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Report

Effect of Taurolithocholate on *In Vivo* Sulfation and Glucuronidation of Acetaminophen in Rats

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Taurolithocholate produces a prompt, complete, and reversible cessation of bile flow in rats. This is associated with impaired hepatic oxidative drug-metabolizing activity. The purpose of this study was to examine the effects of taurolithocholate-induced cholestasis on *in vivo* conjugation. The pharmacokinetics of acetaminophen and the two major processes specifically responsible for its elimination, namely, the formations of acetaminophen sulfate and acetaminophen glucuronide, were used to assess hepatic conjugating activity. A 30-mg/kg bolus of acetaminophen was administered intravenously to rats 2 hr (acute cholestasis) or 20 hr (postcholestasis) after intravenous pretreatment with sodium taurolithocholate, 5 μmol/100 g body weight. Acute cholestasis increased the total clearance of acetaminophen 20%, the partial clearance to acetaminophen sulfate 12%, and the partial clearance to acetaminophen glucuronide 85%. Postcholestasis, these parameters had significantly decreased compared to those during acute cholestasis and were comparable to control values. The results show that cholestasis does not impair acetaminophen conjugation in the rat.

KEY WORDS: cholestasis; bile acids; acetaminophen; conjugation; sulfation.

INTRODUCTION

Sulfation and glucuronidation are prime routes for the conjugation of drugs such as acetaminophen and are quantitatively important pathways of bile acid metabolism in patients with liver disease such as cholestasis. In obstructive jaundice or acute hepatitis, total serum bile acid concentrations increase, and the fraction conjugated with sulfate and glucuronic acid and subsequently excreted in urine also increases (1,2). The ability of humans to form 3-sulfate metabolites of monohydroxy bile acids and to excrete these rapidly appears to protect the liver against the hepatotoxicity of unconjugated bile acids (1–6).

Sulfate conjugation utilizes endogenous inorganic sulfate, the precursor of the cosubstrate for sulfation, phosphoadenosine phosphosulfate (7). Depletion of inorganic sulfate results in dose- and time-dependent elimination of compounds, such as acetaminophen, that are eliminated in large part by sulfation (8). Cholestasis-induced changes in bile salt sulfation by increasing the utilization of inorganic sulfate may alter the elimination kinetics and metabolic fate of drugs that undergo sulfation. Cholestasis also decreases cytochrome P-450-mediated aminopyrine demethylase, NADPH cytochrome *c* reductase, and cytosolic glutathione-S-transferase (1-chloro-2,4-dinitrobenzene, acceptor) activity (9,10). The purpose of this investigation was to examine the effect of taurolithocholate-induced cholestasis on the elimination kinetics and metabolic fate of acetaminophen and on the *in vivo* rates of sulfate and glucuronide conjugation of acetaminophen.

METHODS

Chemicals. Sodium taurolithocholate, acetaminophen, and bovine serum albumin (fraction V) were obtained from Sigma Chemical Company (St. Louis, Mo.). Authentic standards of the sulfate and glucuronide conjugates of acetaminophen were obtained from McNeil Consumer Products Company (Fort Washington, Pa.). All other chemicals were analytical reagent grade.

Animal Studies, Analytical and Data Analyses. Adult male Sprague Dawley rats (Simonsen Laboratories, Gilroy, Calif.) weighing 260 to 325 g received indwelling cannula implants in the right jugular vein (11) 1 day before acetaminophen administration. Animals were housed individually in plastic metabolism cages designed to allow free access to food and water and maximal allowable freedom. Food and water were withdrawn in the morning of each study day and withheld for the duration of blood sampling. Six blood samples (0.25 ml) were collected in disposable plastic syringes and transferred to heparinized glass capillary tubes for plasma separation by centrifugation. One milliliter of 0.9% saline was infused after each blood sample withdrawal to clear the cannula of blood, replace the fluid volume, and stimulate urine flow. Blood samples were collected for 90 min and urine was collected through 24 hr after acetaminophen administration. Plasma and urine samples were stored at −60°C until assay.

