Effects of Bile Salt Infusion on Chlorpromazine-Induced Cholestasis in the Isolated Perfused Rat Liver (43327)

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Abstract. **The present study has demonstrated that tauroursodeoxycholate (TUDC), but not taurocholate, can reverse chlorpromazine (CPZ)-induced cholestasis in the isolated perfused rat liver. At an infusion rate of 1.5 μmol/min, TUDC led to restoration of bile flow in the perfused rat liver made cholestatic by the addition of 250 μM CPZ. This reversal was accompanied by an increased excretion of CPZ and its metabolites. A higher infusion rate of 5.0 μmol TUDC/min, however, led to only a transient increase in bile flow and to no increase in CPZ excretion. In contrast to the effects of TUDC, infusion of taurocholate led to an exacerbation of CPZ-induced cholestasis. The differences in the efficacy of the two bile salts may be due to their relative detergent (hydrophobic) properties.** [P.S.E.B.M. 1992, Vol 199]

Chlorpromazine (CPZ) can cause cholestatic jaundice in humans and impaired bile flow in animals (1). Although its hepatotoxicity in humans has been attributed to the idiosyncratic responses of individual patients, the drug also has been reported to exert toxic effects in both *in vivo* and *in vitro* experimental models. These results may have relevancy to the development of injury in humans (1). Indeed, CPZ interacts extensively with liver plasma membranes, altering their fluidity and the activity of membrane bound Na^+-, K^+-, and Mg-ATPases (2, 3). Moreover, CPZ alters the pericanalicular microfilaments and their function by modifying the actin polymerization (4). Indeed, these changes may be responsible for the cholestatic effects observed in experimental animals (5) and in the *in vitro* perfused rat liver (6–8).

In earlier studies, which examined potential therapeutic approaches to drug-induced hepatic injury, we have demonstrated that, in the *in vitro* perfused rat liver, the infusion of micelle-forming bile salts (BS) was able to prevent the induction of cholestasis by the estradiol 17β-D-glucuronide (E-17G) by enhancing its biliary excretion (9). Moreover, we have also demonstrated the possibility of reversing the cholestasis previously induced by E-17G by administering various BS (10). The present study focuses on the effect of BS administration on the cholestatic effects of CPZ. For this purpose we have used two BS, taurocholate (TC) and tauroursodeoxycholate (TUDC). Both have micelle-forming capacity, but differ in their detergent and choleretic properties.

Materials and Methods

The sodium salts of TC and TUDC (purity greater than 98%) were purchased from Calbiochem Berhinger Diagnostic (La Jolla, CA). Bovine serum albumin, Fraction V, CPZ-HCl, and 3-α-hydroxysteroid dehydrogenase were obtained from Sigma Chemical Co. (St. Louis, MO). [^{3}H]CPZ (benzene ring-^{3}H; sp act 17.1 Ci/mmol) was purchased from New England Nuclear (Boston, MA) with a radiochemical purity greater than 98%. The purities of TUDC and TC and the tritiated and unlabeled CPZ were confirmed by thin layer chromatography. All other chemical were reagent grade.

Male CD rats (250–270 g; Charles River Labs, Wilmington, MA) were used. They were allowed food and water *ad libitum* and were not starved prior to sacrifice. They were housed in wire cages on a 12:12-hr light:dark cycle. Livers were isolated from male CD rats

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(250–270 g) after pentobarbital sodium anesthesia (50 mg/100 g body wt), as described previously (8). After cannulation of the bile duct and portal vein with PE-10 and PE-205 tubing (Intramedic; Clay Adams), respectively, the livers were rinsed free of blood and then transferred to a thermostatic chamber (37 ± 1°C). The livers were perfused with a recycling, erythrocyte-free, Krebs-Henseleit buffer (200 ml; pH 7.45) supplemented with 480 mg of glucose, 2000 units of heparin, and 4 g of albumin. The hydrostatic pressure on the portal vein was 15 cm H_2O and a mixture of O_2 and CO_2 (95/5) was bubbled into the perfusate during the entire experimental period to avoid organ anoxia. At the end of each experiment, all nonhepatic tissue was removed and the liver was blotted on gauze pads and weighed. The mean liver weight of all experiments was 9.1 ± 0.4 g and no treatment altered the wet weight to dry weight liver ratio. Details of the perfusion procedure and its validation, as well as the methods to measure the bile and perfusate flow rates, have been described previously (8, 9, 11).

