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Effect of Chlorpromazine on Hepatic Clearance and Excretion of Bile Acids by the Isolated Perfused Rat Liver[1] (41463)

NICOLA TAVOLONI,[2] JAMES S. REED,[3] AND JAMES L. BOYER

The Polly Annenberg Levee Hematology Center, Department of Medicine, and Department of Physiology, Mount Sinai School of Medicine, New York, New York 10029, and The Liver Study Unit, Department of Medicine, Yale University School of Medicine, New Haven, Connecticut 06510

Abstract. Chlorpromazine (2.5×10^{-4} *M*) significantly diminished the rate of bile acid excretion when added to the perfusate of the isolated perfused rat liver during a constant infusion of sodium taurocholate (40 μmol/hr). The inhibition of bile acid excretion was associated with a significant reduction of the clearance and extraction efficiency of bile acids and with an immediate and progressive increase in the perfusate concentrations of both glutamic pyruvic and glutamic oxalacetic transaminase activities. The reduced hepatic perfusion which followed the drug administration only partly accounted for the diminished bile acid excretion, as these effects of chlorpromazine on bile acid extraction and transaminase release could not be reproduced by comparable mechanical restriction of portal perfusate flow in control livers. The present findings together with previous observations demonstrating alterations in hepatic function and ultrastructure (12, 31), suggest that the inhibition of bile acid excretion by chlorpromazine in the isolated perfused rat liver is related to a generalized effect of the drug on hepatocyte plasma membranes, resulting in impairment of the uptake and eventual excretion of bile acids.

Clinical jaundice is a rare complication of psychotherapy with chlorpromazine (CPZ). Although host idiosyncrasy has long been implicated in the pathogenesis of this form of drug-induced liver injury (1–5), several observations strongly suggest that CPZ exerts a direct toxic effect on the liver. Thus, a high incidence (up to 50%) of an anicteric, mild hepatic dysfunction has been reported among individuals exposed chronically to the drug (1, 4, 6) and a variety of hepatotoxic effects of CPZ have been demonstrated *in vivo* (7–12) and *in vitro* (13–19) experimental models.

Carey *et al.* (16) have demonstrated that CPZ forms insoluble complexes with bile acids *in vitro* when the latter are in solution above their critical micellar concentration. They proposed this physicochemical interaction between the phenothiazine and the bile acids as a possible mechanism of CPZ-induced cholestasis. *In vivo* experimental studies show that CPZ inhibits bile acid excretion both in the Rhesus monkey (10) and in the isolated perfused rat liver (IPRL) (12). However, CPZ simultaneously depresses hepatic perfusate flow in the IPRL and it is unclear whether the diminished bile acid excretion is secondary to impaired hepatic hemodynamics. The primary objective of the present study was to examine the effect of CPZ on the excretion of bile acids in greater detail in the IPRL and to clarify the relationship of the circulatory alterations induced by the drug to its inhibitory effect on bile acid excretion.

Methods. *Liver perfusion.* Male Sprague–Dawley rats (Charles River, Wilmington, Mass.) were used in these studies as liver donors at the weight of 270–320 g. The animals were housed in a temperature-controlled room (22°) with alternating 12-hr light–dark cycles and maintained on Purina Lab Chow and water *ad libitum* prior to use. The liver

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[2] To whom reprint requests should be addressed: The Polly Annenberg Levee Hematology Center, Department of Medicine, Mount Sinai School of Medicine of the City University of New York, 1 Gustave L. Levy Place, New York, N.Y. 10029.

[3] Present address: Kansas City VA Hospital, 4801 Linwood Blvd., Kansas City, Mo. 64128.

