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Biliary Excretion and Choleretic Effect of Cefmetazole in Rats

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The effect of cefmetazole, a broad-spectrum cephalosporin, on bile flow and composition in rats was studied. Intravenous injection of cefmetazole at doses ranging from 40 to 400 μmol/kg of body weight led to an increase in its biliary concentration and excretion rate, with a maximum at 30 min after injection. Excretion of cefmetazole into bile was associated with a marked choleresis. The magnitude of the increase in bile flow was dose dependent, with a maximal increase at a dose of 200 μmol/kg. Cefmetazole administration did not affect the secretion of bile acids or their osmotic activities, whereas the bile acid-independent bile flow increased by 49% at a dose of 200 μmol/kg. Cefmetazole administration at a dose of 200 μmol/kg significantly increased the biliary outputs of sodium, potassium, chloride, and bicarbonate (+36, +56, +28, and +31%, respectively) compared with outputs of controls. A linear relationship was observed between bile flow and cefmetazole excretion, 44 μl of bile being produced per μmol of cefmetazole excreted into bile. Our results demonstrate that cefmetazole induces choleresis by stimulating bile acid-independent bile flow. This effect appears to be partly due to the osmotic properties of cefmetazole transported into bile.

Cefmetazole sodium [7-β-cyanomethylthioacetamide-7-α-methoxy-3-(((1-methyl-1-*H*-tetrazol-5-yl)-thio)methyl)-3-cephem-4-carboxylate] is a semisynthetic derivative of cephamycin. This cephalosporin has a broad antimicrobial spectrum, is efficient against gram-positive and gram-negative bacteria, and shows high resistance to attack by different β-lactamases (23, 31). Several studies have confirmed its usefulness in the treatment of different kinds of infections and in prophylactic administration after surgical wounds (11). Since the drug was first released for clinical use, its pharmacokinetics and metabolism in humans and different animals have been described elsewhere (23, 25, 26). The antibiotic is excreted via urine and bile, with significant species differences in the participation of both routes (18, 26, 27). The biliary excretion of cefmetazole has been shown to be closely related to the bile/plasma ratio of bile acids (27) and dependent on the amount of bile flow and liver function (26).

Appropriate antibiotic therapy in treatment of biliary tract infections must take into account the transfer of the drug into bile. The purpose of this study was to examine the effects of biliary elimination of cefmetazole on the formation of bile in rats. The influences of cefmetazole on the bile acid-dependent and -independent components of bile flow and on bile composition were evaluated.

MATERIALS AND METHODS

Animals and experimental procedure. Male Wistar rats weighing 200 to 250 g that had been maintained on a standard laboratory diet (Panlab, Barcelona, Spain) and under constant light cycle (12 h-12 h, dark-light) were used throughout. Animals were anesthetized with sodium pentobarbitone (50 mg/kg of body weight given intraperitoneally; Claudio Barcia, Madrid, Spain), and a median laparotomy was performed. The bile duct, right jugular vein, and right carotid artery were cannulated with polyethylene tubing. Rectal temperature was monitored with a thermistor probe and maintained at 37°C by a thermostatically controlled heating table.

After two 15-min bile samples were collected in basal conditions, cefmetazole (Antibioticos SA, Madrid, Spain) was injected intravenously (i.v.) at four different doses: 40, 80, 200, and 400 μmol/kg of body weight. Bile was collected for eight additional 15-min periods. Arterial blood samples (200 μl) were collected in heparinized test tubes at 10, 20, 30, 40, 50, and 60 min following administration of cefmetazole.

