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SHORT COMMUNICATIONS

Inhibition by bromsulphthalein of the biliary excretion of its glutathione conjugate

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About 75 per cent of intravenously administered bromsulphthalein (BSP) appears in rat bile as a conjugated form [1, 2]. The biliary excretory system seems to have a greater affinity for the glutathione conjugate of BSP (BSP-GSH) than for BSP itself [3–5]. In addition, the findings of Whelan and Combes [6] suggest that the two compounds share a common transport mechanism and mutually depress the biliary excretion of each other.

The present experiments were designed to investigate the importance of this competition in the overall hepatic transport of BSP. Such experiments can be performed when the conjugation of BSP with GSH is prevented. For this purpose rats were pretreated with diethyl maleate (DEM), which depletes the liver of GSH almost completely [7], without affecting the biliary anion transport system [4].

MATERIALS AND METHODS

Materials. Sodium salt of BSP was purchased from Merck A. G., Darmstadt, and [^{35}S]BSP (10–16 mCi/m-mole) from Radiochemical Center, Amersham-Searle. Diethyl maleate was supplied by Eastman Organic Chemicals, Rochester, and glutathione by Reanal Company, Budapest.

Preparation of BSP-GSH. [^{35}S]BSP (10–16 mCi/m-mole) was diluted with unlabelled BSP 20-fold and used for the synthesis of radiolabelled BSP-GSH. BSP-GSH was synthetized according to the method of Whelan *et al.* [3]. The reaction mixture was applied to a Sephadex G-10 column, and the BSP-GSH was separated from the BSP and BSP-diglutathione conjugate as described previously [4]. The specific activity of the synthetized BSP-GSH varied between 500–800 μCi/m-mole. This preparation was further diluted with unlabelled BSP-GSH. BSP-GSH is stable at 5° for at least 1 month.

Determination of BSP and BSP-GSH tissue concentration. Liver samples were digested with Protosol and the bile samples were added without digestion to Kinard's [8] liquid scintillation solution. Radioactivity was measured in a liquid scintillation spectrometer (Beckman Spectrometer, Model LS-230) using an external standard.

Animal experiments. Male CFY rats (a strain of Sprague-Dawley origin obtained from LATI, Gödöllő, Hungary) of 170–220 g body weight were anaesthetized with urethane (1.2 g/kg i.p.). The common bile duct was cannulated with PE-10 tubing. Half an hour following the administration of DEM (0.7 ml/kg i.p.), BSP and/or BSP-GSH was injected into the femoral vein and the bile collected for 45 min. [^{35}S]BSP (20 μCi/kg) and [^{35}S]BSP-GSH (20 μCi/kg) were diluted with unlabelled BSP and BSP-GSH, respectively. After administration of BSP-GSH to control or DEM-treated rats, 93 per cent of the total BSP-GSH appeared unchanged in the bile, and the major metabolite of BSP-GSH proved to be BSP-cysteinylglycine [2, 4]. This metabolite of BSP-GSH was not separated from BSP-GSH. Thus, the data in the tables on liver concentration and biliary excretion rate of BSP-GSH indicate the sum of BSP-GSH and its metabolite. When the BSP and the BSP-GSH were administered simultaneously, only one of them was radiolabelled, the one whose biliary excretion was to be investigated. Since the molecular weights of BSP (794) and BSP-GSH (1020) are different, their doses and tissue concentrations were expressed in nmoles or μmoles. The body temperature of the rats was maintained at 36.5° by means of a heat pad.

Statistical analysis. Student's *t*-test was used for statistical analysis.

RESULTS AND DISCUSSION

In order to exclude the variations in hepatic dye concentration, due to the different excretion rates of BSP and BSP-GSH, hepatic uptake studies were made on common bile duct-ligated rats. In accordance with earlier findings from this [4] and other laboratories [3, 6, 9], the concentration of BSP in the liver was much higher than that of BSP-GSH (Tables 1 and 2). In bile duct-ligated rats BSP-GSH proved to be a very weak inhibitor of the hepatic uptake of BSP (Table 1). Similarly, BSP depressed the hepatic concentration of BSP-GSH significantly only at a dose of 120 μmoles/kg (Table 2). However, 60 μmoles/kg of BSP did not affect the hepatic uptake of BSP-GSH, although such a dose of BSP very markedly depressed the biliary excretion of BSP-GSH (Table 4). Therefore the competition for hepatic uptake described above may not

