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Metabolism of sulphobromophthalein II. Species differences between rats, guinea-pigs and rabbits

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Abstract

Interesting species differences in the metabolism of sulphobromophthalein sodium have been observed between rats, guinea-pigs and rabbits. The species difference was measured in terms of sulphobromophthalein monoglutathione conjugate (mGSH) positional isomer formation. After an intravenous injection of sulphobromophthalein to rats, 92% of sulphobromophthalein-mGSH excreted into bile was the α-isomer. In contrast, in guinea-pigs the three isomers α, β and δ were excreted in equivalent amounts. In rabbits, the majority of sulphobromophthalein-mGSH was excreted as the β-isomer. The formation ratio of glutathione (GSH) conjugates in-vitro using cytosolic glutathione S-transferases (GSTs) prepared from livers generally accounted for the biliary excretion ratio of α-, β- and δ-monomercaptide isomers in-vivo. GSTs from the livers of rat, guinea-pig, and rabbit were purified and characterized. Although their main GSTs produced different isomers, their 20 amino acid residues showed that they belonged to the same class mu of GSTs. The results suggested differences of the three-dimensional structure of GSTs that formed sulphobromophthalein-mGSH isomers between the three animal species.

Introduction

Sulphobromophthalein sodium has been used in the clinical diagnosis of a chronic cholestatic disease (Vaubourdolle et al 1991). Sulphobromophthalein and its glutathione conjugate are used frequently as useful tools for the hepatobiliary transport mechanisms of organic anions (Takikawa et al 1993; Torres et al 1993; Geng et al 1998). The disposition and metabolism of sulphobromophthalein has been reported in numerous studies (Schwarz et al 1980; Ballatori & Clarkson 1985; Sorrentino et al 1988; Kanai et al 1990; Snel et al 1995). Species differences in sulphobromophthalein metabolism have been reported also (Combes & Stakelum 1961; Whelan & Plaa 1963; Klaassen & Plaa 1967). However, species differences in positional isomers of sulphobromophthalein monoglutathione conjugate (sulphobromophthalein-mGSH) have not been reported.

Previously, we developed an HPLC method for the determination of sulphobromophthalein-mercaptide conjugates based on paired-ion chromatography using triethylamine phosphate (TEA–H_3PO_4) as a pairing agent (Sano et al 1992). Interestingly, three positional isomers (α-, β- and δ-) of sulphobromophthalein-mGSH were identified in-vitro using this method (Sano et al 2001). In this study, we have compared the metabolism of sulphobromophthalein in rats, guinea-pigs and rabbits in detail. Our findings suggested that the species differences in sulphobromophthalein metabolism were due to structural differences in glutathione

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S-transferases (GSTs) between the species. GSTs (EC 2.5.1.18) are a family of multifunctional enzymes involved in the detoxification and excretion of many physiological and xenobiotic compounds (Mannervik et al 1985). They catalyse the conjugation of sulfhydryl group of glutathione to electrophilic substances. Mammalian GSTs can be grouped into at least five classes designated alpha, mu, pi, theta and microsomal one. The basis for substrate discrimination is accounted for by the three-dimensional structure of each GST isoenzyme (Wilce & Parker 1994).

In this study, we have observed species differences in γ-glutamyltranspeptidase (γ-GT) and dipeptidase(s) activities among the three animal models. γ-GT (EC 2.3.2.2) is the enzyme that cleaves the γ-glutamyl bond of glutathione (GSH). This is the initial degradation of GSH. The production of hydrolytic cleavage by γ-GT, the dipeptide cysteinylglycine, is a substrate for dipeptidase(s). Aminopeptidase M (EC 3.4.11.2) and dehydropeptidase I (EC 3.4.13.11) have been thought to be responsible for the hydrolysis of cysteinylglycine S-conjugates. Recently, a novel cytosolic cysteinylglycine S-conjugate dipeptidase was identified in rat and guinea-pig (Jösch et al 1998). The species differences in the distribution of γ-GT and dipeptidase(s) activity between liver and kidney were reported by Hinchman & Ballatori (1990). The results indicated that the two hydrolysis enzymes played an important role in GSH and GSH conjugate metabolism suggesting species-specific inter-organ metabolism of GSH.

Materials and Methods

Chemicals

Sulphobromophthalein was obtained from Aldrich Chemical Co. (Milwaukee, WI). Reduced GSH and γ-glutamylcysteine (Glu-Cys) were purchased from Kozin Chemical Co. (Tokyo, Japan). Cysteinyl glycine (Cys-Gly) was obtained from Bachem Feinchem, AG (Bubendorf, Switzerland). Cysteine (Cys) and N-acetylcysteine (Mer) were from Kanto Chemical Co. (Tokyo, Japan). 3-(Cyclohexylamino) propanesulfonic acid was from Wako Pure Chemical Industries, Ltd (Osaka, Japan). Coomassie Brilliant Blue R-250 was from Gibco BRL (Grand Island, NY). *Staphylococcus aureus* V8 was from Sigma Chemical Co. (St Louis, MO). All other chemicals were of reagent grade without purification.

