Drug-Induced Cholestasis in the Perfused Rat Liver and Its Reversal by Tauroursodeoxycholate: An Ultrastructural Study (43328)

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Abstract. **Chlorpromazine at a concentration of 250 μM and estradiol-17β-D-glucuronide at 17.5 μM on infusion led to a sharp reduction in bile flow by the *in vitro* perfused rat liver. This was accompanied by fragmentation and a loss of canalicular microvilli, dilatation of canaliculi, and thickening of pericanalicular ectoplasm. Less prominent were the smooth endoplasmic reticulum dilatation, lysosomal lamination, and the appearance of amorphous bile in hepatocyte cytoplasm. The bile flow and electron microscopy appearance were restored to normal by infusion of tauroursodeoxycholate in a concentration of 5 μmol/min for the estradiol-17β-D-glucuronide-induced cholestasis and 1.5 μmol/min for the chlorpromazine-induced cholestasis. Changes in ultrastructure paralleled changes in bile flow. These observations demonstrate the feasibility of electron microscopy studies on the perfused liver, and the rapidity with which cholestatic changes appear.** [P.S.E.B.M. 1992, Vol 199]

The isolated perfused rat liver has been employed to study the adverse effects of a number of agents (1–10). It has been particularly useful for the study of factors affecting bile blow (10). The parameters employed have been mainly the volume of bile excreted, its contents (including bile acids), and the ability of the isolated perfused rat liver to clear the perfusate of molecules contained within it. There has been relatively little attention to morphologic changes effected by perfusion *per se* (6, 7) and apparently little effort to correlate morphologic changes with changes in bile flow. The present study was conducted in an effort to determine whether cholestatic effects of estradiol-17β-D-glucuronide (E-17G; 3, 8, 9) and chlorpromazine (CPZ; 4, 5) on the isolated perfused rat liver are mirrored by ultrastructural changes. The morphological reflection of the amelioration of cholestatic effects of the drugs (8) was also examined.

Materials and Methods

Materials. Estradiol-17β-D-glucuronide, chlorpromazine hydrochloride, and bovine serum albumin, Fraction V, were obtained from Sigma Chemical Co. (St. Louis, MO). The sodium salt of tauroursodeoxycholate (TUDC) was obtained from Calbiochem, Behringer Diagnostic (La Jolla, CA).

Procedure. Rats were purchased from Charles River Laboratories, housed in wire cages, and fed standard laboratory chow *ad libitum.* They were kept on a 12:12-hr light:dark cycle and were monitored for 1 week prior to use. Livers isolated from nonfasted animals were employed. Livers were isolated under pentobarbital sodium anesthesia (50 mg/100 g body wt, as described previously (1–4). After cannulation of the portal vein with PE-10 and PE-205 tubing (Intramedic; Clay Adams), respectively, the livers were rinsed free of blood and transferred to a thermostatic chamber (37 ± 1°C). The livers were perfused with recycling, erythro-

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cyte-free Krebs-Henseleit buffer (200 ml, pH 7.45), supplemented with 450 mg of glucose, 2000 units of heparin, and 4 g of albumin. The hydrostatic pressure on the portal vein was 15 cm H_2O, providing a constant flow through the liver of 4.7 ± 0.2 ml/min/g liver. A mixture of O_2 and CO_2 (95/5) was bubbled into the perfusate during the entire experimental period to avoid hepatic anoxia. This perfusion procedure has been validated previously by measurement of enzyme release, oxygen uptake, glycogen synthesis, bile flow, and preservation of normal ultrastructure (2).

Liver perfusion experiments were initiated by infusion with TUDC into the perfusate reservoir at the rate of 25 μmol/min/g liver for the first 45 min of the experiment. At 15 min after the start of the TUDC infusion, E-17G at a concentration of 125 μ*M*, or CPZ at a concentration of 250 μ*M*, was infused. Each of these agents led to a sharp drop in bile flow (Fig. 1) within 20 min. At 45 min, the rate of infusion of TUDC was increased to 1.5 μmol/min for the CPZ experiments and to 5.0 μmol/min for the E-17G experiments. (In separately conducted studies [11], the concentration of TUDC that was most effective in restoring bile flow arrested by CPZ was 1.5 μmol/min and in restoring bile flow arrested by E-17G, 5.0 μmol/min.)

Bile flow was estimated by measuring the time necessary to collect 20 μl of bile. It was measured at 5-min intervals throughout the experiment. For measurement of effects of the drugs on bile flow, five experiments at each concentration were performed.