Analytical methods. Bile flow was determined gravimetrically, assuming a bile density of 1.0 g/ml. Bile acid concentration in bile was determined enzymatically by the method of Talalay (28) as modified by Paumgartner et al. (21). Sodium and potassium concentrations in bile were measured by flame photometry (model Nak II; Meteor, Madrid, Spain) with a lithium standard. Chloride ion concentration in bile was determined with a silver electrode chloridometer (Analytical Control, Milan, Italy). Bicarbonate concentration in bile was determined with an automated gas analysis system (model 168; Corning Medical, Medfield, Mass.). Cholesterol concentration in bile was estimated by an enzymatic esterase-oxidase method (6). Phospholipid concentration in bile was measured by a commercial enzymatic method based on the method of Gurantz et al. (13). Cefmetazole in plasma and bile was measured by a high-performance liquid chromatographic method with a reversed-phase technique. A Waters Powerline chromatograph system (Millipore Corp., Bedford, Mass.) with a UV/visible detector (model 484) set at 254 nm, a peak integrator (model 745B), and a loop injector of fixed volume (20 μl; Waters 700 WISP) was used. Conditions were as follows: column, μBondapak C_{18} (Millipore); mobile phase, methanol-phosphate buffer (0.007 M, pH 7.4, 18/82 [vol/vol]); flow rate, 1.0 ml/min. The detection limit was 0.1 μg of cefmetazole per ml; the variation coefficient varied between 3.7 and 4.2% of the high and low concentrations assayed, respectively (12, 23).

The possible existence of a correlation between the variables studied was investigated by linear regression analysis,

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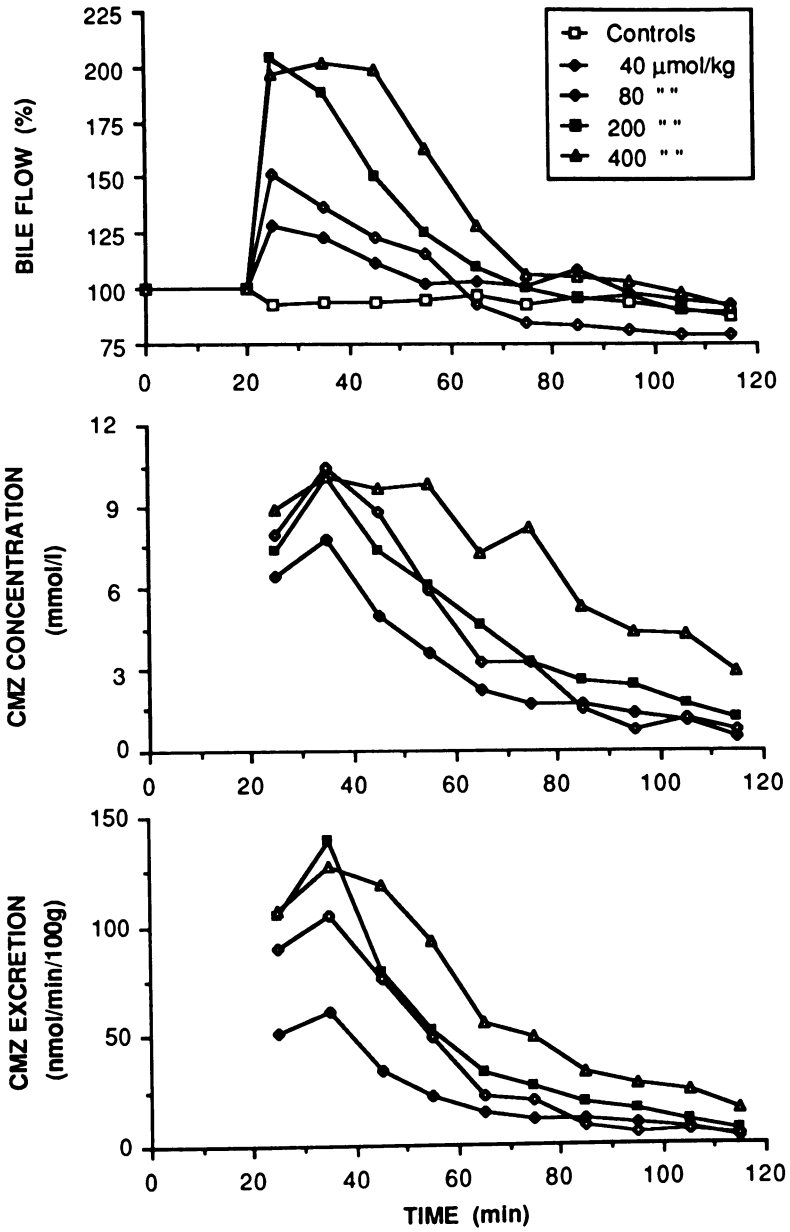

FIG. 1. Bile flow, biliary concentration, and biliary excretion of cefmetazole (CMZ) in rats injected with 40, 80, 200, and 400 μmol/kg i.v. Each point represents the mean of six animals.

assuming homoscedastic variance as the weighting scheme. Values are means ± standard errors of the means. The significance of the differences between means was evaluated by the nonparametric Mann-Whitney U test. A value of $P < 0.05$ was considered significant.