Standard sulphobromophthalein-mercaptides

Authentic sulphobromophthalein monomercaptides including α-, β- and δ-positional isomers (sulphobromophthalein-mGSH, -dGSH, -mGlu-Cys, -mCys-Gly, -mCys and -mMer) were all synthesized using a modification of the method of Whelan et al (1970) and purified as described by Sano et al (1992, 2001).

Animal experiments

Male Wistar rats (230–250 g, 8 weeks of age, Sankyo Labo Service Co., Ltd, Tokyo, Japan), Hartley guinea-pigs (370–390 g, 6 weeks of age, Sankyo Labo, Japan) and Japanese White rabbits (1.5 kg, 7 weeks of age, Sankyo Labo, Japan) were housed in stainless steel cages in groups of four to five, under a 12-h light–dark cycle in a temperature controlled (20–28°C) room. The animals were allowed free access to standard rat or guinea-pig chow (Sankyo Labo, Japan) and water before the experiments. The rats and guinea-pigs were anaesthetized using 50 mg kg^{-1} pentobarbital sodium (i.p.). The right femoral vein of the rats and rabbits was cannulated with PE-10 tubing (Clay Adams, Parsippany, NJ) to instill saline and to inject the drug. The jugular vein was used for guinea-pigs. An abdominal incision was made and the common bile duct was cannulated with PE-10 or PE-50 tubing (Clay Adams) followed by closure with surgical clips. Throughout the experimental procedures, body temperature was maintained at 38 ± 0.5°C using a heating lamp to prevent hypothermic alterations in bile flow. Sulphobromophthalein sodium (50 mg mL^{-1}, 10 μmol kg^{-1}) was injected into the vein over a 15-s period, and bile was collected for 90 min at 10-min intervals. Bile samples were stored at -80°C until analysis.

Preparation of rat, guinea-pig and rabbit liver cytosol

Livers of the rat, guinea-pig and rabbit were quickly excised following perfusion with saline and homogenized with 3 vols 0.01 M Tris-HCl buffer (pH 8.0) containing 3 mM 2-mercaptoethanol. All steps were carried out at 4°C. The debris, nuclei and mitochondria were sedimented at 12000 *g* for 20 min. The major microsomal fraction was removed by centrifugation at 105000 *g* for 60 min. The resultant clear supernatant represented the cytosol. This liver cytosol was stored frozen at -80°C. Protein concentrations were determined according to the method of Lowry et al (1951) with bovine serum albumin used as the standard.

Preparation of liver canalicular membrane samples

Liver canalicular membranes were prepared according to the method of Inoue et al (1983).

Purification of GSTs from rat, guinea-pig and rabbit and amino acid sequence analysis

GSTs were purified from three animal cytosolic fractions according to the method of Oshino et al (1990). After SDS-polyacrylamide gel electrophoresis (SDS-PAGE), the separated peptides were transferred to Immobilon-P membrane (Millipore Co, Bedford, MA), using the TE77 SemiPhor transfer unit (Hoefer Scientific Instruments Co, San Francisco, CA). The transfer was carried out at the constant current of 0.8 mA cm^{-2} for 45 min with 10 mM 3-(cyclohexylamino) propanesulfonic acid at pH 11 containing 10% methanol. After the transfer, the stained bands with 0.025% Coomassie Brilliant Blue R-250 in 50% methanol were cut from the membrane and subjected to the Applied Biosystems protein sequencer (model 473A).

Proteolytic digestion

Partial proteolytic digestion with *S. aureus* V8 was according to Ishii et al (1983). A sample of approximately 1 μg protein was subjected to SDS-PAGE. The gel was stained with Coomassie blue, and regions corresponding to protein bands were cut out. Each gel slice was placed in a well of another SDS-polyacrylamide slab gel consisting of a stacking gel (3% polyacrylamide) and separating gel (20% polyacrylamide). *S. aureus* V8 protease (100 ng) was added to each well and electrophoresis was carried out. Proteins were partially digested during electrophoresis, and resulting peptides were separated on the gel.

