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GLUTATHIONE-DEPENDENT BILIARY EXCRETION OF ARSENIC

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Abstract—This study aimed to clarify whether glutathione (GSH) plays a role in the hepatobiliary transport of arsenic. For this purpose, the biliary excretion of ^{74}As was measured in urethane-anaesthetized rats for 2 hr after the administration of labelled sodium arsenite (50 μmol/kg, i.v.) or arsenate (150 μmol/kg, i.v.) and under the influence of sulfobromophthalein (BSP), indocyanine green (ICG) or diethyl maleate (DEM) which are known to diminish hepatobiliary transport of GSH. Although the biliary excretion of arsenic was different after arsenite and arsenate administration in terms of quantity (19% vs 6% of dose in 2 hr, respectively) and time course, arsenic excretion responded similarly to BSP (50 μmol/kg, i.v.), ICG (25 μmol/kg, i.v.) or DEM (4 mmol/kg, i.p.) irrespective of the injected arsenical. Initially the biliary excretion of arsenic in rats injected with either arsenite or arsenate was significantly reduced, but then moderately increased by BSP and, more lastingly, depressed by ICG, whereas it was virtually abolished by DEM. The responses of arsenic excretion to BSP, ICG and DEM were related, both proportionally and temporally, to the effects exerted by these agents on the hepatobiliary transport of GSH, as assessed by the biliary excretion of non-protein thiols. These findings indicate that the biliary excretion of arsenic after the administration of either arsenite or arsenate is dependent on the hepatobiliary transport of GSH. Transport of arsenic as a GSH complex may account for the GSH dependence of biliary arsenic excretion.

Hepatic glutathione (GSH) is transported into both plasma and bile [1]. Hepatobiliary transport of GSH is thought to play a role in the biliary excretion of the physiologically important copper and zinc [2] as well as the toxic methylmercuric [3], mercuric [4], cadmium [5] and lead [6] ions. It has been shown that excretion of these metals in bile is increased when biliary excretion of endogenous GSH is enhanced [7]. In contrast, biliary metal excretion is diminished when hepatobiliary transport of GSH is decreased by agents that deplete hepatic GSH (e.g. diethyl maleate, DEM) or inhibit transport of GSH from liver to bile (e.g. sulfobromophthalein, BSP; indocyanine green, ICG) [2, 7, 8]. Complexation of metals by hepatic GSH with subsequent hepatobiliary transport of metal–GSH complexes is assumed to be the underlying mechanism for the GSH-dependent biliary excretion of these metals [8, 9].

Arsenic also undergoes significant biliary excretion. Rats excrete 24–37% of injected arsenite in bile within 2 hr [9, 10]. The form of arsenic in bile is unknown, though it has been hypothesized that it is a GSH complex [11]. Whether or not GSH plays a role in the biliary excretion of arsenic is also unclear. Therefore, the present study was designed to address this question by investigating the effect of the GSH depletor DEM, as well as BSP and ICG on the biliary excretion of non-protein thiols (NPSH) and arsenic. Biliary NPSH is composed of GSH and thiol-containing hydrolysis products of GSH (i.e. cysteine and cysteinylglycine). The latter are produced by the γ-glutamyl transferase-initiated intrabiliary hydrolysis of GSH after translocation of GSH from hepatocytes into bile canaliculi [12–14]. Consequently, biliary excretion of NPSH approximates hepatobiliary transport of GSH more closely than the amount of unhydrolysed GSH appearing in bile. The role of GSH in the biliary excretion of arsenic was investigated following administration of arsenic as both sodium arsenite, the trivalent form, and sodium arsenate, the pentavalent form.

MATERIALS AND METHODS

Experiments were performed on urethane-anesthetized (1.2 g/kg, i.p.), bile duct-cannulated, 12–16-week-old female Wistar rats (LATI, Gödöllő, Hungary). The body temperature of the anesthetized animals was maintained at 37° by means of heating lamps. Separate groups of rats were injected intraperitoneally with DEM (Koch-Light Lab., Colnbrook, U.K.) dissolved in sunflower oil or intravenously with BSP (Fluka, Buchs, Switzerland) or ICG (Hynson, Wescott and Dunning, Baltimore, MD, U.S.A.), both dissolved in water at dosages and times indicated in the figure legends.