At the end of each experiment, the liver was removed and three blocks, each measuring about 1 cm in length and 3 mm in thickness, were removed from the right, middle, and left portions of the liver. They were fixed in 2.5% glutaraldehyde, dehydrated in graded alcohols, cleared in propylene oxide, and impregnated and embedded in epon resin. Thin sections were mounted on copper grids, stained with 4% uranyl acetate and lead citrate, and examined in a Zeiss EM 109 electron microscope. Liver sections were coded and morphology was evaluated by K. G. I. without awareness of the treatment.

For the study of the effects of perfusion with each drug with and without TUDC, four to six perfusions were performed for each group (Table I). For the study of the effects of the drugs and TUDC on ultrastructure, two perfusions were performed for each group.

Results

Effects of Drugs and TUDC on Bile Flow. Both E-17G and CPZ led promptly to a sharp reduction in bile flow (Fig. 1). The flow dropped by 70–80% during the infusion of E-17G and by 60% during infusion of CPZ. Infusion of TUDC at the increased rate of 1.5 μmol/min of the CPZ-treated liver led to a prompt increase in the rate of bile flow (Fig. 1). A return of bile

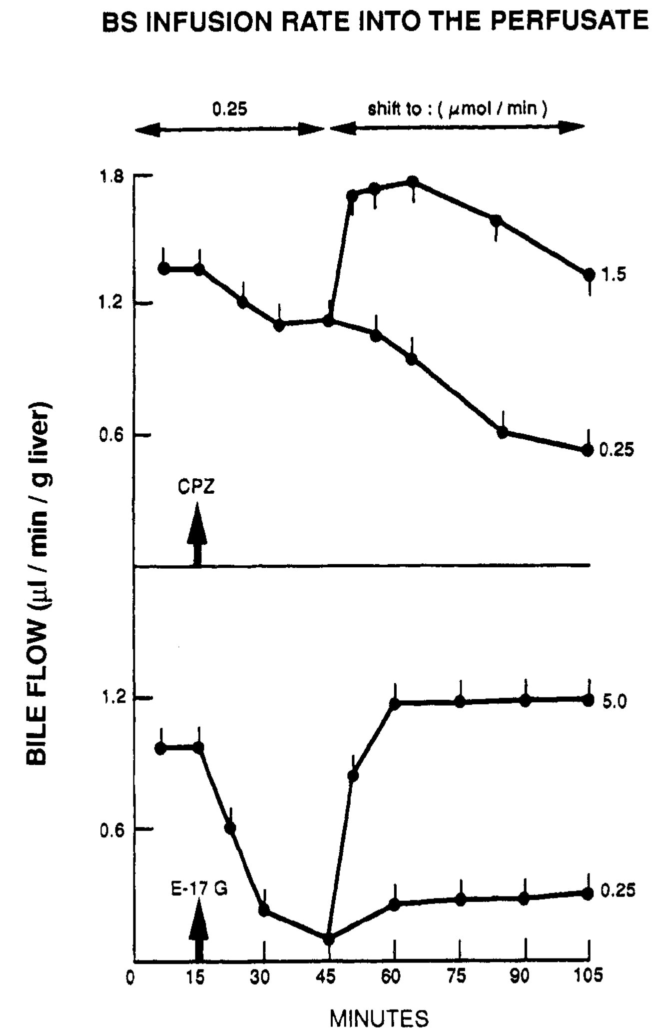

Figure 1. Effects of E-17G and CPZ on bile flow during infusion of TUDC at 0.25 μmol/min and after addition of TUDC at μmol/min. Rate of infusion shown by each line. Note the sharp reduction in bile flow imposed by each drug and the restoration of flow by the addition of the bile salt (BS) TUDC.

flow by the E-17G-treated liver to control values also was effected by perfusion with TUDC at the increased concentration of 5 μmol/min (Fig. 1).

Ultrastructural Changes. The appearance of the liver by electron microscopy, at the end of the perfusion, is shown in Figures 2 and 3 and the details are tabulated in Table I.

After each of two control perfusions, the appearance was normal. The canalicular lumens were empty, microvilli appeared normal, and cytoplasmic spaces and organelles appeared normal (Fig. 2A). Cholestasis effected by E-17G led to the fragmentation and disappearance of canalicular microvilli, dilatation of canaliculi, and widened intercellular spaces. There was also thickening of the pericanalicular ectoplasm, dilatation of the smooth endoplasmic reticulum, lamination of lysosomes (Fig. 2B), and amorphous bile in the cyto-