Analytical methods

HPLC assay of sulphobromophthalein and its conjugates in bile

Sulphobromophthalein and its conjugates in bile were analysed according to Sano et al (1992). Two isocratic mobile phases (solvents A and B) were performed using a Capcell Pak C18 column (5 μm, 6×150 mm; Shiseido Co. Ltd, Tokyo, Japan). Solvent A (0.1 M TEA–H_3PO_4 buffer (pH 9.90 at 22°C): acetonitrile, 90:13, v/v) was used for the separation of sulphobromophthalein metabolites. For the analysis of unconjugated sulphobromophthalein, solvent B (0.1 M TEA–H_3PO_4 buffer (pH 9.90 at 22°C): acetonitrile, 73:27, v/v) was used. After the elution of solution A for 30 min, the mobile phase was changed to solution B. Sulphobromophthalein and its metabolites were detected at 580 nm. The flow rate was 1.0 mL min^{-1} and all separations were carried out at room temperature (22–23°C).

Assay of enzyme activity

GST activity toward sulphobromophthalein (sulphobromophthalein-GST) was measured according to the method of Habig et al (1974). The reaction mixtures contained 0.1 μM sulphobromophthalein and 10 μM GSH in a final volume of 1.0 mL, at pH 7.5. The 105000-*g* supernatant fraction of rat liver was the source of the enzyme. Sulphobromophthalein-GST activity was calculated using an extinction coefficient at 332 nm of 6.2 cm^2 μmol^{-1} for sulphobromophthalein-mGSH produced at 25°C min^{-1}. γ-Glutamyltranspeptidase (γ-GT) activity was assayed using γ-glutamyl-*p*-nitroanilide as the substrate and glycylglycine (Gly-Gly) as the acceptor. γ-GT activity was determined by incubation of liver canalicular membranes at 25°C in the presence of triethanolamine-HCl (0.1 M, pH 9.0), γ-glutamyl-*p*-nitroanilide (4.0 mM), Gly-Gly (50 mM) and $MgCl_2$ (10 mM). γ-GT activity was calculated using an extinction coefficient of 9.95 cm^2 μmol^{-1} for *p*-nitroaniline produced at 410 nm and 25°C for 1 min. Dipeptidase activity was assayed using L-leucine-*p*-nitroanilide as a substrate. Dipeptidase activity was calculated using the same extinction coefficient for *p*-nitroaniline produced at 25°C min^{-1} as used for γ-GT activity.

Assay of concentration of hepatic GSH

Bile samples collected into perchloric acid were diluted with an appropriate volume of 5% perchloric acid before analysis. All samples were kept at −80°C before analysis. GSH concentration was determined according to the method of Tietze (1969) and reduced GSH was determined according to the method of Hissin & Hilf (1976).

Statistical analysis

Results are expressed as means±s.e. of separate experiments. The data were compared by the unpaired Student's *t*-test; $P < 0.05$ was considered significant.

Results and Discussion

Biliary excretion of sulphobromophthalein metabolites in rats, guinea-pigs and rabbits

Figure 1 shows typical chromatograms of sulphobromophthalein metabolites excreted into bile 0–20, 20–40