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was removed and perfused as previously described (20) following pentobarbital anesthesia (50 mg/kg, ip). Perfusions were carried out through the portal vein with 100 ml of Ringer–Krebs bicarbonate solution containing 0.8 g bovine serum albumin fraction V (Armour Pharm. Co., Chicago, Ill.), 150 mg glucose, 1000 units heparin, and 5 mg hydrocortisone–Na succinate. The perfusate was oxygenated with a humidified mixture of 5% CO_2 and 95% O_2 at a gas flow rate of 300–500 ml/min and maintained at a constant temperature of 37°. The pH of the perfusate was adjusted to 7.44 with 1 *N* NaOH prior to initiation of the perfusion and automatically maintained throughout each study with 0.75 *N* $NaHCO_3$ by means of a titrator-autoburette device (Radiometer, Copenhagen). In all experiments, perfusion pressure was constantly kept at 15 cm of perfusate. To minimize production of photooxidative metabolites of CPZ (21–24), perfusions were carried out in a darkened room. Illumination was provided by a dark room type 15-W incandescent bulb with red filter. CPZ was diluted with an appropriate volume of normal saline prior to use and stored in an aluminum-covered flask at 4° until added to the perfusate. All experimental livers received 1 ml of the CPZ solution to achieve the desired perfusate concentration, whereas control perfusions were given 1 ml of normal saline. Bile was collected in small vials immersed in an ice bath and stored at −20° until analyses were done. Hepatic blood flow rate was measured by timed collections of the vena cava outflow.

Design of the experiments. The effect of CPZ on bile acid excretion, bile acid clearance from perfusate, and hepatic perfusate flow was studied in the IPRL while a constant infusion of sodium taurocholate was maintained at 40 μmol/hr. The taurocholate infusion was initiated after a 20-min equilibration period and was continued until the end of the perfusion. Twenty minutes after the bile acid infusion was started, when a steady state in the biliary excretion of bile acids was achieved, CPZ was added to the perfusate as a bolus injection to obtain a concentration of 2.5×10^{-4} *M*. Two groups of experiments were performed. The first was designed to study the effect of CPZ on bile acid excretion and hepatic perfusate flow throughout the study. In the second group of experiments, perfusate samples from portal inflow and cava outflow were obtained simultaneously 10 min after CPZ (2.5×10^{-4}) was administered, at the time maximal inhibitory effects of the drug were obtained. Perfusate samples from cava outflow were obtained by timed collections in preweighted 25-ml beakers so that the rate of hepatic perfusion could be determined accurately. Portal and cava perfusate samples averaged 5 and 10 ml, respectively. The perfusion was terminated after the perfusate samples were obtained. Since CPZ markedly depressed the rate of hepatic perfusion, two sets of control experiments were also conducted. In one group, perfusate flow was maintained at a constant high rate (approximately 7 ml/min/g liver) throughout the experiment. In the second control group, hepatic blood flow was mechanically reduced by partially clamping the portal inflow tubing and progressively diminishing perfusate flow from 7 to 0.7–1.1 ml/min/g liver during a 10-min period, comparable to the reduction in flow produced by CPZ. Thereafter, perfusate flow was progressively increased in increment during the next 25 min to previous control values. An additional set of experiments was also carried out to study the effect of CPZ (2.5×10^{-4}M) on glutamic-pyruvic (GPT) and glutamic-oxalacetic (GOT) transaminase release from the IPRL. In these studies CPZ was added as a bolus to the perfusate 20 min after a sodium taurocholate infusion (40 μmol/hr) was initiated and the experiment was continued for an additional 180-min period. Perfusate samples (0.5 ml) were obtained at 20-min intervals and analyzed for transaminase activities.

Analyses. Bile acids in bile were determined by the hydroxy-steroid dehydrogenase procedure (25). Bile acids in perfusate were determined by a spectrofluorometric method reported previously (26). GOT and GPT activities in perfusate were measured by the methods of Karmen (27) and Wroblewski and LaDue (28) respectively, using a reagent kit from Sigma (St. Louis, Mo.).

Statistic and kinetic analyses. Experi-

mental data were analyzed for statistical differences by Student's t test. When more than two groups of data were compared, the analysis of variance followed by the Bonferroni's t statistics (29) were used. The hepatic extraction (E_{ss}) and clearance (C_{ss}) of bile acids at steady state were calculated as follows:

$$E_{ss} = HPF\ (C_p - C_c), \quad [1]$$

$$C_{ss} = HPF\ (1 - C_c/C_p), \quad [2]$$

where HPF = hepatic perfusate flow and C_p and C_c are the concentrations of bile acids in the portal inflow and cava outflow tubing, respectively. Both Eqs. [1] and [2] hold on the assumption that the rate of HPF leaving the liver equals that entering the organ and that C_p and C_c are constant during the time portal and cava samples are taken.