Liver perfusion experiments were initiated 3–5 min after cannulation of the bile duct and portal vein. Then, the BS (TC or TUDC) was infused into the perfusate at a rate of 0.25 μmol/min for the first 45 min of the experiment. Fifteen minutes after the initiation of the experiment, [^{3}H]CPZ (10 μCi diluted with the unlabeled compound) was added to the perfusate at an initial concentration of 150 or 250 μ*M*. CPZ was injected slowly into the perfusate over a 3-min period to minimize its depressant effect on hepatic perfusate flow (7). At 45 min (i.e., 30 min after CPZ addition), a 10-μmol bolus of the BS to be studied was injected into the perfusate and then the infusion rate of that BS was increased. For TC, the rate was increased to 0.75 or 1.5 μmol/min and for TUDC, to 1.5 or 5.0 μmol/min. Perfusion experiments were then prolonged for an additional 60 min (total experimental time, 105 min). Control experiments were run under the same conditions, but with no change in the initial infusion rate of the BS (0.25 μmol/min). Previous experiments in this model (10) have shown that the maximal secretory rates of TC and TUDC were 180 ± 10 and 390 ± 12 nmol/min/g liver, respectively.

Although CPZ is extensively metabolized in the liver to more or less toxic metabolites (2, 12), no attempts were made to identify them in perfusate or bile samples. Accordingly, the specific activity of radioactive samples was used to calculate the drug and its metabolite concentration, expressed in terms of the parent compound. Biliary BS concentration was measured by the hydroxysteroid dehydrogenase method, as described previously (11), and correction was made for transit time through biliary tree dead space (9).

All results are expressed as the mean ± SD of three to five experiments in each group. The Student's *t* test was used to compare means and the level of 5% was used to denote significance (13).

Results

Within 5 min after the initiation of infusion, bile flow and bile acid excretion had become stable. They remained constant until a bolus dose of 10 μmol was added and the BS infusion rate increased. This procedure increased both bile flow and TC excretion rates, which again equilibrated at the higher rates (Fig. 1). Similar effects were seen with TUDC (data not shown).

After the first 15 min of equilibrium with each BS infused into the perfusate at 0.25 μmol/min, the basal bile flow rates were 1.66 ± 0.15 μl/min/g liver for TC and 1.48 ± 0.10 for TUDC experiments ($P < 0.05$; Fig. 2). At this time, basal BS excretion was 19.8 ± 4 nmol/min/g liver for TC and 24 ± 5 for TUDC. These rates were similar to the rate of BS infused (approximately 20 nmol/min/g liver) and indicated that equilibrium had been achieved (Fig. 3). In the same experiments, perfusate flow rates through the liver were 5.6 ± 0.8 and 5.6 ± 0.9 ml/min/g liver for TC and TUDC, respectively.

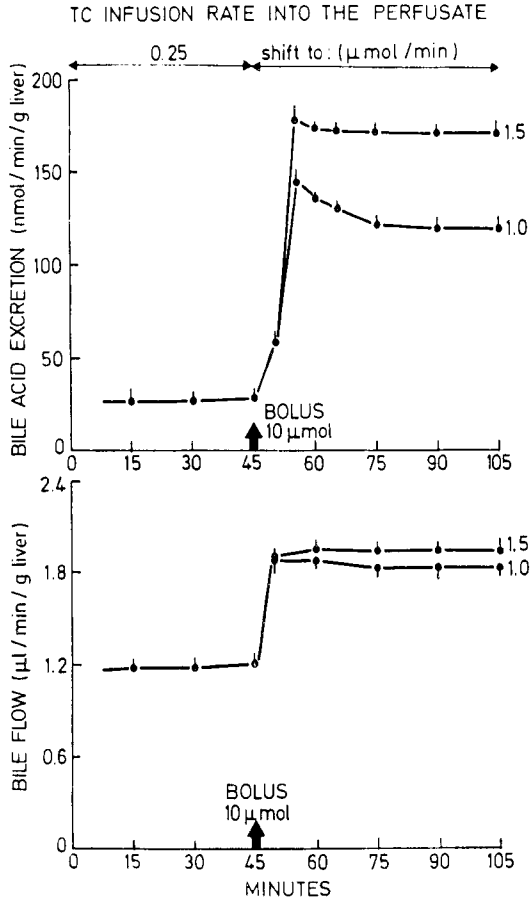

Figure 1. Effects of increasing the infusion rate of taurocholate on bile flow and biliary excretion of TC. A bolus of TC (10 μmol) was added at 45 min and the infusion rate was increased from 0.25 μmol/min to either 1.0 or 1.5 μmol/min.

