (50 μmol/kg) in saline (3 ml/kg) into the left saphenous vein. These arsenic doses are high enough to permit sensitive analysis of biliary and urinary arsenic metabolites throughout the experiments but do not cause signs of overt toxicity. Bile and urine samples were then collected in 20 min periods into pre-weighed 1.5 ml microcentrifuge tubes. The
In order to study the effect of phosphate analog drugs on the disposition of inorganic arsenic, fosfomycin or foscarnet was injected into rats 1 min before administration of AsV or AsIII in a therapeutically applicable human dose (500 μmol/kg, iv). To study the effect of entacapone (30 mg/kg, ip) or PAD (50 μmol/kg, ip), either of these compounds was administered 40 min before injection of AsIII. Entacapone was dissolved in 2% of Tween 80 in saline (Model 501, Waters, Milford, MA) through an injector disposition, rats were exposed to nitralgin gas for 2 h in a 5–10 ml/kg of saline, depending on the actual concentration of PAD in the solution (Tandon et al. 1986). To examine the influence of nitrous oxide on arsenic disposition, rats were exposed to nitralgin gas for 2 h in a 2.5 liter desiccator, which was continuously rinsed through with a steady stream of the gas. Thereafter the rats were removed, surgically prepared, and injected with AsIII within 20 min after termination of nitrous oxide exposure. Rats serving as controls for the nitrous oxide-prepared animals were also kept for 2 h in a desiccator, which was continuously purged with air from a tank containing compressed air.

**Analysis.** Arsenic in bile and urine was speciated and quantified by HPLC hydride-generation, atomic-fluorescence spectrometry (HPLC-HG-AFS) based on the procedure of Gomez-Ariza et al. (1998), as described recently by Gregus et al. (2000). The eluents containing 10 mM (A) or 60 mM (B) K₂HPO₄·KH₂PO₄ (pH 5.75) were pumped at a combined flow rate of 1 ml/min with 2 HPLC pumps (Model 501, Waters, Milford, MA) through an injector (Rhenodyne 7125) equipped with a 20 μl sample loop onto a strong anion-exchange guard column linked to an analytical column (both Hamilton PRP X-100 with dimensions of 20 × 4.1 mm and 250 × 4.1 mm, respectively). To the effluent exiting from the analytical column, first 1.5 M HCl, then 1.5% m/v sodium borohydride in 0.1 M sodium hydroxide were mixed using 2 tees and a mixing coil. Both solutions were pumped with a peristaltic pump (Type 313S, Watson-Marlow Ltd., Falmouth, UK) at flow rates of 1 ml/min. These reagents converted the arsenic compounds eluted from the HPLC column into gaseous arsenic hydrides, which were subsequently separated from the combined liquids by a gas-liquid separator (A type, PS Analytical, Kent, U.K.). The hydrides were conveyed with a mixed stream of argon (200 ml/min) and hydrogen gas (70 ml/min) through a hygrosopic membrane drying tube (Perma Pure Products, Farmingdale, NJ) into the hydrogen-argon diffusion flame inside the atomic fluorescence spectrometer (PSA Excalibur, PS Analytical, UK) equipped with an arsenic-booster discharge hollow cathode lamp (Photron Pty. Ltd., Victoria, Australia). The fluorescence signal of the detector was recorded by computer using Millennium Chromatography Manager (Waters) which also controlled the HPLC pumps.

Immediately after collection, the bile and urine samples were deproteinized by mixing with 9 volumes of 80% methanol in water and centrifuged for 2 min in a Beckman Microfuge E. The resultant supernatant was subsequently diluted with water 10–25 fold. Both diluents were purged with argon to minimize oxidation of trivalent arsenic in the samples. Recovery of arsenic in methanol-precipitated bile samples obtained from AsIII-injected rats (which contained predominantly AsIII) and AsV-injected rats (which contained predominantly MMAAsIII) was tested by comparing the quantity of total arsenic in water-diluted bile samples with the quantity of total arsenic in the supernatant of the bile samples precipitated with 80% methanol. Total arsenic was analyzed using the equipment described above from which the guard and analytical columns were omitted. These tests revealed that the supernatant of methanol-precipitated bile samples collected from AsIII-injected and AsV-injected rats contained, respectively, on the average 95.4 and 98.5% of arsenic present in the same bile samples that were diluted with water. Thus, our deproteinization procedure results in almost complete recovery of biliary arsenic.