Results. CPZ significantly diminished the rate of bile acid excretion when added to the perfusate of the IPRL during a constant infusion of sodium taurocholate (40 μmol/hr). As previously demonstrated for bile flow (12), the inhibitory effect of CPZ was dose related in a concentration range of 1×10^{-4}–5×10^{-4} M and was associated with a parallel diminution of hepatic perfusate flow. Both effects were reversible after 10 min. Figure 1A illustrates the effects of CPZ when added to the perfusate at 2.5×10^{-4} M. To determine whether the impaired hepatic hemodynamics were responsible for the diminished bile acid excretion, portal perfusate flow was mechanically reduced to similar levels and for the same duration as observed in the CPZ livers. As illustrated in Fig. 1B, bile acid excretion significantly declined when hepatic perfusate flow was diminished from 6.9 to 0.9 ml/min/g liver. In these blood flow restricted controls, however, bile acid excretion only decreased by 21 and 30% during the first and second 10-min period, respectively, as opposed to a 64 ($P < 0.001$) and 41% reduction in the same periods following CPZ administration.

To further assess the role of the circulatory alterations induced by CPZ on the inhibition of bile acid excretion, we studied the

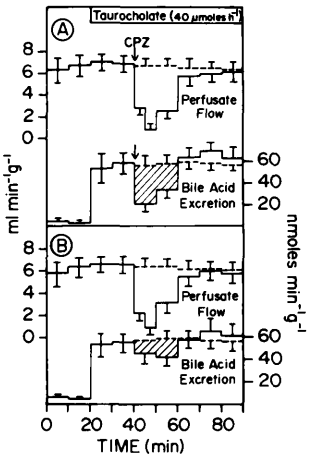

FIG. 1. (A) Effect of CPZ (2.5×10^{-4} M) on hepatic perfusate flow and bile acid excretion in the IPRL. CPZ was added as a bolus to the perfusate 20 min after the taurocholate infusion was initiated. Values are means ± SD of six controls (dashed line) and five CPZ (solid line) experiments. (B) Effect of mechanically restricting hepatic perfusate flow on bile acid excretion. Portal blood flow was artificially reduced by partially clamping the inflow tubing to mimic the effect of CPZ at 2.5×10^{-4} M on hepatic perfusion (see Methods for details). The dashed line refers to the high-flow-perfused controls shown in A; the solid line refers to the blood flow-restricted controls ($n = 5$).

perfusate clearance of sodium taurocholate in high-flow- and low-flow-perfused controls and in CPZ-treated livers. Table I summarizes the results obtained in the three groups of experiments. In high-flow-perfused controls (6.92 ml/min/g liver), sodium taurocholate was efficiently removed from perfusate when constantly infused at 40 μmol/hr. At steady state, its plasma clearance was 5.27 ml/min/g liver and 77% of the portal load was extracted after a single pass. As expected, when hepatic perfusate flow was reduced mechanically to 0.95 ml/min/g liver, the removal of taurocholate from perfusate substantially declined. Portal delivery diminished to 23.65 nmol/min/g liver and, although the extraction efficiency increased from 77 to 97%, bile acid clearance dropped considerably to 0.92 ml/min/g liver. Ten minutes after the addition of CPZ to the perfusate (2.5×10^{-4} M) the plasma clearance of bile acids diminished even further when com-