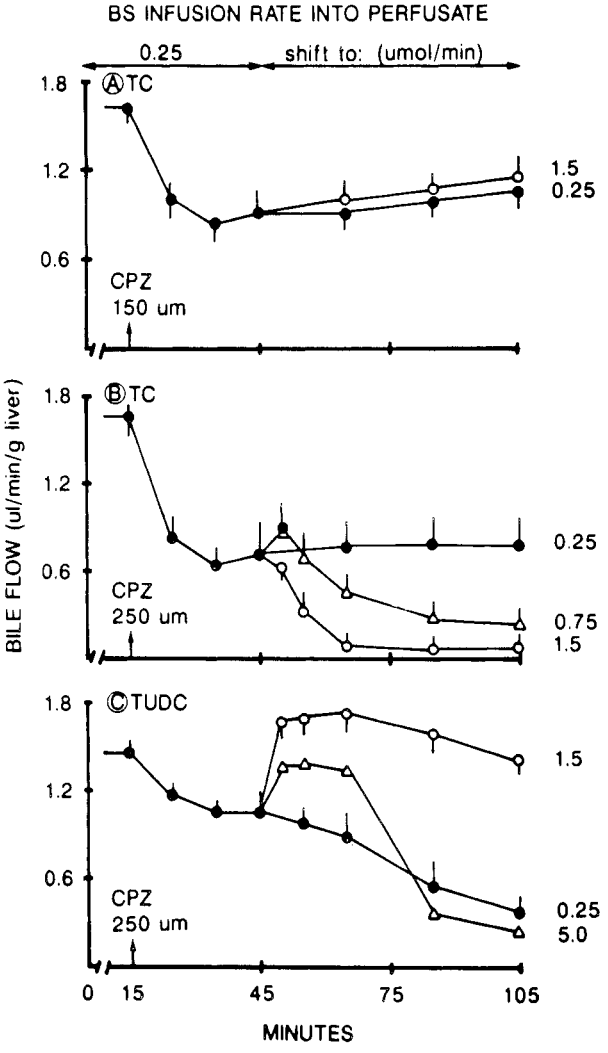

Figure 2. Effects of varying the infusion rate of taurocholate or tauroursodeoxycholate on bile flow in the chlorpromazine-treated liver. CPZ was added 15 min after initiation of the perfusion experiment, while the shift in the bile salt infusion rate occurred at 45 min. (A) TC (●) was infused into the perfusate at 0.25 μmol/min for the entire experimental time; the open circle (○) indicates those experiments in which a bolus of 10 μmol of TC was added at 45 min and a shift in the TC infusion rate to 1.5 μmol/min. (B) Closed circle (●), as in panel A. In other experiments, a bolus of 10 μmol of TC was infused into the perfusate, and was followed by an increase in the TC infusion rate to 0.75 (△) or 1.5 (○) μmol/min. Differences of bile flow rates in the three sets of experiments were significantly different ($P < 0.001$) from 50 to 90 min. (C) TUDC (●) was infused at 0.25 μmol/min for the entire experiment. In the other experiments, a bolus of 10 μmol of TUDC was added to the perfusate, and was followed by an increase in the TUDC infusion rate to 1.5 (○) or 5.0 (△) μmol/min. Differences in the bile flow rates in the three sets of experiments were statistically significant ($P < 0.001$) from 50 to 90 min.

At 15 min, CPZ was slowly added into the perfusate. In our experiments, the slow infusion of CPZ caused only a transient (10–15 min) reduction of the perfusate flow by 20–30%; in no instance did perfusate flow fall below the 3 mg/min/g liver necessary to avoid organ anoxia with an erythrocyte-free medium (14).

The addition of CPZ at 150 μ*M* decreased bile flow by approximately 50%. It remained depressed both in the experiments in which TC infusion was maintained at 0.25 μmol/min or was raised to 1.5 μmol/min (Fig. 2A). In all experiments, BS excretion was only temporarily decreased by CPZ, whereas BS concentration rose significantly (Fig. 3A), indicating an inhibition of the bile salt-independent flow (7). Although the shift to a greater infusion rate of TC induced a rise in BS excretion and biliary concentration, it did not lead to a choleretic response.

When CPZ was added at 250 μ*M* and TC was infused at 0.25 μmol/min for the entire experimental period, bile flow declined to 35–40% of the basal value and remained depressed. In these experiments, BS excretion was temporarily decreased and biliary BS concentration rose to an extent greater than that observed with the lower CPZ concentration. The addition, at 45 min, of a TC bolus of 10 μmol, followed by an increase in the TC infusion rate to 0.75 or 1.5 μmol/min, led to a dose-dependent decrease of bile flow. At the highest TC infusion rate (1.5 μmol/min), bile flow virtually ceased (Fig. 2B). Under these circumstances, although the biliary TC concentration was still higher than the basal value, TC excretion was markedly reduced, despite the higher TC infusion (Fig. 3B).