RESULTS

Biliary concentration and excretion of cefmetazole are shown in Fig. 1. Greater amounts of the antibiotic were excreted as the dose increased, until a maximum was reached at a dose of 200 μmol/kg. Figure 2 represents cumulative biliary excretion of the antibiotic at the four tested doses. The recovery of cefmetazole from bile ranged from 46 to 16% of the dose for injections of 40 and 400 μmol/kg, respectively. The ratio of the area under the concentration-time curve (AUC) with serum to the AUC with bile for cefmetazole concentrations was 0.019 for the 200-μmol/kg dose.

Cefmetazole administration caused a choleretic effect, with a peak increase of bile flow 30 min after injection. The maximal rate of bile flow achieved was observed after a 200-μmol/kg dose. A subsequent dose of 400 μmol/kg did not cause additional increases in bile flow (Fig. 1).

A linear relationship between bile flow and bile acid secretion was found both in the control and in the different groups of cefmetazole-treated rats. Linear regression analysis in controls and in animals receiving cefmetazole at 200 μmol/kg revealed that the slopes of the two lines were not

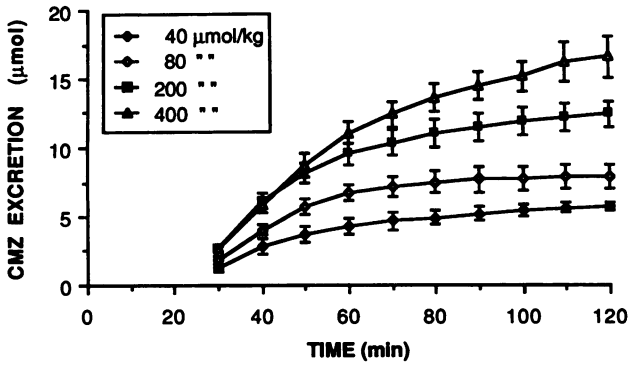

FIG. 2. Cumulative biliary excretion of cefmetazole (CMZ) in rats injected with 40, 80, 200, and 400 µmol/kg i.v. Each point represents the mean ± standard error of the mean of six animals.

significantly different. However, the y intercept increased significantly from 4.91 ± 1.18 (95% confidence limits) to 7.32 ± 1.32 (95% confidence limits) (Fig. 3).

Bile compositions in controls and in cefmetazole-treated (200 µmol/kg) rats are shown in Table 1. The concentrations of sodium, potassium, chloride, and bicarbonate were unchanged, but bile acids, cholesterol, and phospholipid concentrations were significantly lower in animals injected with cefmetazole. Phospholipid and cholesterol excretion were lower; the excretion of inorganic electrolytes, especially cations, was significantly higher after cefmetazole administration, but the secretion rate of bile acids was not significantly modified by the drug (Table 1).

When bile flow was plotted against cefmetazole excretion into bile (Fig. 4), a linear relationship was found. The slope of the regression line indicated that 44 µl of additional bile was produced per µmol of cefmetazole excreted into bile.

DISCUSSION

Our data show that a high percentage of cefmetazole is excreted into bile in rats. Previous studies have demonstrated that after subcutaneous or intramuscular administration of cefmetazole, there are significant interspecific differences in the biliary recovery of this antibiotic, with excretion rates higher in rats than in dogs, rabbits, or monkeys (27). This fact does not necessarily imply interspecific differences in the hepatocellular mechanism of excretion of the antibiotic, although such differences have been demonstrated for a large number of xenobiotics (10). The hepatic transport of cefmetazole appears to be saturable, reaching an apparent maximal excretion into bile after injection at 200 µmol/kg in our experiments. An AUC with serum/AUC with bile ratio of 0.019 was found after drug administration, indicating that cefmetazole was concentrated in bile. These data support the hypothesis of excretion by active, carrier-mediated systems, as occurs for other compounds undergoing biliary excretion that are included in the class B of Brauer (29). Elimination of cefmetazole into bile was accompanied by a choleretic effect with marked increases in bile flow rates.