After injecting the appropriately diluted samples into the HPLC column, the arsenic compounds were eluted by pumping 100% eluent A for 2 min, then 100% eluent B till 8.6 min, after which the eluent was changed to 100% A again till minute 12. Quantification of arsenic compounds was based on peak areas of samples and authentic standards (AsIII, DMAsV, MMAAsV, AsV). Because pure MMAAsIII is not available, the biliary arsenic compound identified as MMAAsIII was quantified based on the MMAAsIII peak area in the bile sample and the AsIII peak area in the standard (Gregus et al., 2000). With 20 μl sample injection volume, the lowest detectable concentrations of AsIII, DMAsV, MMAAsV and AsV were 20, 25, 25, and 35 nM, respectively. Within-day variations of repeated analyses of the same sample were less than 6%.

**Statistics.** Data were analyzed by ANOVA followed by Dunnett’s test with p < 0.05 as the level of significance.

**RESULTS**

**Effect of phosphate analog drugs on excretion of AsV and its metabolites.** Biliary and urinary excretion of AsV and its metabolites in rats injected with fosfomycin or foscarnet and AsV are presented in Figures 2 and 3. The saline-injected control rats receiving AsV (50 μmol/kg, iv) excreted approximately 10% of the arsenic dose into bile within 2 h. Two arsenic metabolites appeared in bile consistently: AsIII and MMAAsIII. No new arsenic metabolite was detected in the bile of rats injected with AsV plus fosfomycin or foscarnet (500 μmol/kg, iv). In the first hour after administration, foscarnet significantly decreased the biliary excretion rates of AsIII (Fig. 2, top left) and MMAAsIII (Fig. 2, bottom left) by 68–73% and 32–56%, respectively. The cumulative 2 h biliary excretion of AsIII, MMAAsIII and total arsenic was also diminished signif-
significantly (by 66, 54, and 56%, respectively) in fosfarnet-treated rats as compared to the saline-injected control animals (Fig. 2, right). Fosfomycin failed to influence the biliary excretion of AsV metabolites.

As much as 40% of the dose of the injected arsenic was excreted into urine by the vehicle-injected control rats receiving AsV. Three arsenic compounds appeared in urine, specifically the unchanged AsV, AsIII, and from the third period in some rats, DMAsV (less than 5 nmol/kg/min, not shown). No new arsenic metabolite was found in the urine of rats injected with AsV and fosfomycin or foscarinet. Foscarinet increased the urinary excretion of AsV as much as 2.5 fold in the first 20 min after administration, but not later (Fig. 3, top left). In contrast, foscarinet persistently diminished the urinary AsIII excretion by 41–75% (Fig. 3, bottom left and right). This drug did not significantly influence the urinary excretion of either DMAsV (not shown) or total arsenic (Fig. 3, right). Fosfomycin also failed to influence the urinary excretion of AsV and its metabolites.

Effect of phosphate analog drugs on excretion of AsIII and its metabolites. Because foscarinet markedly diminished the excretion of AsIII and MMAsIII in AsV-injected rats, the effects of the phosphate analog drugs on arsenic disposition were also investigated in rats receiving AsIII, in order to determine whether foscarinet influenced the fate of AsV-derived AsIII directly or indirectly. Figures 4 and 5 depict the biliary and urinary excretion of AsIII (50 μmol/kg, iv) and its metabolites in rats given fosfomycin and foscarinet. Within 2 h, control animals excreted 28% of the injected AsIII into bile as AsIII and MMAsIII. Other arsenic metabolites did not appear in the bile of rats injected with AsIII plus fosfomycin or foscarinet (500 μmol/kg, iv). The phosphate analog drugs did not significantly influence the biliary excretion of AsIII and MMAsIII in rats receiving AsIII (Fig. 4).

