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CHENODEOXYCHOLIC ACID-3-SULFATE

METABOLISM AND EXCRETION IN THE RAT AND HAMSTER AND EFFECTS ON HEPATIC TRANSPORT SYSTEMS*

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Abstract—The metabolism and excretion of chenodeoxycholic acid-3-sulfate were determined in rats and hamsters. Constant intravenous infusions of 1, 2, and 3 μmoles/min in rats gave a maximum excretion in bile of 1.25 μmoles/min. Simultaneous infusions of sodium taurocholate at 2.0 μmoles/min and sulfobromophthalein at 0.2 μmole/min had no effects on the maximum excretion rate of chenodeoxycholic acid-3-sulfate. However, the bile acid ester sulfate caused a dose-related reduction in the excretion rate of BSP without affecting bile acid excretion rate and without a reduction in total bile flow. Chromatographic analysis of ester sulfate, a bile acid recovered in bile and urine, indicated that more than 95% had not undergone further metabolic transformation.

Disturbances in hepatic excretory function generate complex changes in the distributions of bilirubin and bile acids in plasma, bile and urine [1]. To understand the pathophysiologic basis for these changes, it is necessary to evaluate systematically the effect of each of the individual species of bile acids on the transport of other bile acids and other organic anions. In this study, we evaluate the effect of chenodeoxycholic acid-3-sulfate on the transport of sodium taurocholate and sulfobromophthalein (BSP).

METHODS

For the synthesis of the specific 3-sulfate ester of chenodeoxycholic acid, the methyl ester-7-acetate was prepared using the method described by Haslewood and Haslewood [2]. The sulfation reaction was then done using dicyclohexylcarbodiimide as described by Mumma [3], and the product was isolated from other components of the reaction mixture by DEAE-cellulose column chromatography. After elution from the column as the ammonium salt, the methyl ester was removed by methanolysis in 5% methanolic KOH. After acidification with HCl, the ester sulfate was adsorbed onto a column of XAD-2 (Mallinckrodt). The column was washed with water to remove inorganic salts, and then the chenodeoxycholic acid-3-sulfate was eluted using methanol. Chenodeoxycholate-3-sulfate was then recrystallized from ethanol and gave a single spot by thin-layer chromatography using plates coated with silica gel G and a solvent system of chloroform–methanol–H_2O (65:24:9) which resolves completely glycochendeoxycholate and taurochenodeoxycholate from their sulfate esters and the 3- and 7-monosulfate esters of chenodeoxycholic acid. For the preparation of radioactive [^{35}S]chenodeoxycholic acid-3-sulfate, a microscale of the same synthesis was used. Thus, 5 mg of the 7-acetate of chenodeoxycholic acid methyl ester was dissolved in 0.3 ml of dimethylformamide in a minivial followed by 0.01 ml of dicyclohexylcarbodiimide and 0.01 ml of 36 N H_2 $^{35}SO_4$. After allowing it to react for 15 min at 0°, the reaction mixture was placed on a silica gel G plate and run in a solvent system of ethyl acetate–hexane (50:50). In this system, the chenodeoxycholic acid-3-sulfate remains at the origin and all other components migrate up the plate except for the inorganic sulfate. The silica at the origin is removed, and the compound is eluted with hot methanol which is then reduced in volume by a stream of nitrogen to approximately 0.5 ml to which is added an equal volume of 10% KOH in methanol. Following overnight hydrolysis, the free acid is separated from other constituents by thin-layer chromatography as described previously. The radioactive chenodeoxycholic acid-3-sulfate is then recrystallized together with the nonradioactive compound to give a final specific activity of 2.36 μCi/μmole. Radioactivity was determined using a Beckman cpm 200 liquid scintillation counter with external standardization and correction to constant efficiency. Chenodeoxycholic acid disulfate was prepared as described above using the methyl ester instead of the specific monosulfates. Chromatographic analysis of the reaction mixture as described above indicated the formation of three derivatives all of which yielded chenodeoxycholic acid after solvolysis. The derivative with the lowest R_f was considered to be the disulfate. The 7-sulfate was distinguished from the

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3-sulfate using the specific 3-sulfate derivative prepared from chenodeoxycholic-7-acetate.

BSP was obtained from Hynson, Westcott & Dunning, Baltimore, MD, and analyzed in plasma and bile as described previously [4]. Sodium taurocholate was obtained from Supelco, Bellefonte, PA. Bile acids were determined enzymatically as described previously [5].