Different responses were observed in the experiments involving TUDC. When TUDC was infused at 0.25 μmol/min for the entire experimental period, 250 μ*M* CPZ led to a progressive decline in bile flow (Fig. 2C). The TUDC concentration in the bile rose progressively despite no increase in its excretion rate (Fig. 3C). Increasing the TUDC infusion rate to 1.5 μmol/min at 45 min after the 10 μmol bolus led to a prompt reversal of CPZ cholestasis. Bile flow rose immediately to values above or equal to the pre-CPZ value and remained elevated (Fig. 2C). The increase in bile flow was also accompanied by an increase in the excretion and biliary concentration of TUDC (Fig. 3C). However, maximal TUDC excretion by the CPZ-treated liver was only one third of the infused dose.

The infusion of TUDC at 5 μmol/min also led to the rapid reversal of CPZ cholestasis (Fig. 2C). However, this effect was transient and was not accompanied by a significant increase in TUDC excretion (Fig. 3C). From 65 min (20 min after the shift) to 105 min, bile flow and TUDC excretion declined progressively.

The reversal of CPZ cholestasis by the infusion of TUDC at 1.5 μmol/min was accompanied by a significant increase of CPZ (and its metabolites) excretion in bile (Table I). On the other hand, the adverse effect of higher rates of TC infusion was accompanied by a significant, dose-dependent decrease in CPZ excretion in the bile.

Discussion

The present study has demonstrated that the cholestasis induced by CPZ in the perfused rat liver can be

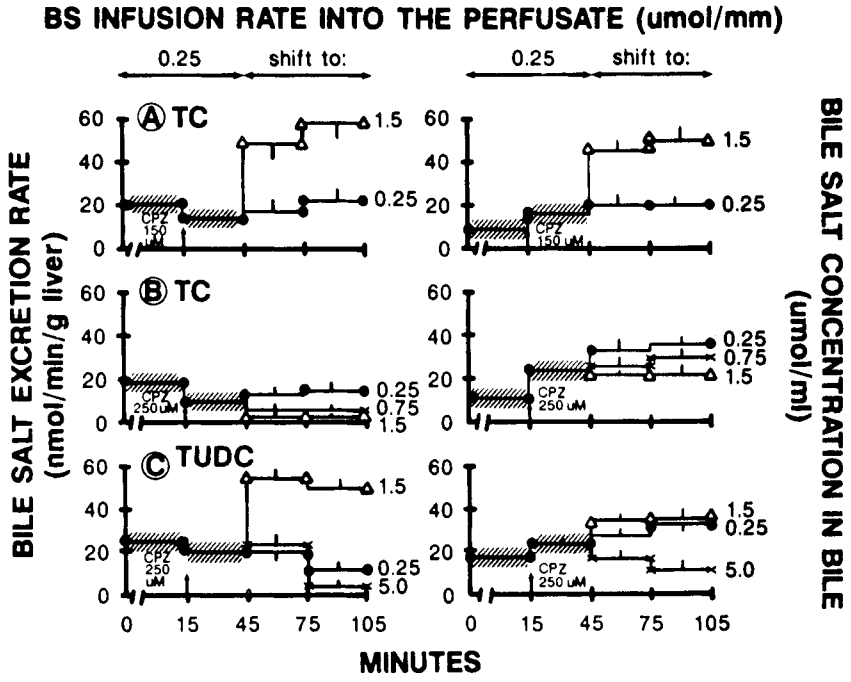

Figure 3. Effects of increasing the infusion rate of taurocholate or tauroursodeoxycholate on bile salt excretion and biliary concentration during cholestasis induced by administration of chlorpromazine. CPZ and TC or TUDC were added 15 min and 45 min, respectively, after the start of the perfusion. CPZ was added at 15 min. TC (●) or TUDC was infused at 0.25 μmol/min for the entire experimental period. In other experiments, a bolus of 10 μmol of either TC or TUDC was added at 45 min, followed by a shift of infusion rate to 1.5 μmol/min TC (A, B; △) or TUDC (C; △) or to 0.75 μmol/min TC (B; x) or 5.0 μmol/min TUDC (C; x).