Acetaminophen was administered as an intravenous

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bolus injection of 30 mg/kg (5 mg/ml dissolved in 0.15 *M* sodium chloride) at time zero. Some of the animals received an intravenous injection of taurolithocholate, 5 μmol/100 g body weight [3.6 mg/ml of sodium taurolithocholate dissolved in 0.15 *M* sodium chloride containing 8% (w/v) bovine serum albumin], at 2 or 20 hr prior to acetaminophen administration. Taurolithocholate was solubilized in the saline–albumin vehicle solution by continuous stirring for 6 hr at 25°C. The slightly cloudy solution was then filtered through an 0.8-μm PVC copolymer filter (Metricel-type membrane filter, DM-450; Gelman Sciences, Inc., Ann Arbor, Mich.). Some animals received an intravenous injection of the saline–albumin vehicle solution and served as controls. This dose of taurolithocholate produces an almost immediate cessation of bile flow, which remains below 50% of the initial rate for up to 4 hr and which is completely reversed after 20 hr (12).

Acetaminophen was administered between 0930 and 1100 hr to control for diurnal variation in serum sulfate (13) and hepatic glutathione concentration (14), bile salt- and phenol sulfotransferase activity (15,16), and other hepatic drug-metabolizing activity (15,17). Administration of acetaminophen at 2 hr after taurolithocholate pretreatment is defined as during acute cholestasis, whereas at 20 hr it is defined as postcholestasis (12). The drug disposition studies were performed in free-moving, unanesthetized animals. They remained calm in the cages, where their behavior was placid during the experimental procedures of drug administration and blood sampling. The experimental protocol was approved by the University of Utah Institutional Animal Care and Use Committee.

Acetaminophen and its sulfate and glucuronide conjugates in plasma and urine were separated and quantitated by the high-performance liquid chromatographic method of Corcoran *et al.* (18). The apparent volume of distribution (V_d) and plasma half-life ($T_{1/2}$) of acetaminophen were calculated from the zero-time intercept and the slope of the line regressing log plasma concentration versus time data by the method of linear least squares. Total clearance was calculated as the product of V_d and $0.693/T_{1/2}$. Partial clearance to metabolites was calculated as the product of total clearance and the fraction of the administered dose recovered in urine as that metabolite. Pharmacokinetic methods are those previously described (8,19). Statistical analysis employed the Kruskal–Wallis test for nonparametric analysis of variance. Differences between groups were determined by Wilcoxon–Mann Whitney test using the Bonferroni correction (20,21). Differences were not attributed to chance variation when $P < 0.05$.

RESULTS

The effect of taurolithocholate pretreatment on acetaminophen concentrations in plasma of rats following an intravenous acetaminophen dose of 30 mg/kg is shown in Fig. 1. Acetaminophen concentrations declined monoexponentially with time in all three groups. Acute cholestasis slightly increased the total clearance and decreased the half-life of acetaminophen, compared to controls (Table I). The partial clearance of drug to glucuronide increased 85% and that to sulfate increased 12%, compared to control values (Table II). Twenty hours after taurolithocholate pretreatment, the acetaminophen clearance was significantly decreased compared to that during acute cholestasis. Postcholestasis, the partial clearance to metabolites had significantly decreased compared to the minus 2-hr pretreatment (Table II). The renal clearance of unchanged drug increased 34% during acute cholestasis and decreased 14% at 20 hr compared to control animals.