TABLE I. PERFUSATE CLEARANCE OF SODIUM TAUROCHOLATE BY THE ISOLATED PERFUSED RAT LIVER

	Controls (HF, $n = 4$)	Controls (LF, $n = 4$)	P^a	CPZ ($n = 4$)	P^b	P^c
LW (g)	9.89 ± 0.83	10.31 ± 0.97	0.2	10.07 ± 0.78	0.2	0.2
HPF (ml/min/g)	6.92 ± 1.05	0.95 ± 0.18	0.001	0.77 ± 0.35	0.001	0.2
C_p (nmol/ml)	9.92 ± 2.21	24.82 ± 3.75	0.001	34.15 ± 8.63	0.01	0.1
L (nmol/min/g)	70.23 ± 22.05	23.65 ± 5.24	0.01	27.65 ± 7.11	0.01	0.2
C_c (nmol/ml)	2.33 ± 0.62	0.83 ± 0.31	0.01	23.78 ± 8.28	0.01	0.01
E (nmol/min/g)	53.72 ± 7.11	22.91 ± 3.08	0.001	6.82 ± 1.84	0.001	0.001
EE (%)	76.78 ± 7.40	96.53 ± 1.79	0.001	28.16 ± 10.12	0.001	0.001
Cl (ml/min/g)	5.27 ± 0.75	0.92 ± 0.19	0.001	0.20 ± 0.07	0.001	0.001

Note. Values are means ± SD and correspond to 10 min after CPZ (2.5×10^{-4} *M*) was administered or 10 min after hepatic perfusate flow was mechanically reduced (see Fig. 1A, B). Number of experiments in parentheses. *LW*, liver weight; *HPF*, hepatic perfusate flow; C_p and C_c, bile acid concentrations in the portal and cava outflow tubings, respectively; *L*, bile acid load; *E*, bile acid extraction; *EE*, extraction efficiency $[(1 - C_c/C_p) \times 100]$; *Cl*, bile acid clearance; HF, high-flow-perfused livers; LF, low-flow-perfused livers.

[a–c] P^a = LF vs HF controls; P^b = CPZ vs HF controls; P^c = CPZ vs LF controls. Underlined values indicate significant differences which were assessed by the analysis of variance followed by the Bonferroni's *t* statistics (29).

pared to both high-flow- and low-flow-perfused controls. The rate of hepatic perfusion and the portal load of bile acids in the CPZ-treated livers were quite comparable to those observed in the blood flow restricted controls. However, the extraction efficiency in the CPZ livers was reduced to 28% and bile acid clearance dropped to 0.20 ml/min/g liver.

To determine whether the inhibitory effect of CPZ on the perfusate clearance and excretion of bile acids occurred in association with nonspecific hepatic injury, we studied the effect of the drug on GOT and GPT release from the IPRL in a separate group of experiments. In control livers release of transaminases was minimal until after 2 hr of perfusion, when perfusate levels of both GPT and GOT increased progressively with time (Fig. 2). At the end of the 200-min perfusion period, GPT and GOT concentrations in perfusate averaged 41.5 and 89.8 IU/liter, respectively. This low rate of transaminase release in the IPRL is in agreement with previous reports using this experimental model (20, 30). However, addition of CPZ (2.5×10^{-4} *M*) led to an immediate and progressive increase in the perfusate concentrations of both GPT and GOT, although differences from control values were not significant until 100 min after administration of the drug. At the end of the 220 min perfusion,

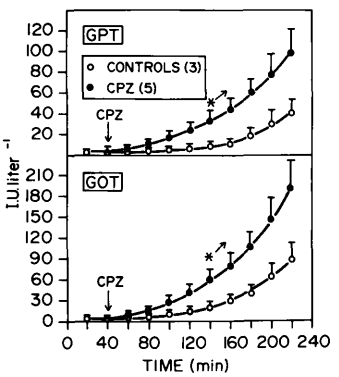

FIG. 2. Transaminase activities in the perfusate of controls and CPZ-treated livers (2.5×10^{-4} *M*). Sodium taurocholate was continuously infused throughout the study at 40 μmol/hr. In control livers, hepatic perfusate flow was mechanically reduced to mimic the effect of CPZ on hepatic perfusion. Perfusate volume (100 ml) was maintained constant by adding the same volume (0.5 ml) of perfusate each time a sample was withdrawn for GPT and GOT assay. Asterisk denotes significantly different from controls ($P < 0.05–0.001$); arrow refers to all values thereafter.

perfusate levels of GPT and GOT averaged 98.3 and 191.4 IU/liter, respectively, more than twice the values observed at this time in the control livers.