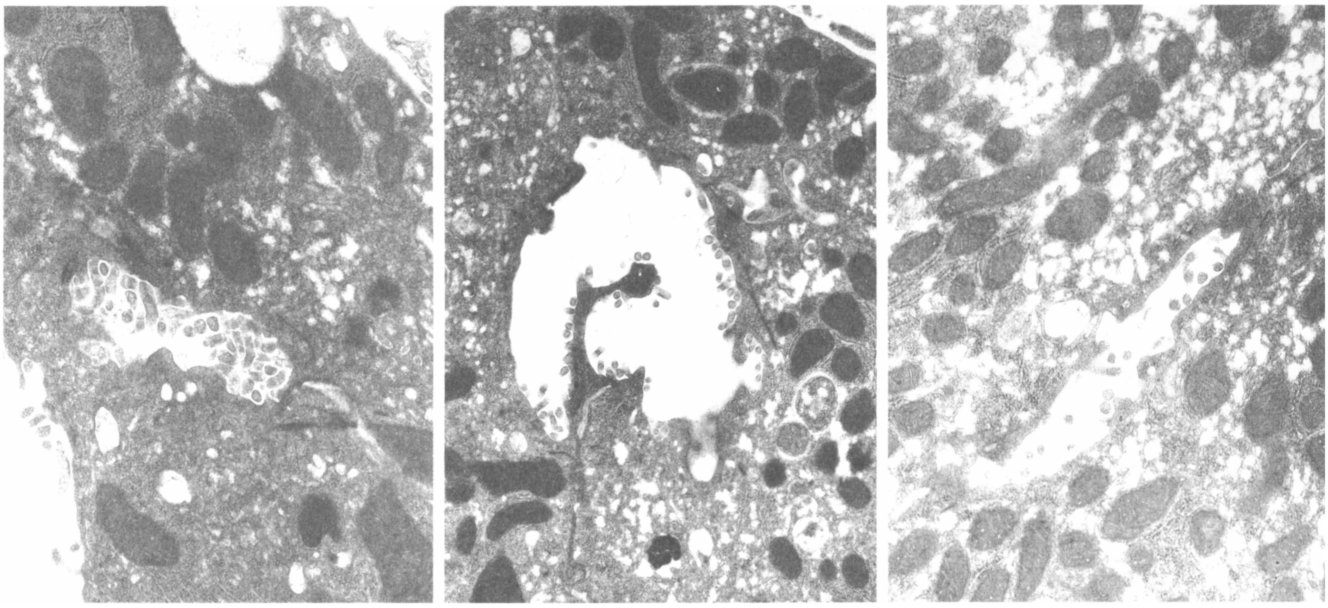

Figure 2. (A) Electron micrograph of control liver. Note normal canaliculi with empty lumen and normal microville (original magnification ×40,670). (B) Electron micrograph of liver perfused with E-17G. Note fragmentation and decrease in microvilli (especially in the left half), dilatation of the canaliculus, and lamination of lysosomes (original magnification ×24,300). (C) Electron micrograph of liver perfused with E-17G *and* TUDC. Note striking decrease in abnormalities shown in Figure 2B (original magnification ×37,700).

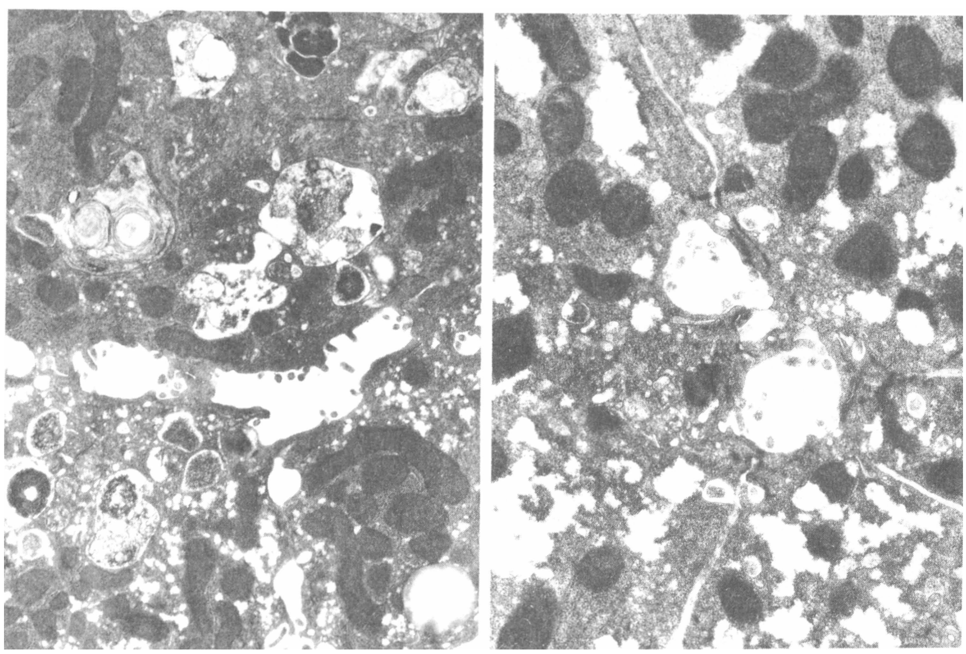

Figure 3. (A) Electron micrograph of liver perfused with CPZ. Striking accumulation of dense, amorphous, and laminated inclusions in lysosomes (original magnification ×22,600). (B) Electron micrograph of liver perfused with CPZ *and* TUDC. Note striking decrease in degree of abnormality (original magnification ×20,835).

plasm, but these changes were of a somewhat lesser degree than the canalicular changes.