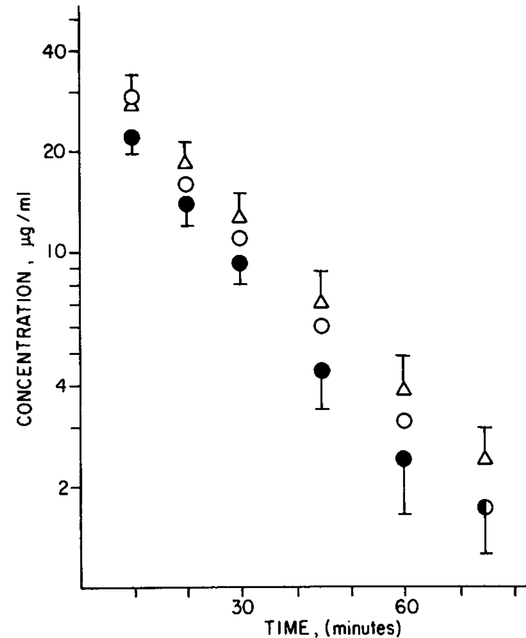

Fig. 1. Acetaminophen concentrations in plasma of rats following intravenous injection of 30 mg/kg at time zero. Rats were pretreated with taurolithocholate, 5 μmol/100 g body weight at minus 2 hr (●; $N = 6$) or at minus 20 hr (△; $N = 6$). Control animals received vehicle injection (○; $N = 6$). Data are expressed as the mean ± SD.

The composition of urinary excretion products is shown in Table III. The majority of the dose was recovered as the

Table I. Effect of Taurolithocholate Pretreatment on the Elimination Kinetics of Acetaminophen in the Rat[a]

Pretreatment (*N*)	Half-life (min)	Apparent volume of distribution (ml/kg)	Total clearance (ml/min/kg)
Saline–albumin (6)	17.9 ± 2.5	817 ± 103	31.7 ± 5.4
Taurolithocholate at minus 2 hr (6)	16.0 ± 3.0 (−11%)	899 ± 147 (+10%)	38.1 ± 4.0 (+20%)
Taurolithocholate at minus 20 hr (6)	18.0 ± 1.0 (+1%)	747 ± 84 (−8%)	28.9 ± 4.1* (−9%)

[a] Taurolithocholate, 5 μmol/100 g body weight, as the sodium salt, was injected intravenously 2 or 20 hr prior to intravenous administration of 30 mg/kg acetaminophen. Data are expressed as mean ± SD. Control animals received vehicle solution. Percentage indicates relative mean change from control.

* $P < 0.05$ versus minus 2-hr taurolithocholate pretreatment.

Table II. Effect of Taurolithocholate Pretreatment on the Partial Clearance of Acetaminophen (A) to A Glucuronide and A Sulfate and on the Renal Clearance of A in Rats[a]

	ml/min/kg		
Pretreatment (N)	Partial clearance to A glucuronide	Partial clearance to A sulfate	Renal clearance of acetaminophen
Saline–albumin (6)	2.0 ± 0.4	24.0 ± 2.7	2.9 ± 1.4
Taurolithocholate at minus 2 hr (6)	3.7 ± 1.0** (+85%)	26.8 ± 2.2 (+12%)	3.9 ± 1.5 (+34%)
Taurolithocholate at minus 20 hr (6)	2.5 ± 0.4* (+25%)	22.0 ± 0.4* (−8%)	2.5 ± 0.9 (−14%)

[a] Taurolithocholate, 5 μmol/100 g body weight, as the sodium salt was injected intravenously 2 or 20 hr prior to intravenous administration of 30 mg/kg acetaminophen. Data are expressed as mean ± SD. Control animals received vehicle solution. Percentage indicates mean relative change from control.
* $P < 0.05$ versus minus 2-hr taurolithocholate pretreatment.
** $P < 0.05$ versus saline–albumin pretreatment.

sulfate metabolite. This was decreased by 8%, while recovery of the glucuronide metabolite increased 34% in the acute cholestasis group. Postcholestasis, the fraction recovered as the sulfate was unchanged from the control and that of the glucuronide decreased 14% compared to control values. None of these changes was statistically significant.