To examine the effects of these chemicals on the biliary excretion of NPSH (Fig. 1) bile was collected in 20-min consecutive intervals into 0.4 mL ice-cold 5% metaphosphoric acid (Alfa Products, Danvers, MA, U.S.A.) in order to prevent oxidation of GSH [15, 16].

To examine the effects of DEM, BSP or ICG on the biliary excretion of arsenic ^{74}As-arsenite (50 μmol, 0.2 MBq/kg) or ^{74}As-arsenate (150 μmol, 0.2 MBq/kg) was injected (i.v.) at the start of bile collection. For preparation of labelled arsenate solution, sodium arsenate (Reanal, Budapest, Hungary) was mixed with ^{74}As-arsenic acid (1.3 MBq/

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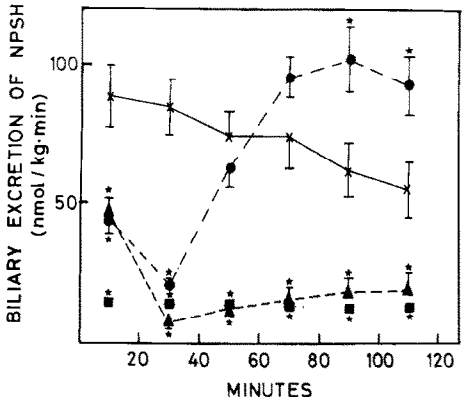

Fig. 1. Effect of sulfobromophthalein (BSP), indocyanine green (ICG) and diethyl maleate (DEM) on the biliary excretion of endogenous non-protein thiols (NPSH). BSP (●; 50 μmol/kg, i.v.), ICG (▲; 25 μmol/kg, i.v.) and DEM (■; 4 mmol/kg, i.p.) were given to separate groups of rats 1, 1 and 45 min prior to starting bile collection at time zero, respectively. Control animals (×) were given saline (3 mL/kg, i.v.) 1 min before starting the bile collection. Symbols represent means ± SE of 5–7 rats. * Indicates values significantly different ($P < 0.05$) from control.

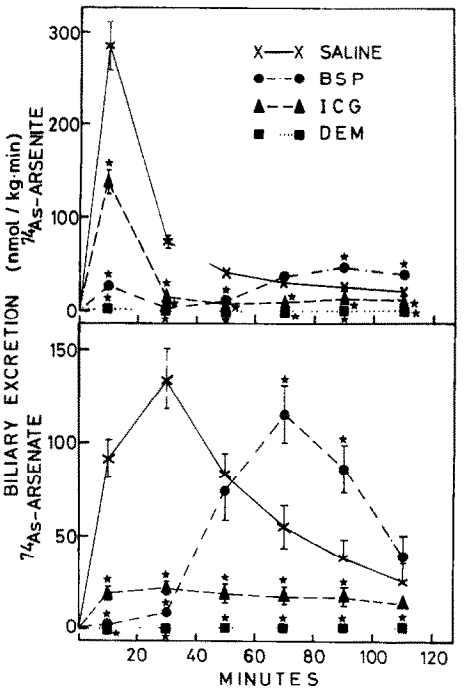

Fig. 2. Effect of sulfobromophthalein (BSP), indocyanine green (ICG) and diethyl maleate (DEM) on the biliary excretion of ^{74}As administered as ^{74}As-arsenite (upper panel) or ^{74}As-arsenate (lower panel). BSP (50 μmol/kg, i.v.), ICG (25 μmol/kg, i.v.) and DEM (4 mmol/kg, i.p.) were injected to separate groups of rats 1, 1 and 45 min prior to injection of ^{74}As-arsenite (50 μmol/kg, i.v.) or ^{74}As-arsenate (150 μmol/kg, i.v.). Control animals were given saline (3 mL/kg i.v.) 1 min prior to one of the arsenicals. Immediately after injection of the arsenicals (time zero) bile was collected after 20-min intervals for 2 hr. Symbols represent means ± SE of 5–7 rats. * Indicates values significantly different from control ($P < 0.05$).