The saline-injected control rats excreted predominantly un-
changed AsIII in urine (Fig. 5) together with little amounts of DMAsV after the third period (less than 5 nmol/kg/min, 0.120 ± 0.046 μmol/kg within 2 h, not shown). AsV did not appear in the urine of any of the control rats. Neither fosfomycin, nor foscarnet influenced the urinary excretion of AsIII and total arsenic. Interestingly, all foscarnet-treated animals excreted AsV into the urine (Fig. 5, bottom left). AsV also appeared in the urine of some, but not all, fosfomycin-injected rats.

Effect of entacapone, nitrous oxide, and PAD on excretion of AsIII and its metabolites. Figures 6 and 7 present the effect of COMT inhibitor entacapone, the methylcobalamin inactivator, nitrous oxide, and the general S-adenosylmethionine-dependent methylation inhibitor, PAD, on the excretion of AsIII and its metabolites in AsIII-injected rats. For clarity, these figures display the values of only one control group because the values of the air-exposed controls for the nitrous oxide-exposed rats were similar to the values of the saline-injected controls. The control and treated rats receiving AsIII excreted arsenic into bile as unchanged AsIII together with MMAsIII (Fig. 6). In the first 20 min, entacapone, nitrous oxide, and PAD decreased the AsIII excretion by 34, 28, and 38%, respectively. However, later the biliary excretion rate of AsIII decreased precipitously in each group, and the methylation inhibitors did not decrease the excretion of AsIII when compared to controls. Moreover, the biliary AsIII excretion was increased 2.5–3.5 fold by PAD from the 80th minute (Fig. 6, top left). Each methylation inhibitor reduced the 2 h cumulative AsIII excretion, entacapone, by 29%, nitrous oxide by 28%, and PAD by 34% (Fig. 6, right).

The bottom left panel of Figure 6 depicts the time courses of the biliary excretion of MMAsIII in rats pretreated with entacapone, PAD, or nitrous oxide and injected with AsIII. PAD reduced the excretion rate of MMAsIII by 97% compared to control animals. Entacapone, however, only delayed the excretion of MMAsIII (Fig. 6, bottom left) without influencing the cumulative MMAsIII excretion significantly (Fig. 6, right). Nitrous oxide only slightly, but not significantly, decreased the MMAsIII excretion (Fig. 6, right). The cumulative biliary excretion of total arsenic decreased by 22, 25, and 53%, respectively, in rats treated with entacapone, nitrous oxide, or PAD when compared to the control animals.

AsIII-injected rats excreted AsIII into the urine unchanged, and from the fourth period, minute amounts of DMAsV (Fig. 7, left). None of the methylation inhibitors influenced the urinary excretion of AsIII and total arsenic (Fig. 7, right). PAD completely abolished the renal excretion of DMAsV (Fig. 7, bottom left, right), however, entacapone and nitrous oxide did not affect it.

DISCUSSION

AsV itself is relatively innocuous compared to AsIII and MMAsIII (Petrick et al., 2000; Styblo et al., 2000). The latter arsenicals, however, can be formed from AsV in the liver (and possibly other tissues as well) by incompletely characterized reductases and arsenite methyltransferase (Radabaugh and Aposhian, 2000; Wildfang et al., 1998).

Strong circumstantial evidence indicates that cellular uptake
of AsV is mediated by phosphate transporters such as the universally expressed Na\(^+\) phosphate cotransporter and the phosphate-anion exchanger in the erythrocytes (Wehrle and Pedersen, 1989). For example, AsV competitively inhibits the transport of phosphate across the luminal membrane of proximal renal tubules (Hoffmann et al., 1976), the sinusoidal membrane of hepatocytes (Ghishan and Dykis, 1993), and the plasma membrane of erythrocytes (Kenney and Kaplan, 1988). The view that AsV is also transported by the phosphate carrier(s) is supported by the observation that AsV induces an inward current in Xenopus oocytes expressing the human Na\(^+\)-phosphate cotransporter (Busch et al., 1995), and that phosphate inhibits the uptake and metabolism of AsV by kidney slices (Lerman and Clarkson, 1983), cultured human epidermal cells (Huang and Lee, 1996), and hepatocytes (Petrick et al., 2000). Furthermore, whereas the cytotoxic potential of AsV approaches that of AsIII in a phosphate-free incubation medium, the toxicity of AsV, but not that of AsIII, is markedly ameliorated in phosphate-supplemented medium (Huang and Lee, 1996; Petrick et al., 2000). Thus, these in vitro studies lend strong support to the hypothesis tested here that phosphate transporter inhibitors would counter toxification of AsV in vivo.