The canalicular secretion of bile is a complex process attributed to osmotic water flow in response to the active transport of solutes. In all species studied until now, bile acids are considered the major solutes generating bile flow and responsible for the so-called bile acid-dependent fraction of bile flow. Furthermore, several findings point to the existence in all species studied of a bile acid-independent fraction of bile flow which is apparently mediated by transport of inorganic ions. Some bile is also formed by secretion or reabsorption in the bile ducts (10, 19).

Cefmetazole-induced choleresis could be explained by enhancement in the biliary secretion or osmotic activity of bile acids, stimulation of inorganic ion transport into bile, osmotic water flow accompanying biliary excretion of cefmetazole, or changes in ductular reabsorption or secretion or both.

Bile acid-independent bile flow has usually been estimated by extrapolation to a zero bile acid excretion rate from the plot of bile flow versus bile acid secretion. This relation is not necessarily best represented by a single line in the entire range of bile acid excretion rates (3). However, our data fit a single regression line, and the results obtained suggest that cefmetazole enhances bile acid-independent flow and has little effect on the osmotic activity or biliary secretion of bile acids.

Previous investigations have demonstrated that bile acid administration significantly modifies biliary excretion of cefotiam and other cephalosporins in both humans and rats (15, 20), and the existence of a common excretory mechanism for bile acids and antibiotics has been suggested (14). However, even when saturated, hepatic transport of cefmetazole did not affect biliary secretion of bile acids, which suggests that transport mechanisms are not necessarily shared by both kinds of compounds. Recent investigations of the biliary excretion of piperacillin and ampicillin, which have demonstrated an absence of effects of both antibiotics on the

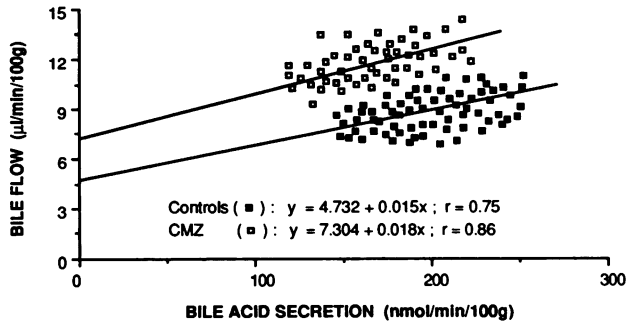

FIG. 3. Relationship between bile flow and bile acid secretion in control rats and in animals injected with cefmetazole at 200 µmol/kg i.v.

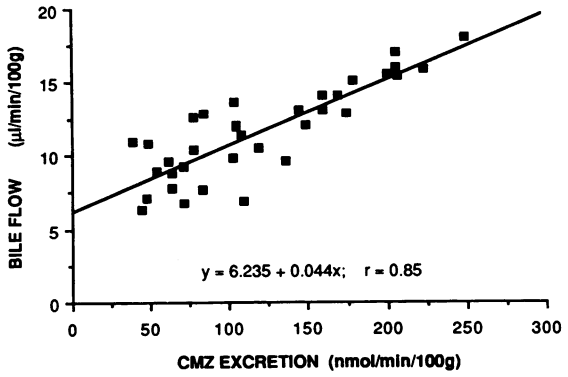

FIG. 4. Relationship between bile flow and cefmetazole (CMZ) excretion into bile in rats injected with cefmetazole at 200 µmol/kg i.v.