Table 1. Effect of simultaneously administered BSP-GSH on the hepatic uptake (nmoles/g) of BSP in rats pretreated with diethyl maleate*

Dose of BSP (μmoles/kg i.v.)	Control†	With BSP-GSH (μmoles/kg i.v.)† 60	120
30	582 ± 49	556 ± 48	412 ± 29‡
60	1130 ± 160	1059 ± 141	992 ± 41
120	2082 ± 153	1985 ± 181	1782 ± 130

* The determinations were performed in liver specimens 15 min after the i.v. administration of BSP control or BSP and BSP-GSH. The common bile duct was ligated prior to the injection of the dyes.
† Mean value ± S.E. of 5 rats.
‡ Significantly different ($P < 0.05$) from the control value.

Table 2. Effect of simultaneously administered BSP on the hepatic uptake (nmoles/g) of BSP-GSH in rats pretreated with diethyl maleate*

Dose of BSP-GSH (μmoles/kg i.v.)	Control†	With BSP (μmoles/kg i.v.)† 60	120
30	333 ± 28	300 ± 24	173 ± 14‡
60	489 ± 51	549 ± 27	231 ± 14‡
120	643 ± 42	585 ± 37	421 ± 64‡

* The determinations were performed in liver specimens 15 min after the i.v. administration of BSP-GSH control or BSP-GSH and BSP. The common bile duct was ligated prior to the injection of the dyes.
† Mean value ± S.E. of 5–10 rats.
‡ Significantly different ($P < 0.01$) from the control value.

Table 3. Effect of simultaneously administered BSP-GSH on the biliary excretion rate (nmoles/min/kg) of BSP in rats pretreated with diethyl maleate*

Dose of BSP (μmoles/kg i.v.)	Control†	With BSP-GSH (μmoles/kg i.v.)† 60	120
30	170 ± 12	192 ± 19	168 ± 5
60	289 ± 23	261 ± 21	288 ± 17
120	333 ± 32	391 ± 28	312 ± 18

* The determinations were performed in bile samples collected 0–45 min after the administration of BSP control or BSP and BSP-GSH.
† Mean value ± S.E. of 5 rats.

Table 4. Effect of simultaneously administered BSP on the biliary excretion rate (nmoles/min/kg) of BSP-GSH in rats pretreated with diethyl maleate*

Dose of BSP-GSH (μmoles/kg i.v.)	Control†	With BSP (μmoles/kg i.v.)† 7.5	15	30	60
30	677 ± 24	650 ± 21	587 ± 41	197 ± 14‡	113 ± 18‡
60	1138 ± 101	1031 ± 85	763 ± 64‡	316 ± 56‡	141 ± 30‡
120	1872 ± 87	1688 ± 94	1265 ± 87‡	437 ± 30‡	244 ± 38‡

* The determinations were performed in bile samples collected 0–45 min after the administration of BSP-GSH control or BSP-GSH and BSP.
† Mean value ± S.E. of 5 rats.
‡ Significantly different ($P < 0.001$) from the control value.

be significant in the overall transport of BSP or BSP-GSH from blood to bile.

BSP-GSH did not significantly depress the biliary excretion of BSP, even after the administration of a dose as high as 120 μmoles/kg (Table 3). However, the excretion rate of BSP-GSH was markedly depressed by a dose as small as 15 μmoles/kg of BSP (Table 4). Moreover, a significant inhibition of BSP-GSH excretion could be observed in the first 15 min of bile collection with the simultaneous administration of 7.5 μmoles/kg of BSP. But the excretion of BSP-GSH in the following 30 min of bile collection masked this inhibition.

After following the injection of BSP in normal rats the total biliary excretion of BSP plus its main metabolite (BSP-GSH) is lower than that of an equimolar dose of BSP-GSH [3]. Based on this observation it was postulated that the rate limiting step in the overall transport of BSP was the rate of conjugation of BSP with GSH [3, 10]. Our results indicate that the inhibitory effect of free BSP on BSP-GSH excretion may also have an important role in the transport rate of total dye excretion.

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