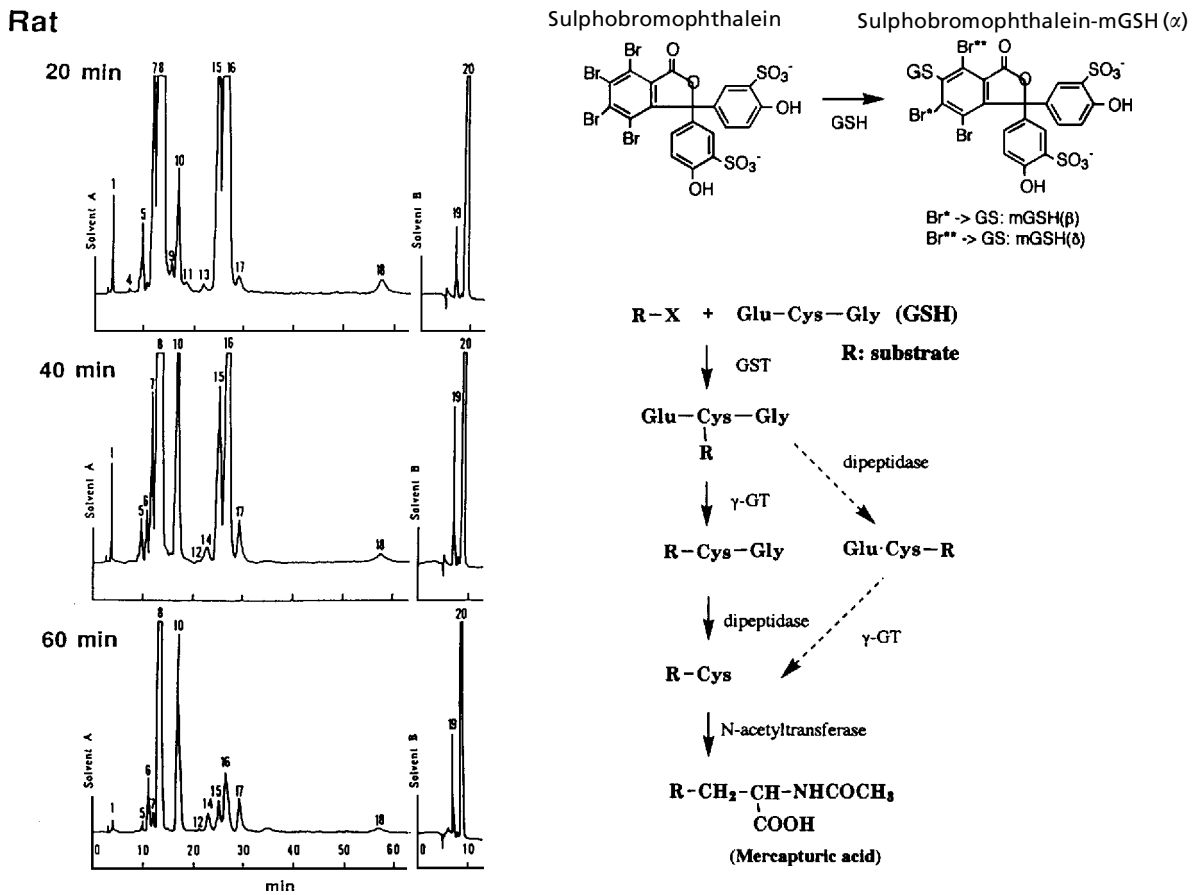

Figure 1 Typical chromatograms of rat bile collected 0–20, 20–40 and 40–60 min after intravenous injection of sulphobromophthalein, 9.5 μmol kg^{-1}. Peaks: 1 = a mixture of sulphobromophthalein-dGSH and sulphobromophthalein-dCys; 2 = X-1; 3 = X-2; 4 = X-3; 5 = sulphobromophthalein-mGluGSH; 6 = sulphobromophthalein-mGluCys(α); 7 = sulphobromophthalein-mGSH(δ); 8 = sulphobromophthalein-mGSH(α); 9 = sulphobromophthalein-mCys(δ); 10 = sulphobromophthalein-mCys(α); 11 = sulphobromophthalein-mMer(δ); 12 = sulphobromophthalein-mGluCys(β); 13 = sulphobromophthalein-mCysGly(δ); 14 = sulphobromophthalein-mMer(α); 15 = sulphobromophthalein-mCysGly(α); 16 = sulphobromophthalein-mGSH(β); 17 = sulphobromophthalein-mCys(β); 18 = sulphobromophthalein-mCysGly(β) and sulphobromophthalein-mMer(β); 19 = X-4; 20 = sulphobromophthalein.

and 40–60 min after the intravenous injection of sulphobromophthalein (9.5 μmol kg^{-1}) to rats. Cumulative amounts (%) of biliary excretion of metabolites during a 90-min period after intravenous injection of sulphobromophthalein to rats, guinea-pigs and rabbits are given in Figure 2.

In the rat, sulphobromophthalein-mGSH(α) (peak 8) was a major metabolite in biliary excretion. The amount of sulphobromophthalein-mCysGly(α) (peak 15) excreted into bile was greatest in the first 20 min after sulphobromophthalein injection and sulphobromophthalein-mCys(α) (peak 10) was excreted at a later stage than mCysGly, mostly in the 20–40-min bile (Figure 1). These results showed that the mCysGly form was an intermediate of mCys synthesis. In guinea-pigs, unchanged sulphobromophthalein was mainly excreted and the major metabolites were sulphobromophthalein-mGSH(α), sulphobromophthalein-mGSH(β) and sulphobromophthalein-mGSH(δ). Various hydrolysed products (sulphobromophthalein-mCysGly, -mCys and -mGluCys) delivered from the corresponding sulphobromophthalein-mGSH conjugates were excreted into bile. This suggested that the activity of γ-GT and dipeptidase in the guinea-pig was markedly higher compared with the rat.