μg As; Amersham International, Amersham, U.K.) that had been kept under UV light for 4 hr in order to ensure that all the arsenic was in the pentavelent form. For preparation of labelled arsenite, sodium arsenite (Merck, Darmstadt, F.R.G.) was mixed with ^{74}As-arsenous acid that had been prepared from ^{74}As-arsenic acid by reduction with bisulfite and thiosulfate according to the method of Reay and Asher [17].

The volumes of the bile samples were measured gravimetrically assuming unity for specific gravity. NPSH concentration in bile was determined spectrophotometrically with Ellman's reagent [18]. The radioactivity of the bile samples obtained from rats injected with ^{74}As-labelled arsenicals was measured by a well-type gamma-scintillation counter. Standard solutions containing a known amount of labelled arsenite or arsenate were also counted to calculate the dosimetry.

Data were analysed by analysis of variance followed by Duncan's test with $P < 0.05$ as the level of significance.

RESULTS AND DISCUSSION

Biliary excretion of endogenous NPSH

The cholephilic organic acids BSP and ICG that do not cause significant depletion of hepatic GSH but inhibit its hepatobiliary transport [3], both decreased NPSH excretion in bile markedly but with different time-courses. BSP exerted a biphasic effect on biliary thiol output (Fig. 1). After a significant reduction (up to 20% of the control value) during the first 40 min of bile collection, an enhancement in NPSH excretion was observed in the second hour of the experiment resulting in a 40% higher NPSH excretion rate in BSP-injected rats than in saline-injected animals. Early recession of the BSP-induced inhibition of NPSH excretion may be due to rapid elimination of BSP, the inhibitory parent compound, both by conjugation with GSH and by biliary excretion. The mechanism of the later increase in NPSH excretion after BSP administration is unknown, however, it probably is a rebound phenomenon evoked by the initial inhibition of hepatobiliary GSH transport.

ICG inhibited thiol excretion by only 50% in the initial 20 min after its injection. Later its inhibitory effect became more pronounced resulting in NPSH excretion rates approximately 10% of the control value 20–120 min after ICG injection. This potent and prolonged ICG-induced inhibition of biliary NPSH excretion may be related to the liver's high capacity to accumulate, but low capacity to eliminate (i.e. by excretion into bile) this cholephilic organic acid [19].

DEM, a depletor of hepatic GSH, markedly and steadily decreased biliary excretion of NPSH in accordance with earlier observations [7, 20].

Biliary excretion of arsenic

Excretion of arsenic in bile in control rats was more efficient and rapid after injection of arsenite

(Fig. 2, upper panel) than of arsenate (Fig. 2, lower panel). This is reflected by the finding that both the cumulative 2-hr excretion and the maximal excretion rate of ^{74}As was larger following administration of arsenite (19% of dose, 290 μmol/kg · min) than arsenate (6% of dose, 130 μmol/kg · min) despite the fact that the dosage of arsenate was three times greater than that of arsenite (150 vs 50 μmol/kg, i.v.). In addition, the apparently instantaneous and then rapidly receding biliary excretion of arsenic after arsenite injection contrasts with the gradually increasing and slowly declining excretion of arsenic following administration of arsenate. Differences in chemical properties [21, 22], biotransformation [23] and hepatic uptake (which is rapid for arsenite but slow for arsenate) [23, 24] between arsenite and arsenate may contribute to the observed differences in biliary arsenic excretion following administration of these arsenicals.

Despite the variations described above, biliary excretion of ^{74}As responded similarly to inhibitors of hepatobiliary GSH transport and hepatic GSH depletion, irrespective of the injected arsenical. Soon after both arsenic and arsenate administration, BSP, ICG and DEM all dramatically diminished the excretion of arsenic (Fig. 2). This inhibitory effect of ICG and DEM lasted till the end of the second hour. In contrast, BSP no longer inhibited arsenic excretion after either arsenite or arsenate administration after 60 min; moreover, arsenic excretion rates during this period exceeded those in control rats (Fig. 2). The similar responsiveness to these agents in the biliary excretion of arsenic following arsenite or arsenate administration may suggest that the arsenic species that is actually transported from the liver cells to the bile canaliculi is the same, irrespective of the arsenic species (i.e. arsenite or arsenate) administered. It is possible that the excreted arsenic species is arsenite as it can be rapidly formed *in vivo* from arsenate [23, 25, 26] and is unlike arsenate, thiol-reactive [21, 27].