TABLE 1. Effect of cefmetazole on bile composition[a]

Bile composition	Amt (mmol/liter [nmol/min per 100 g])	
	Control	Cefmetazole-treated
Bile acid	38.3 ± 3.7 (285 ± 56)	24.5 ± 2.2[b] (256 ± 52)
Sodium	156 ± 3 (1,135 ± 41)	152 ± 3 (1,546 ± 77)[b]
Potassium	5.97 ± 0.20 (41.3 ± 5.1)	6.23 ± 0.40 (64.1 ± 8.2)[b]
Chloride	88 ± 4 (713 ± 29)	90 ± 5 (915 ± 41)[b]
Bicarbonate	26.2 ± 1.8 (197 ± 11)	24.8 ± 2.2 (258 ± 26)[b]
Phospholipid	3.09 ± 0.44 (20.4 ± 2.4)	1.28 ± 0.13[b] (13.1 ± 1.7)[b]
Cholesterol	0.40 ± 0.03 (2.64 ± 0.25)	0.08 ± 0.02[b] (0.87 ± 0.25)[b]

[a] Values are means ± standard errors of the means of six animals and correspond to 15 to 30 min following i.v. injection of cefmetazole at 200 μmol/kg.
[b] $P < 0.05$. Significantly different from control value.

secretion of bile acids, point to a similar conclusion (7). The effects of bile acids on the biliary excretion of cephalosporins could be explained, as suggested for other organic anions, by a direct effect of increased bile flow, intracellular interaction with mixed micelles, or cotransport via a vesicular system (9).

Choleresis originating at the ducts and ductules is thought not to occur in rats. Ductular reabsorption is almost nonexistent in this species (11), and secretin causes only a slight increase of bile flow, apparently originating at the hepatocellular level (22). Choleresis produced by cefmetazole in our experiments could be explained by stimulation of an electrolyte transport mechanism or by the osmotic activity of cefmetazole in bile.

Cefmetazole was concentrated in bile, and each micromole excreted was associated with 44 μl of bile, which strongly suggests that cefmetazole-induced choleresis is directly related to the biliary excretion of cefmetazole. Because the increase in bile flow is readily reversible and related to biliary levels of cefmetazole, it is not probable that this compound has effects similar to agents such as SC 2644, which stimulates bile acid-independent bile flow by activation of Na-K ATPase (30).

Several xenobiotics such as ethacrynic acid (8), diethyl maleate (4), valproic acid (29), ioglycamide (16), and piperacillin (7) stimulate bile flow in different species, including rats, by a mechanism that is thought to be predominantly due to the osmotic activity of these compounds or their metabolites. However, the volume excreted per micromole exceeds the theoretical maximal increment in bile flow anticipated for the osmotic activity of these agents (29). Apparently, other determinants of secretion are stimulated, a situation that would also be present in the case of cefmetazole-induced choleresis.

The increase in bile flow was accompanied in our experiments by a higher biliary excretion of inorganic electrolytes. Recent experimental data suggest that the active transport of bicarbonate through the canalicular membrane could play an important role in the bile acid-independent fraction formation (10, 19). However, cefmetazole does not stimulate the bile acid-independent fraction of bile flow through a concentrative mechanism of this kind, since the bicarbonate concentration in bile during cefmetazole-induced choleresis was similar to that observed in the controls and to preadministration levels.

Since cefmetazole is an amphoteric organic compound, highly ionized at physiological pH values, it can be coupled with inorganic cations during its transport into the biliary space. This could explain why biliary outputs of sodium plus potassium are higher than outputs of chloride plus bicarbonate, as observed by us. Anyway, further work is required to elucidate the mechanism of enhanced bile flow above that dependent on the osmotic activity of cefmetazole in bile.

Additionally, the present study indicates that cefmetazole choleresis is associated with decreased cholesterol and phospholipid biliary excretion without affecting bile acid secretion. This uncoupling of biliary lipid secretion has been previously reported for different agents such as bilirubin (1), sulfobromophthalein (24), ampicillin (2), ioglycamide (5), and cefoperazone (20). The effects are apparently not due to impairment of mixed micelle formation but rather to presecretory events (20).

In summary, our data indicate that the biliary excretion of cefmetazole not only depends on bile flow and liver function, as previously indicated, but also modifies bile flow and composition, causing a choleretic effect that is dose related and saturable. The increase in bile flow appears to be partly due to the osmotic properties of cefmetazole excreted into bile.

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