In rabbits, sulphobromophthalein-mGSH(β) was the major metabolite (40.9%) and sulphobromophthalein-mCysGly(β) and sulphobromophthalein-mCys(β) derived from this sulphobromophthalein-mGSH(β) were excreted into the 20-min bile sample (sum total 15.1%) after a single intravenous injection. Rabbits showed a different tendency from rats and guinea-pigs wherein

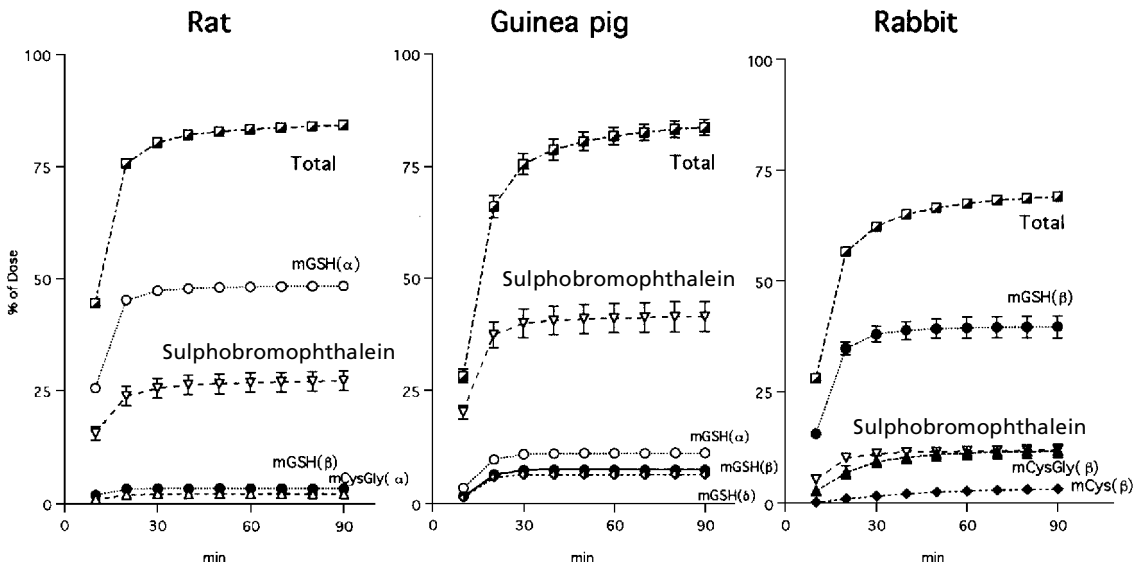

Figure 2 Time course for biliary excretion of sulphobromophthalein-mercaptide conjugates after intravenous injection of sulphobromophthalein (9.5 μmol kg^{-1}) in rats, guinea-pigs and rabbits. Data are expressed as the mean$\pm$s.e. of four animals.

the 'β-form' accounted for 56.0% of the dose (93% of total biliary metabolites).

Comparison of biliary excretion of sulphobromophthalein metabolites

Figure 3 shows the percentage of biliary excretion for each metabolite against the dose after sulphobromophthalein administration. In the rats and rabbits, the grand total of mGSH conjugates ($\alpha+\beta+\delta$ and their hydrolysed products) excreted in bile accounted for 57.0 and 59.5% of the dose, respectively. In contrast, the value of tal mGSH conjugates ($\alpha+\beta+\delta$ of mGSH conjugates except hydrolysed products) for guinea-pigs (25.1%) was approximately half that of rats and rabbits (52.6 and 41.3%, respectively). Guinea-pigs excreted 41.5% of the dose as unchanged sulphobromophthalein, and this value was significantly higher than that for rats and rabbits (27.3 and 11.9%, respectively). This suggested that a high excretion rate of unconjugated sulphobromophthalein produced low excretion rates of sulphobromophthalein metabolites and high hydrolysis activity existed in guinea-pigs. These results indicated that rats and rabbits showed a higher level of GST activity toward sulphobromophthalein than guinea-pigs, whereas guinea-pigs showed high rates of bile flow relative to their body weight and excreted a high amount of unchanged sulphobromophthalein.

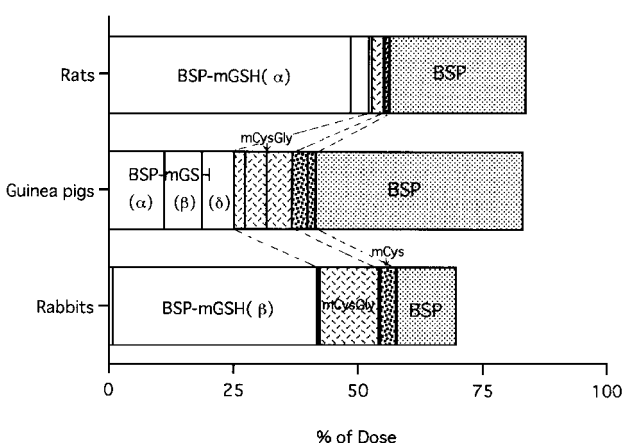

Figure 3 Comparison of sulphobromophthalein (BSP) metabolites (% of dose) between rats, guinea-pigs and rabbits.

The total amount of sulphobromophthalein biliary excretion over 90 min was similar between rats and guinea-pigs. In contrast, rabbits showed a low level of recovery. This trend was illustrated by the much larger distribution amount observed for rabbits than in the other two species.