Both arsenite and arsenate increased bile production by approximately 50% (data not shown) in the initial phase of their excretion. This choleretic effect is most likely of osmotic origin and is due to excretion of arsenic and/or its GSH complex as inhibition of biliary excretion of arsenic by BSP, ICG or DEM completely abolished the effect (data not shown).

Following administration of BSP, ICG or DEM the relative changes, and their time courses, in NPSH and arsenic excretion are largely similar (Fig. 3). This similarity was most apparent with BSP. In BSP-injected rats, biliary excretion rates of both NPSH and arsenic (administered as either arsenite or arsenate) markedly decreased initially, however, later the relative excretion rates of NPSH and arsenic rose in parallel and attained 150–200% of control rates (Fig. 3, upper panel). Similar parallel changes can be observed with ICG (Fig. 3, middle panel). However, the GSH-depletor DEM decreased consistently the biliary excretion of arsenic (both after arsenite and arsenate administration) more so than that of NPSH (Fig. 3, bottom panel). The reason for this discrepancy is uncertain. However, it is speculated that a decreased biliary GSH

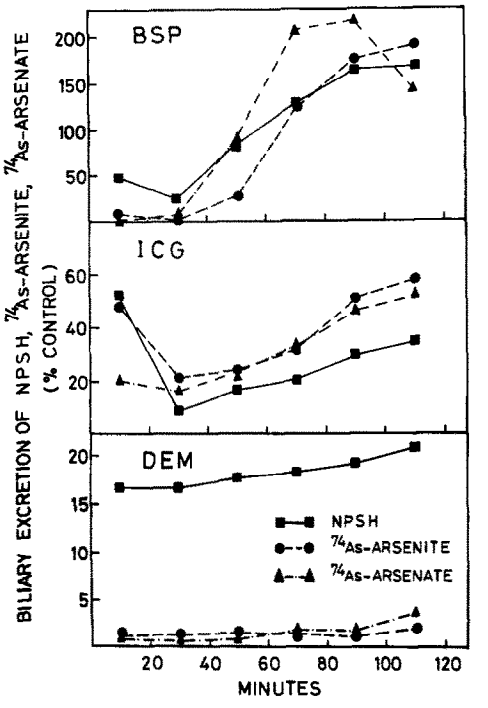

Fig. 3. Relative changes in the biliary excretion of endogenous non-protein thiols (NPSH) and ^{74}As after administration of ^{74}As-arsenite or ^{74}As-arsenate in response to administration of sulfobromophthalein (BSP; upper panel), indocyanine green (ICG; middle panel) and diethyl maleate (DEM; bottom panel). Experimental conditions are described in the legends of Figs 1 and 2. Symbols represent biliary excretion rates of NPSH or ^{74}As in BSP-, ICG, and DEM-treated rats expressed as percentage of biliary excretion rates of NPSH or ^{74}As, respectively, in control rats.

excretion evoked by a GSH-depletor reduces hepatobiliary transport of a metal as a GSH-complex not only because it results in decreased transport rate of the complexing GSH molecule, but also because it increases the chance for the metal ion to associate with other intracellular ligands due to limited availability of GSH.

In summary, this study indicates that agents that decreased hepatobiliary transport of GSH also diminished, in a largely proportional and temporally associated manner, the biliary excretion of arsenic administered as either arsenite or arsenate. This observation supports the GSH-dependence of biliary arsenic excretion. Since the GSH-dependence of other metallic compounds (e.g. methylmercury) is apparently based upon formation and hepatobiliary transport of their GSH complexes, it is likely that arsenic is also transported in bile as a GSH complex. However, direct evidence for formation and biliary excretion of such a complex remains to be presented.

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