Table I. Effects of Varying the Infusion Rates of Taurocholate or Tauroursodeoxycholate on Excretion of Chlorpromazine by the Isolated Rat Liver

Initial CPZ concentration in perfusate	μmol CPZ excreted (Min post-CPZ addition)	
	30	90
CPZ—150 μM		
TC 0.25-0.25[a]	656 ± 62 (2.1)[b]	2740 ± 253 (9.1)
TC 0.25—1.5	752 ± 75 (2.5)	2438 ± 312 (8.1)
CPZ—250 μM		
TC 0.25—0.25	1260 ± 224 (2.5)	4912 ± 146 (9.8)
TC 0.25—0.75	1012 ± 172 (2.0)	2953 ± 358 (5.9)[c]
TC 0.25—1.50	1069 ± 281 (2.1)	1201 ± 147 (2.4)[c]
CPZ—250 μM		
TUDC 0.25-0.25	1392 ± 217 (2.7)	3694 ± 745 (7.4)
TUDC 0.25-1.50	1398 ± 72 (2.8)	6565 ± 1025 (13.2)[d]
TUDC 0.25-5.00	1403 ± 159 (2.8)	2687 ± 129 (5.4)

[a] TC or TUDC was infused at 0.25 μmol/min from 0 to 45 min. The second number represents the infusion rate, in μmol/min, of the bile salt from 45 to 105 min. There were three to five experiments in each group.
[b] The numbers in parentheses are the percentages of the infused CPZ dose excreted in the bile.
[c] $P < 0.01$ from TC controls.
[d] $P < 0.05$ from TUDC controls.

reversed by increasing the infusion rate of TUDC. Reversal occurred following the infusion of a 10 μmol bolus of TUDC and a shift to a higher infusion rate (from 0.25 to 1.5 μmol/min); it lasted for the entire experimental period (Fig. 2C). A significantly greater excretion of CPZ and metabolites in the bile accompanied this effect (Table I). A higher rate (5 μmol/min) temporarily reversed the cholestasis.

Comparable infusion rates of the more hydrophobic, yet more choleretic, TC not only did not ameliorate the cholestasis induced by 150 μM CPZ, but even enhanced it at 250 μM.

In this same model, we have found that BS infusion can reverse the cholestasis caused by E-17G and that the relative beneficial versus adverse effects seemed related to the physicochemical properties of the BS (10).

With E-17G, TC appeared to be the most effective, since lower doses were able to reverse cholestasis, but it had a lower margin of safety, since the highest dose did not ameliorate the impaired bile flow. In contrast, the more hydrophilic TUDC was able to ameliorate the E-17G cholestasis at all doses employed (from 1 to 6 μmol/min) and showed no adverse effects (10).

To reconcile the results of the two studies, one must consider that the transport capacity of each BS by the hepatocyte is inversely related to its detergent (hydrophobic) and toxic effects. Therefore, TC, which is more hydrophobic than TUDC, has a lower maximal secretory rate than TUDC. When TC is infused at rates that supersaturate its transport capacity, it becomes toxic, as reflected by a marked decline in bile flow and BS excretion (15).

In agreement with other studies (5, 7), we have demonstrated that CPZ decreased the BS output and raised the BS biliary concentrations. Indeed, studies in isolated hepatocytes have demonstrated that CPZ inhibits the efflux of TC to a greater extent than its uptake, thus markedly increasing the intracellular TC content (16). Moreover, CPZ and TC may form intracellular precipitates that may further impair TC transport (17).

It is possible, therefore, that the higher rates of TC supersaturate the decreased ability of the CPZ-treated liver to handle TC. Accordingly, it accumulates in toxic amounts and causes additional toxicity. This may also apply to TUDC. Infusion of TUDC at 1.5 μmol/min reverses the cholestasis. However, at a higher rate (5.0 μmol/min), it has only a transient effect. Then, it also becomes toxic.

The reversal of cholestasis by TUDC was accompanied by an increase in biliary excretion of CPZ. One may speculate that removal of CPZ (and, perhaps, toxic metabolites) from the liver into the bile alleviated the cholestasis caused by CPZ administration. At effective doses, TUDC may facilitate CPZ excretion through micellar solubilization (18). Alternatively, TUDC may counteract the adverse effects of CPZ on membrane fluidity (19), restoring the membrane-ATPases activity and, thereby, promoting bile flow and CPZ transport.

This study supports the concept that the administration of micellar-forming BS with hydrophilic properties, such as TUDC, may be beneficial in the reversal of the hepatotoxic effects of certain drugs (e.g., CPZ, E-17G) with amphipathic structures that interfere with hepatocytic membrane function.

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