The inhibitory effect of foscarnet on the Na\(^+\)-dependent phosphate transport has been well documented (Ghishan and Dykis, 1993; Szczepanska-Konkel et al., 1986; Timmer and Gunn, 1998; Yusufi et al., 1986), but the influence of this drug on the disposition of AsV has not been studied. Foscarnet is absorbed from the gut via transport by the Na\(^+\)-phosphate cotransporter (Swaan and Tukker, 1995; Tsuji and Tamai, 1989), eliminated from the body by renal glomerular filtration in an unchanged form (VanScoy et al., 1988), and is acutely well tolerated by experimental animals (Swaan and Tukker, 1995; Szczepanska-Konkel et al., 1986).

The present work demonstrates that the intravenously injected foscarnet significantly influences the fate of simultaneously administered AsV in two ways: it enhances the excretion of AsV into urine and decreases the excretion of the major AsV metabolites, AsIII, and MMAAsIII. The increased urinary clearance of AsV is analogous to the phosphaturia elicited in rats by administration of foscarnet (Szczepanska-Konkel et al., 1986; VanScoy et al., 1988), which has been attributed to direct inhibition by this drug of the Na\(^+\)-phosphate symporter in the brush-border membrane of proximal tubular cells. Thus, inhibition of this transporter by foscarnet most certainly accounts also for the diminished tubular reabsorption, and in turn, increased urinary excretion of the injected AsV. In contrast, foscarnet does not exert a direct effect on the disposition of AsIII because it did not influence the excretion of AsIII and MMAAsIII in rats receiving AsIII (Figs. 4 and 5). Therefore, foscarnet must have decreased the excretion of AsIII and MMAAsIII in AsV-exposed rats (Figs. 2 and 3) indirectly, i.e., by interfering with the formation of these toxic trivalent metabolites from AsV. Na\(^+\)-phosphate cotransporters located in both the sinusoidal membrane of the hepatocytes (Ghishan and Dykis, 1993) and the luminal membrane of proximal tubular cells (Szczepanska-Konkel et al., 1986) are inhibited by foscarnet in vitro. Therefore, it is most likely that foscarnet inhibits the hepatic uptake of AsV through the phosphate carrier, thus decreasing the amounts of AsIII and MMAAsIII produced from AsV in the liver and delivered into bile. It probably also inhibit uptake of AsV into the renal tubular cells, resulting in less AsV being converted into AsIII in these cells and less AsIII being secreted into the urine (Ginsburg, 1965; Tsukamoto et al., 1983). In addition, the profound loss of AsV into urine early after injection of foscarnet may well contribute to diminished formation and excretion of the AsV metabolites later.

Surprisingly, all foscarnet-treated, AsIII-injected rats excreted AsV into the urine, despite the fact that AsV was not detected in the urine of AsIII-exposed animals not receiving foscarnet (Fig. 5, bottom left). This unexpected finding suggests that some AsIII is converted in the rats into AsV, which however, is completely reabsorbed from the renal tubules, escaping excretion. Indeed, AsV is readily detectable in the blood of rats injected with AsIII (our unpublished observation). Foscarnet apparently inhibits reabsorption of the AsIII-derived AsV; therefore AsV appears in urine.

In review, the antiviral drug foscarnet influences the disposition of inorganic arsenic in rats. The observed alterations can be attributed to the drug’s inhibitory effect on the phosphate transporter-mediated renal reabsorption and tissue uptake of AsV. This effect of foscarnet could also be manifested in humans, especially because foscarnet is 7 times more potent an inhibitor of the human than the rat Na\(^+\)-phosphate cotransportor at the brush border membrane of renal tubules (Yusufi et al., 1986).