Livers perfused with both TUDC at 5 μmol/min and E-17G showed much less abnormality. Canalicular dilatation and microvillar loss was barely apparent. Other changes were also less marked (Fig. 2C).

Perfusion with CPZ led to changes similar to those effected by E-17G (Fig. 3A). The canalicular abnormalities seemed ameliorated by perfusion with TUDC at the lower concentration of 1.5 μmol/min (Fig. 3B). Grading the severity of the abnormalities for each experiment (Table I) permitted examination of the relationships between the effects of drugs on bile flow and on morphologic features.

Table I. Effects of Treatment on Electron Microscopy Morphology[a]

Treatment[b]	Canaliculi			Cytoplasmic organelles				
	Dilatation	MV	Ectoplasm thickening	Bile in cytoplasm	Lamination of lysosomes	Golgi	SER	Mitochondrial changes
Control	0	0	0	0	0	0	0	0
Control	0	0	0	0	0	0	0	0
E-17G	3+	3+	2+	2+	2+	Pr	2+	2+
E-17G	2+	2+	2+	2+	1+	Pr	2+	2+
E-1⁷G, TUDC	1+	1+	1+	2+	1+	Pr	1+	2+
E-17G, TUDC	1+	0	1+	1+	1+	No	1+	2+
CPZ	3+	3+	3+	3+	2+	Pr	1+	2+
CPZ	2+	2+	2+	3+	2+	Pr	2+	3+
CPZ, TUDC	2+	1+	2+	2+	1+	Pr	2+	2+
CPZ, TUDC	1+	1+	1+	2+	1+	No	2+	2+

[a] Abbreviations and symbols used in table: MV, microvilli loss and fragmentation; SER, smooth endoplasmic reticulum changes; Pr, prominence; 0, no change; 1+, minimal change; 2+, moderate change; 3+, marked change.
[b] Concentrations described in text.

The functional and morphologic changes appeared to parallel each other. The striking reduction in bile flow effected by E-17G and by CPZ was accompanied by marked ultrastructural abnormalities, especially of the canaliculi. The reversal of the cholestatic effects of these drugs by simultaneous perfusion of the liver with TUDC in high concentrations seemed to be paralleled by lesser degrees of the electron microscopy canalicular abnormalities.

Discussion

Cholestasis is characteristically associated with canalicular and cytoplasmic organelle changes. Canalicular changes include distortion, fragmentation, and loss of microvilli, accompanied by pericanalicular ectoplasmic thickening (6, 7, 11). Organelle changes have included a prominence and an altered appearance of Golgi apparatus, lamellar changes in lysosomes, an increase in smooth endoplasmic reticulum, and mitochondrial abnormalities. All of these changes, as well as the presence of bile in the hepatocyte cytoplasm, were observed in livers perfused with E-17G and with CPZ. Particularly prominent were the canalicular changes. Most of these changes, especially the canalicular ones, have been considered to be the result, at least in part, rather than the cause, of cholestasis, since they are seen in extrahepatic as well as intrahepatic cholestasis. The appearance of these changes in the briefly perfused rat liver was reported by Hruban *et al.* (6) and observed in the current study.

The rapid development of the abnormalities in livers perfused with cholestasis-producing drugs, in concert with the rapid development of impaired bile flow, suggests that the canalicular changes may lead to, as well as result from, cholestasis. Indeed, the rapidity of onset suggests that the changes were the result of an effect of the E-17G and of the CPZ presumably involving alteration of hepatic plasma membranes, changes apparently attributable to the effects of the drugs on both canalicular and basolateral membranes (6, 7, 10). The reversal of cholestasis, as well as the canalicular structural abnormalities, presumably involves reversal of the physicochemical changes in the membranes.

The infusion of TUDC at a high concentration led to an improved bile flow, virtually returning it to the level seen before E-17G or CPZ was introduced. The infusion of TUDC also appeared to prevent the adverse effects of the drugs on the canalicular ultrastructure.

The reversal by TUDC of the structural effects of CPZ and E-17G suggests that the prevention of cholestasis reflects the prevention of tissue injury. It is of particular interest that TUDC, which reverses functional and morphological evidence of cholestasis in this *in vitro* model, offers promise of effectiveness in the treatment of chronic cholestasis in humans.

The opinions and assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army, the Department of Defense, or the Evironmental Protection Agency.

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