Biliary excretion after sulphobromophthalein conjugate administration in rats, guinea-pigs and rabbits

To confirm whether this species difference in biliary excretion of sulphobromophthalein was due to the amount of biliary excretion, we administered sulphobromophthalein-mGSH(α). Figure 4 shows the cumu-

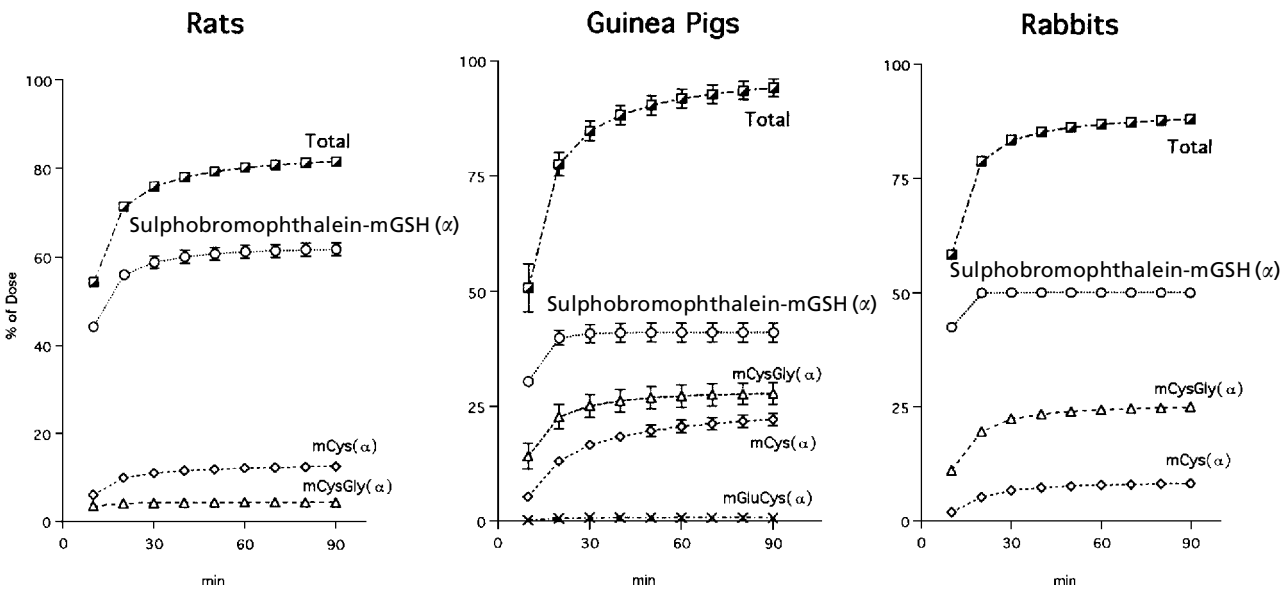

Figure 4 Time course for biliary excretion of sulphobromophthalein-mercaptide conjugates after intravenous injection of sulphobromophthalein-mGSH(α) (equivalent to 9.5 μmol sulphobromophthalein kg^{-1}) in rats, guinea-pigs and rabbits. Data are expressed as the mean$\pm$s.e. of four animals.

Table 1 $\alpha:\beta:\delta$ isomer ratios of sulphobromophthalein-mono mercaptides in bile after administration of sulphobromophthalein.

Species	Total metabolites (μmol/90 min)			% of total mercaptides		
	α	β	δ	α	β	δ
Rat	1.24$\pm$0.02	0.09$\pm$0.00	0.01$\pm$0.00	92	7	1
Guinea-pig	0.79$\pm$0.04*	0.64$\pm$0.04*	0.48$\pm$0.05*	42	33	25
Rabbit	0.75$\pm$0.23	10.10$\pm$0.16*	0.05$\pm$0.01	6	93	1

α, sum of sulphobromophthalein-mGSH(α), -mCys(α), -mCysGly(α) and mMer(α); β, sum of sulphobromophthalein-mGSH(β), mCys(β) and -mCysGly(β); δ, sum of sulphobromophthalein-mGSH(δ), -mCys(δ) and -mCysGly(δ). *$P < 0.05$ compared with the rat value.

lative biliary excretion of sulphobromophthalein mercaptides.

Following sulphobromophthalein-mGSH(α) administration, sulphobromophthalein-mGSH(α) was excreted into the bile at a faster rate and at a greater amount than the other metabolites in all three animals. The ratio of the CysGly and Cys conjugates to the total amount of biliary excretion in the guinea-pig bile were 5.3- and 1.5-times higher than that in the rat, respectively. These in-vivo results demonstrated much higher γ-GT and dipeptidase activity in guinea-pigs than in rats.