DISCUSSION

Bile acid sulfation and glucuronidation serve as important processes to prevent the accumulation of naturally occurring bile acids that exhibit hepatotoxic properties. Sulfate and glucuronide conjugation of bile acids reduces terminal ileal reabsorption, thus preventing enterohepatic circulation of unconjugated bile acids (3,4). Fecal excretion of sulfated lithocholic acid in humans, for example, limits accumulation of this toxic bile acid to less than 5% of the total bile acid pool. During chenodeoxycholic acid therapy for dissolution of gallstones, the exchangeable pool of lithocholate increases four- to fivefold over baseline and serum concentrations double (4,5). In species unable to sulfate bile acids as efficiently as humans, chronic chenodeoxycholate therapy results in cholestatic liver damage (22). Variations in the efficiency of bile acid sulfation have been cited as responsible for the toxic effects noted in patients treated with chenodeoxycholic acid therapy. Moreover, a relative deficiency in bile acid sulfation in infants and adults with cholestatic liver disease may also explain bile salt accumulation in these patients (23).

The results of the present investigation demonstrate that acute cholestasis, induced by taurolithocholate, slightly increased sulfation, glucuronidation, and the overall elimination of acetaminophen. The relative increase in the partial clearance by glucuronidation was greater (85%) than the increase by sulfation (12%). This is in contrast to the reported decrease in P-450 activity. Several explanations may account for these effects of taurolithocholate-induced cholestasis in rats. The total clearance of acetaminophen following the 30-mg/kg dose approached the estimated hepatic perfusion rate of 40–60 ml/min/kg (24). The mean clearance in control animals of 31 ml/min/kg is sufficiently high enough to be influenced by any effect of taurolithocholate producing an increase in hepatic blood flow. In addition, Vermeulen *et al.* (25) report that the addition of bile acids to microsomes increases UDP-glucuronyltransferase activity three- to sevenfold and suggest that this is due to intramembranous reverse micelle formation resulting in increased substrate access to enzyme. Non-micelle-forming bile acids had no effect on microsomal glucuronidation, while non-bile salt, micelle-forming detergents increased activity. Further, the disproportionate increase in glucuronidation relative to sulfation is probably not due to limited cofactor (sulfate) availability because the 30-mg/kg dose of acetaminophen produces only a slight and transient decrease in serum inorganic sulfate concentrations (7).

Increased sulfation of bile acids in cholestatic syndromes may lead to increased competition for the limited stores of inorganic sulfate. An increase in hepatic glutathione concentration has also been reported in bile duct-ligated, cholestatic rats (9), which could indicate an increase in glutathione turnover and increased utilization of endogenous cysteine. Cysteine is the common precursor for both

Table III. Effect of Taurolithocholate Pretreatment on the Composition of Urinary Excretion Products of Acetaminophen (A) in Rats[a]

	Percentage of administered dose			
Pretreatment (N)	A glucuronide	A sulfate	A	Total
Saline–albumin (8)	7.4 ± 1.7	76.1 ± 8.2	9.7 ± 3.6	93.7 ± 7.0
Taurolithocholate at minus 2 hr (7)	9.7 ± 2.7 (+31%)	70.1 ± 2.5 (−8%)	10.5 ± 4.2 (+8%)	90.3 ± 8.1 (−4%)
Taurolithocholate at minus 20 hr (8)	8.4 ± 0.9 (+14%)	75.2 ± 5.9 (−1%)	8.0 ± 3.2 (−17%)	91.6 ± 5.2 (−2%)

[a] Taurolithocholate, 5 μmol/100 g body weight, was injected intravenously 2 or 20 hr prior to intravenous administration of 30 mg/kg acetaminophen. Control animals received vehicle solution. Data are expressed as mean ± SD. Percentage indicates mean relative change from control.

inorganic sulfate and glutathione and increased glutathione turnover appears to decrease sulfation by decreasing the amount of cysteine available for oxidation to inorganic sulfate (19).

The results of this study show that sulfation and glucuronidation of acetaminophen are not impaired by cholestasis. In human cholestatic disease sulfate and glucuronide conjugation of bile acids is enhanced. Administration of acetaminophen or other drugs eliminated in part by conjugation may rate- and capacity-limit sulfate and glucuronide conjugation of bile acids by competing for cosubstrates. This may lead to accumulation of unconjugated bile acids and could increase the risk for the development of hepatotoxicity.

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