Male Sprague–Dawley rats weighing between 250 and 300 g were anesthetized with intraperitoneal pentobarbital, and polyethylene cannulas were inserted into the common bile duct and a femoral artery and vein. Animals were placed in restraining cages and offered food and water. After overnight depletion of the bile acid pool, infusion studies were done the following day with the animals at preoperative body temperature.

Male Syrian hamsters weighing 100–115 g underwent bile duct ligation under pentobarbital anesthesia and received an intravenous injection of chenodeoxycholic acid-3-sulfate. They were placed in metabolic cages, and stool and urine were collected separately. In addition, bile was aspirated from the gallbladder at the time of sacrifice.

Compounds were infused intravenously after being dissolved in 5% dextrose in 0.45% NaCl except for sodium taurocholate which was prepared in 3% human serum albumin to a concentration of 45 mM as described previously [6]. BSP and sodium taurocholate were infused at rates close to their known maximum excretion rates in bile [7–9].

RESULTS

As shown in Fig. 1, infusions of chenodeoxycholic acid-3-sulfate at rates of 1, 2, and 3 μmoles/min had no effect on the excretion rate of non-sulfated bile acids. In contrast, at all infusion rates there was a dose-dependent decrease in the excretion rate of BSP. Chromatographic analysis of the BSP in bile [4] indicated no change in the proportion of conjugated BSP. In one study the dibrom analog phenoldibromphthalein disulfonate, which is not conjugated in the rat [10], was substituted for BSP, and a similar reduction in excretion rate occurred. As shown in Table 1, chenodeoxycholic acid-3-sulfate, when infused alone or during an infusion of sodium taurocholate, caused an increase in bile flow.

Infusion of chenodeoxycholic acid-3-sulfate at 3 μmoles/min gave a maximum biliary excretion rate of 1.25 μmoles/min. The bile to plasma concentration ratio at this time was 34.8 per 1.83 μmoles per ml or 19 to 1. In a separate study, chenodeoxycholic acid-3-sulfate was infused alone at 3 μmoles/min (Table 1). It was found that both the maximum excretion rate and bile/plasma concentration ratio were not different from that found when it was administered during an infusion of sodium taurocholate and BSP. Recovery of infused chenodeoxycholic acid-3-sulfate was greater than 70% in 24 hr of which more than 80% was found in bile (Table 2). Chromatographic analysis of the radioactivity found in the bile and urine of rats and hamsters

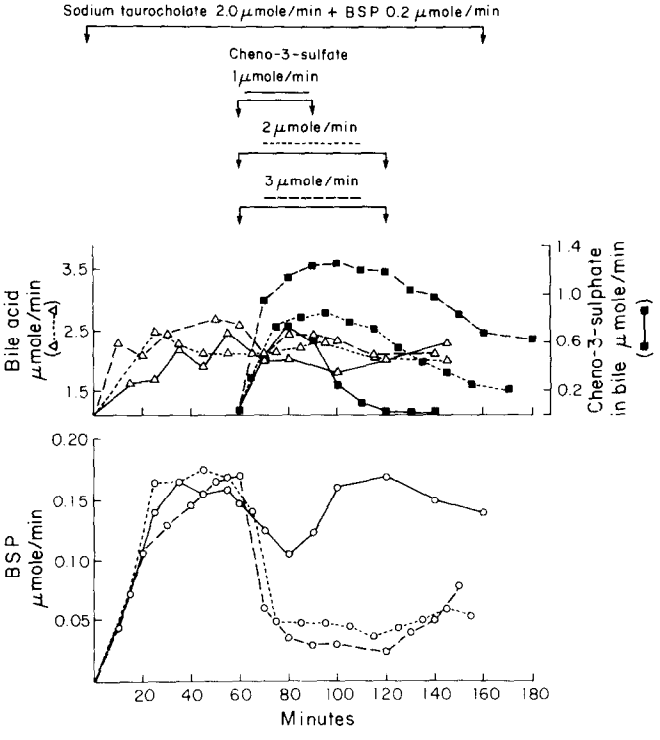

Fig. 1. Effects of chenodeoxycholic acid-3-sulfate on taurocholate and sulfobromophthalein excretion. Chenodeoxycholic acid-3-sulfate (■) was infused intravenously at 1 (——), 2 (.....) and 3 (– – –) μmoles/min for the time periods shown. Key: (△) bile acid excretion, and (○) BSP excretion.