In a similar manner, sulphobromophthalein-mCys(α) and sulphobromophthalein-mMer(α) were administered intravenously to all three animals and the total biliary excretion of sulphobromophthalein-mercaptides was compared (data not shown). In these animals, a faster rate of excretion of sulphobromophthalein-mercaptides was observed compared with that of sulphobromophthalein, indicating that all three animals had biliary excretion systems of sulphobromophthalein-mercaptides. These results suggested that the species difference was not due to the specificity of the corresponding biliary excretion system or the transporter on the canalicular membrane, but rather was caused by glutathione conjugation ability.

Comparison of isomer ratio in bile and isomer synthesis ratio by liver cytosolic GST

The in-vivo biliary excretion ratios of $\alpha:\beta:\delta$ isomers in the rat, guinea-pig, and rabbit, were 92:7:1, 42:33:25, and 6:93:1, respectively (Table 1). The in-vitro synthesis ratios of $\alpha:\beta:\delta$ isomers by rat, guinea-pig, and rabbit liver cytosolic GST, were 94:5:1, 31:16:53, and 2:98: not detected, respectively (Table 2).

The in-vitro biosynthesis ratio of isomers of rats and rabbits corresponded to the biliary excretion ratio of isomers in-vivo. These results suggested that the species differences in biliary excretion of sulphobromophthalein metabolites might be due to the molecular diversity of GST. On the other hand, guinea-pigs showed a higher level of GST activity for sulphobromophthalein-mGSH(δ), rather than for sulphobromophthalein-mGSH(α) and (β). Therefore, the findings cannot explain the biliary ratio of mono GSH conjugate isomers perfectly.

In particular, in guinea-pigs the results suggested the need for considering the substrate specificity of γ-GT and dipeptidase, bile flow, characteristics of canalicular membrane and chemical properties of intermediary products, as well as the molecular diversity of GST to explain the properties of biliary excretion of sulphobromophthalein.

Comparison of hepatic GST, γ-GT and dipeptidase activities in three animals

As expected, determination of in-vitro activity revealed that GST activity was 4.4-times higher in the rat than in the guinea-pig. In addition, γ-GT activity was 4.6-times higher in the guinea-pig than in the rat (Table 3). Bile

Table 2 $\alpha:\beta:\delta$ isomer ratios of sulphobromophthalein conjugation activities in liver cytosol.

Species	Sulphobromophthalein-mGSH conjugates formed (nmol min^{-1} mg^{-1})			% of total sulphobromophthalein-mGSH conjugates formed		
	α	β	δ	α	β	δ
Rat	2.01±0.07	0.11±0.00	0.02±0.00	94	5	1
Guinea-pig	0.18±0.00*	0.09±0.02	0.31±0.01*	31	16	53
Rabbit	0.02±0.01*	1.13±0.14*	N.D.	2	98	N.D.

N.D., not detected. *$P < 0.05$ compared with the rat value.

Table 3 Hepatic GST, γ-GTP and dipeptidase activities and physiological parameters.

	μmol min^{-1} (mg protein)$^{-1}$			μmol min^{-1} (g liver)$^{-1}$		
	Rat	Guinea-pig	Rabbit	Rat	Guinea-pig	Rabbit
GST (cytosol)						
toward sulphobromophthalein	4.22±0.50	0.96±0.23	4.23±0.75	0.36±0.01	0.07±0.02	0.24±0.05
γ-GTP						
25% homogenate	0.27×10^{-3}	0.95×10^{-3}*	1.61×10^{-3}*	0.002	0.19	0.30
canalicular membrane	12.0×10^{-3}	88.1×10^{-3}*	132×10^{-3}*	0.002	0.18	0.25
Dipeptidase						
25% homogenate	2.0×10^{-3}	10.0×10^{-3}*	8.9×10^{-3}*	0.1	1.9	1.6
canalicular membrane	0.003	0.121*	0.196*	0.01	0.27	0.38
Bodyweight (kg)	0.233±0.004	0.379±0.006	2.68±0.15			
Liver weight (g)	7.8±0.5	18.3±0.4	103.2±5.8			
Bile flow (mL/10 min)	0.14±0.01	1.38±0.10	1.82±0.34			
Bile flow/BW (mL g^{-1})	0.600	3.641	0.680			

Each value represents the mean of three determinations. *$P < 0.05$ compared with the rat value.

flow in guinea-pigs was greater than rats and rabbits. This result explains that unconjugated sulphobromophthalein was excreted preferentially in guinea-pig bile.