Fosfomycin is another phosphate analog drug with phosphate transporter-mediated intestinal absorption (Ishizawa et al., 1990, 1991, 1992). After intravenous dosing, this antibiotic is almost completely eliminated from the body by urinary excretion in unchanged form (Bergan, 1990). Because phosphate, arsenate, or foscarnet inhibited Na\(^+\)-dependent uptake of fosfomycin into small intestinal brush border-membrane vesicles prepared from rats (Ishizawa et al., 1990), we hypothesized that fosfomycin may inhibit the phosphate transporter-mediated AsV transport and thus it may influence the disposition of AsV in rats, similar to the influence of foscarnet. The negative findings presented in Figures 2 and 3 fail to confirm this hypothesis. Nevertheless, fosfomycin might be a very weak inhibitor of AsV transport in the kidney, because some, but not all, rats receiving fosfomycin and AsIII excreted appreciable amounts of AsV into urine (Fig. 5, bottom left). Indeed, studies on intestinal brush-border vesicles indicate that fosfomycin has much lower affinity for the phosphate transporter than foscarnet, as the $K_m$ of fosfomycin is 15 mM (Ishizawa et al., 1990), whereas the $K_m$ of foscarnet as an inhibitor of phosphate uptake is 1.3 mM (Loghman-Adham et al., 2000).
Experiments with the methylation inhibitor candidate drugs have yielded negative results as neither the COMT inhibitor entacapone nor the methylcobalamin inactivator, nitrous oxide, significantly decreased the biliary excretion of MMAsIII. In contrast, PAD virtually abolished the biliary excretion of MMAsIII. PAD inhibits S-adenosylhomocysteine hydrolase, resulting in accumulation of S-adenosylhomocysteine, which is a potent inhibitor of S-adenosylmethionine-dependent methyltransferases (Hoffman, 1980). Because entacapone, given at a dose of 10 mg/kg, po, lowered hepatic COMT activity by 70% at 1 h after administration (Nissinen et al., 1992), the activity of COMT in the livers of our rats receiving 30 mg/kg, ip, must have decreased significantly during the course of this study. Similarly, methylcobalamin was expected to have been inactivated in the livers of our rats inhaling nitrous oxide for 2 h, because hepatic methionine synthetase activity approached zero in rats exposed to 50% nitrous oxide for 2 h. Even at 24 h after returning the rats to ambient air, activity was only approximately 25% of normal (Deacon et al., 1980). Thus, this work supports the significance of S-adenosylmethionine-dependent methyltransferase(s), but not of COMT or methylcobalamin, in the formation of MMAsIII and DMAVs from AsIII in rats. It is most likely that inhibition of arsenic methylation by pyrogallol, based on the role of COMT in arsenic methylation (De Kimpe et al., 1999), did not result from pyrogallol-induced COMT inhibition, but rather from methylation of pyrogallol by COMT and the consequential consumptive depletion of S-adenosylmethionine and formation of S-adenosylhomocysteine (Baldessarini, 1966; Waldmeier and Feldtrauer, 1987).

For unknown reasons, the biliary excretion of AsIII was approximately 30% lower in rats pretreated with entacapone, nitrous oxide, or PAD when compared to the controls. Only because of the slight inhibitory effect of entacapone on the hepatobiliary transport of AsIII can we offer ready explanation. Elimination of this drug in rats involves a glucuronidation, with the glucuronides being excreted partly into bile (Wikberg and Vuorela, 1994). Glucuronides are typically transported into bile by the bile canalicular MRP2, which also transports the labile glutathione conjugates of AsIII and MMAsIII (Kala et al., 2000). Thus, competition for the same transporter between entacapone-glucuronide and arsenic-glutathione conjugates is expected to result in mutual inhibition of the hepatobiliary transport of these compounds.

In summary, this study has demonstrated that phosphate transporter inhibitors, such as the antiviral drug foscarinet, can increase the elimination of AsV by urinary excretion and decrease its conversion into the harmful AsIII and MMAsIII; therefore, such drugs can be considered as potential antidotes of AsV. In addition, this work supports the view that MMAsIII is formed by PAD-inhibitable, S-adenosylmethionine-dependent methyltransferase(s) but not via methylation involving COMT or methylcobalamin.

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