Table 1. Effect of chenodeoxycholic acid-3-sulfate on bile flow

	Rate of infusion of cheno-3-sulfate (μmoles/min)	Bile flow before cheno-3-sulfate (ml/min)	Bile flow after cheno-3-sulfate (ml/min)	Increase (ml)	Percent increase
(A)*	3.0	0.016	0.034	0.018	112.5
(B)†	1.0	0.030	0.039	0.009	30
	2.0	0.031	0.044	0.013	42
	3.0	0.035	0.046	0.011	31

* Rat with bile acid pool depleted by overnight bile drainage.
† Rats with bile acid pool repleted by a constant infusion of sodium taurocholate, 2.0 μmoles/min.

Table 2. Recovery of chenodeoxycholic acid-3-sulfate administered to rats

Infused cheno-3-SO_4 (μmoles)	Percent recovery of infused dose in 24 hr	Percent recovered from bile	Percent recovered from urine
30	83.0	98.6	1.4
120	79.1	83.4	16.6
180	92.0	97.0	3.0
180	71.0	93.0	7.0

indicated that no further metabolism had occurred (Table 3).

DISCUSSION

Esterification of conjugated and unconjugated bile acids with sulfate or glucuronic acid does not occur to any major extent unless there is impairment of bile acid excretion [11]. Under these conditions, the kidney becomes the major excretory route for bile acid esters that are synthesized mostly in the liver. However, the clearance of esterified bile acids by the kidney does not occur at rates sufficient to prevent an increase in sulfated bile acids in plasma [11, 12]. Under these circumstances, the esterified bile acids can become competitive inhibitors of other compounds that are normally transported by the liver.

Although monohydroxy bile acids such as 3α-hydroxy-5β-cholanoic acid (lithocholic acid) are found only in esterified forms in urine, the total amount present is considerably less than chenodeoxycholic acid esters of which the 3-sulfate is considered to be the predominant derivative in human urine [13]. For this reason we evaluated the effect of esterification at the 3-hydroxy position of chenodeoxycholic acid on the excretion of a bile acid, sodium taurocholate, and an organic anion, BSP. It is known that bile acids do not competitively inhibit the excretion of BSP or conjugated bilirubin which are considered to be transported and excreted by similar mechanisms [8]. Actually the choleretic effect of bile acids has been shown to increase the excretion rate of BSP and other organic anions [14].

The findings in this study demonstrate that esterification of the 3-hydroxy position of bile acids generates a species which now becomes a competitive inhibitor of organic anion excretion. Thus, a marked reduction in the concentration of BSP in bile occurred with no reduction in bile flow, leading to a more than 80% reduction in excretion rate. In contrast, under the conditions of the study, no competition for bile acid excretion could be demonstrated even though the infusion rate of chenodeoxy-

Table 3. Distribution of radioactivity in bile and urine following administration of chenodeoxycholic acid-3-^{35}sulfate

Compound	R_f*	Percent radioactivity			
		Rat		Hamster	
		Bile	Urine	Bile	Urine
Glycochenodeoxycholic acid-3-sulfate	0–0.25	0	0	0	0
Taurochenodeoxycholic acid-3-sulfate	0–0.25	0	0	0	0
Taurochenodeoxycholic acid	0.30	0	0	0	0
Chenodeoxycholic acid-7-sulfate	0.50	0	0	0	0
Chenodeoxycholic acid-3-sulfate	0.65	98.5	99.2	98.6	96.4
Glycochenodeoxycholic acid	0.82	0	0	0	0

* Silica gel G, solvent system: chloroform–methanol–water (65:24:9, by vol.).

cholic acid-3-sulfate was greater than that of sodium taurocholate.

From our findings it is reasonable to speculate that, when impairment of bile acid excretion occurs and esterification becomes proportionally greater, considerable amounts of chenodeoxycholic acid, which represent pool sizes ranging from approximately 1100 to 2500 mg [15], are esterified and compete competitively for biliary excretion of the approximately 350 mg of bilirubin that is synthesized and conjugated per day. Under these circumstances, the occurrence of conjugated hyperbilirubinemia may reflect, at least in part, competition for excretion rather than a primary defect in the transport system for organic anions.

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