Furthermore, the hepatic GSH concentration was found to be 8.6-times higher in the rat (8.15 ± 0.34 μmol (g liver^{-1})) than in the guinea-pig (0.95 ± 0.17 μmol (g liver^{-1})). These results may explain also in part why there was a higher rate of excretion of GSH conjugates in the rat.

Purification and characterization of glutathione S-transferases

We purified and characterized GSTs from the rat, guinea-pig, and rabbit liver (Table 4). Although their main GSTs produced different isomers, their 20 amino acid residues showed that they belonged to the same class mu according to the classification by Mannervik et al (1985). To test the structural similarity of main GSTs, we compared the patterns of their partial proteolytic digests by *S. aureus* V8 (Figure 5). The electrophoretic patterns of these peptides on SDS-polyacrylamide gels were significantly different. These results suggested that their primary and tertiary structures were different. GSTs have two active sites per dimer that behave independently of one another. Each active site consists of at least two ligand-binding regions for GSH and the electrophilic substrate. The C-terminal tail forms part of the substrate binding site. The basis for substrate discrimination is accounted for by the three-dimensional structure of each GST isoenzyme (Wilce & Parker 1994). GSTs towards sulphobromophthalein from the three animal species that have similar N-terminal domains might form a different substrate binding site on their three-dimensional structure.

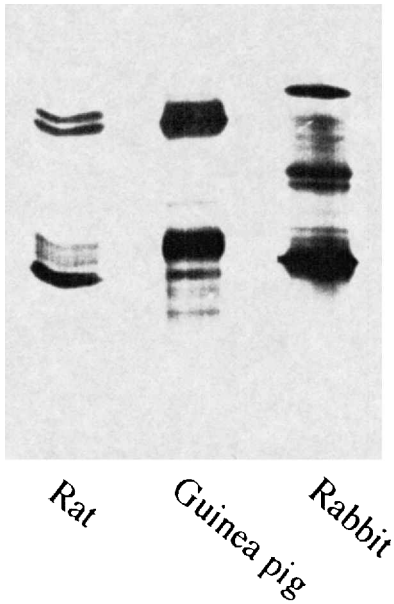

Figure 5 Partial proteolytic digestion of purified GST from livers of rat, guinea-pig and rabbit with *S. aureus* V8 protease. GST from each animal liver was digested with 100 ng of the *S. aureus* V8. Gels were stained with the Bio-Rad silver stain kit.

Conclusion

The three positional isomers (α, β and δ) of sulphobromophthalein-mGSH have been isolated in-vivo and in-vitro. Differences of metabolism of sulphobromophthalein in terms of sulphobromophthalein-mGSH positional isomer formation were seen between rats, guinea-pigs and rabbits. The formation ratio of GSH conjugates in-vitro using cytosolic fraction prepared from these animal livers generally reflected the biliary excretion ratio of α-, β- and δ-monomercaptide isomers in-vivo. Although their main GSTs produced different isomers, their 20 amino acid residues showed that they

Table 4 N-Terminal amino acid sequences of main glutathione S-transferase towards sulphobromophthalein.

GST isozyme #	−1 1	5	10	15	20	25	30	35	40
Rat	P-M-I-L-G-Y-W-D-V-R-G-L-A-H-A-I-R-L-L-L-								
Guinea-pig	P-M-T-L-G-Y-W-N-I-R-G-L-T-H-P-I-R-L-I-L-E-Y-N-S-G-Y-N-M-G-D-A-P-D-Y-								
Rabbit	P-M-T-L-G-Y-W-D-L-R-G-L-A-?-?-I-R-I-L-								
Class α*	A-G-K-P-V-L-H-Y-F-N-A-R-G-R-M-E-S-I-R-W-L-L-A-A-A-G-V-E-F-E-E-K-F-I-K-S-P-E-D-L-E-K-								
Class μ*	M-P-M-T-L-G-Y-W-D-I-R-G-L-A-H-A-I-R-L-L-L-E-Y-T-D-S-S-Y-E-E-K-R-Y-T-M-G-D-A-P-D-Y-								
Class π*	P-P-Y-T-I-V-Y-F-P-V-R-G-R-C-E-A-M-R-M-L-L-A-D-Q-G-Q-S-W-K-E-E-V-T-I-D-T-W-L-Q-G								
Guinea-pig (b)#	P-M-T-L-G-Y-W-N-I-R-G-L-T-H-P-I-R-L-I-L-E-Y-T-N-S-G-Y-E-E-K-R-Y-N-M-G-D-A-P-D-Y-								

*Wilce & Parker (1994). #Kamei et al (1990).

belonged to the same class mu. The results of partial proteolytic digests by *S. aureus* V8 suggested that their primary and tertiary structures were different. These findings suggested that the species differences in sulphobromophthalein metabolism were due to structural differences in GSTs between these three animal species.

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