Discussion. The results of the present studies indicate that CPZ is capable of inhibiting bile acid excretion independently of any concomitant alterations in hepatic hemodynamics. Reduction of portal hepatic perfusate flow to a degree similar to that induced by CPZ does indeed diminish bile acid excretion, but the perfusate flow reduction accounts for no more than 50% of the diminished bile acid excretion obtained with CPZ and results in a reduced portal delivery of bile acids to the IPRL. On the other hand, CPZ reduces the extraction of bile acids from perfusate and produces biochemical evidence of liver cell injury. These effects of CPZ, together with those previously reported on hepatic membrane structure (31) and function (12), suggest that the inhibition of bile acid excretion induced by CPZ is related to a generalized adverse effect of the drug on hepatocyte plasma membranes resulting in impairment of the uptake and eventual excretion of bile acids.

Some reserve, however, may exist in comparing the effect of mechanically reducing perfusate flow on bile acid excretion to that produced by CPZ. We have previously demonstrated, in fact, that the transient hemodynamic effect of CPZ in the IPRL is associated with profound segmental perfusion defects which cannot be reproduced by mechanical restriction of portal perfusate flow (12). During CPZ vasoconstriction perfusate shunting may occur and bile acid distribution to the hepatic parenchyma may be significantly altered. Consequently, bile acid uptake may be reduced. However, in the CPZ livers the extraction efficiency of bile acids was only 28%, a value less than one-third of that observed in the restricted controls (96.5%). Since in both groups of livers portal concentrations of bile acids were essentially the same, such a drastic decline in bile acid extraction could be achieved only if perfusate shunting resulted in increasing sinusoidal concentrations of bile acids more than three times the saturation region of bile acid uptake, a possibility which seems highly unlikely. It is therefore reasonable to conclude that only part of the reduced clearance and excretion of bile acids induced by CPZ is secondary to the diminished hepatic perfusion; the remainder is independent of the hemodynamic changes and presumably results from a direct effect of CPZ on events underlying transport of bile acids from perfusate into bile.

Several mechanisms can be considered for this direct effect of CPZ on bile acid transport. First, CPZ may interact physicochemically with bile acids. Such an interaction may occur in the perfusate or within the hepatocyte and may result in the formation of insoluble complexes as has been demonstrated *in vitro* (16). Alternatively, CPZ may interfere with the process of bile acid excretion at the canalicular membrane and the reduced perfusate clearance of bile acids may be a secondary manifestation of their impaired excretion. Inhibition of bile acid excretion by CPZ has been demonstrated in the Rhesus monkey (11) and this effect has been attributed to impairment of synthesis and excretion of bile acids. Third, CPZ may directly inhibit the uptake of bile acids across the sinusoidal membrane. We have previously demonstrated that CPZ, added to the IPRL at doses similar to those employed here, produces widespread alterations of hepatocyte membrane structure, including formation of sinusoidal blebbing, cytoplasmic vacuoles at both sinusoidal and biliary poles, and myeloid bodies (31). CPZ inhibits bile acid independent bile flow and the activity of liver plasma membrane enzymes, Mg^{2+}-ATPase and 5′-nucleotidase in the IPRL (12) and that of Na^+, K^+-ATPase *in vitro* (18, 19). Thus, it is likely that CPZ inhibits both hepatic uptake and transcellular and excretory steps in bile acid transport. We have also shown in the present studies that perfusate concentrations of CPZ that inhibit bile acid excretion also release GPT and GOT from the IPRL. Release of transaminases is a sensitive indicator of liver cell

damage (32) and has been associated with addition of CPZ to liver slices (15), Chang liver cells (13, 14), and isolated hepatocytes (33). These observations support a generalized effect of CPZ on hepatocyte plasma membranes and suggest that the morphological, biochemical and physiological changes produced by the drug are all likely to be related to an interaction of CPZ with hepatocyte membranes. CPZ may bind to membrane phospholipids, perhaps phosphatidylserine (16) or alter calcium binding (34), examples where the functional properties of the liver cell membranes are likely to be altered and result in changes in permeability, transport, and secretory activity that might also inhibit bile acid transport. CPZ also interferes with microfilament function (35), which has been shown to play an important role in the uptake and excretion of bile acids (36, 37). Therefore, the CPZ-induced inhibition of bile acid excretion observed in the present studies presumably results from both impairment of the uptake and excretion of bile acids, as both processes involve active mechanisms and most likely require structural and functional integrity of the respective sinusoidal and canalicular plasma membranes and cytoskeleton.

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