Lack of Benefit of Ursodeoxycholic Acid in Drug-Induced Cholestasis in the Rat (43403)

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Abstract. **The administration of ursodeoxycholic acid (UDCA) has been reported to improve cholestasis in patients with primary biliary cirrhosis or sclerosing cholangitis. In the present study, we tested the hypothesis that UDCA similarly might reduce cholestasis induced by drugs. Rats were treated with three different drugs reported to induce cholestasis: 17α-ethynylestradiol, α-napthylisothiocyanate, and cyclosporine A. UDCA administration (0.4 $\cdot$ g/day$^{-1}\cdot$k^{-1} before and during administration of the cholestatic drug) did not improve survival, food intake, or serum indicators of cholestasis in any of these three animal models of cholestasis. To the extent that drug-induced cholestasis in rats mimics the human situation, we conclude that UDCA probably will not be beneficial in drug-induced cholestasis in humans.** [P.S.E.B.M. 1992, Vol 200]

Intrahepatic cholestasis can result from a variety of anatomical and biochemical derangements that interfere with secretion of bile within the liver. Therapy for this problem has been limited to withdrawal of the causative agent (i.e., drugs) or symptomatic treatment with bile acid-sequestering agents. No therapy directed at the cholestatic process presently is available, although *S*-adenosylmethionine has shown promise in several forms of drug-induced cholestasis (1, 2).

Recently, French investigators reported that administration of ursodeoxycholic acid (UDCA) to 15 patients with primary biliary cirrhosis resulted in significant improvement in pruritus as well as serum indicators of cholestasis (3). The authors postulated that the improvement resulted from replacement of the more toxic endogenous bile salts with the less membrane-toxic UDCA. Based on this encouraging study, as well as subsequent reports confirming the value of UDCA in biliary cirrhosis (4–7) and sclerosing cholangitis (8–9), we hypothesized that UDCA might also provide a safe and effective means of treating drug-induced cholestasis. In the present study, we tested the efficacy of UDCA in rats with cholestasis induced by each of three drugs: 17α-ethynylestradiol (EE), α-naphthylisothiocyanate (ANIT), and cyclosporine A. Unfortunately, in these animal models, we found no evidence to support the concept that UDCA may be beneficial in drug-induced cholestasis.

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Received July 19, 1991. [P.S.E.B.M. 1992, Vol 200]
Accepted January 13, 1992.

0037-9727/92/2001-0122$3.00/0

Materials and Methods

Male Sprague-Dawley rats (BioLab Inc., St. Paul, MN) weighing approximately 250 g were used in all studies. Animals were allowed to eat and drink *ad libitum* during the course of the study. UDCA was obtained from Reid-Howell Inc (Baudette, MN), EE and ANIT from Sigma Chemical Co. (St. Louis, MO), and cyclosporine A from Sandoz (East Hanover, NJ).

EE Study. For 3 days prior to administration of EE, eight animals received 100 mg of UDCA in 1 ml of distilled water via gavage, while eight control animals received 1 ml of water via gavage. The technique employed to produce cholestasis was identical to that described by Elias and co-workers (10). One day prior to EE administration, the rats were castrated under Metofane anesthesia. Just prior to the first dose of EE (Day 1), a baseline blood sample was obtained, and all animals then received intraperitoneal injections of EE (5 mg/kg/day) dissolved in propylene glycol for 14 days. The UDCA-treated animals continued to receive UDCA daily by gavage, while controls received water. On Day 8 and Day 15, blood samples were obtained and BSP clearance was measured by analysis of a serum

sample obtained 40 min after an intravenous infusion of 10 mg of bromosulfalein (BSP).

ANIT Study. Forty-eight rats were divided into an experimental group that received UDCA (1%) in chow for 4 days prior to receiving ANIT and a control group that received regular chow. The animals ingested about 20 g of chow/day or about 100 mg of UDCA/day. Just prior to gastric instillation of 200 mg/kg of ANIT in olive oil, baseline blood samples were obtained from all animals. Because of poor oral intake following ANIT, the experimental animals received 100 mg/day of UDCA via gavage, while the controls received water by gavage. Blood samples were obtained from eight animals in each group (and the animals were then sacrificed) at 1, 3, and 5 days after ANIT administration.

Cyclosporine A Study. The technique described by Stone *et al.* (11) was used to produce cholestasis. Sixteen experimental rats received UDCA in rat chow (1%) for 3 days, while 16 controls were fed regular chow. A baseline blood sample was then obtained just prior to administration of the first dose of cyclosporine A (10 mg/kg/day) in miglyol (Dyanimid Nobel Chem, Stony Point, NY). The UDCA animals continued to receive UDCA in their chow; however, if food intake fell to less than 10 g/day, the animals received 100 mg/day of UDCA via gavage. Blood was obtained after 7 days of cyclosporine therapy.

Bile Composition during UDCA Therapy. To determine the influence of the UDCA feeding regimen on the bile acid composition of bile, four rats were fed 1% by weight of their diet as UDCA. Since the rats ingested about 20 g/day of chow, the dose of UDCA was about 200 mg/day. After the fourth day of this regimen, a sample of bile was obtained under pentobarbital anesthesia. Bile acid composition was determined by gas chromatographic analysis of methyl ester acetate derivatives (12).

Assays. Serum bilirubin, alanine transferase (ALT), and alkaline phosphatase were determined by an Ektachem 700 thin-film technique. Total bile acids were measured by an enzymatic fluorometric method (13). BSP was determined by absorbance at 565 nm using an acidified blank to minimize error due to specimen turbidity.

Results

Analysis of bile of four rats fed a diet containing 1% UDCA for 4 days showed that UDCA constituted 66 ± 2% of the total bile acids.

Each of the three cholestatic agents caused a decrease in food intake with a resultant appreciable loss of weight (see Table I). Treatment with UDCA had no significant ($P > 0.20$) influence on this weight loss. Figures 1, 2, and 3 summarize the results of determinations of the serum analytes.

EE Studies. Six of eight UDCA-treated animals died prior to completion of the 2-week study as compared with three of eight controls. As shown in Figure 1, administration of EE did not cause significant elevations of the serum bilirubin, bile acids, alkaline phosphatase, or ALT above the baseline (pre-estrogen) value in either the UDCA-treated animals or the controls. Administration of EE caused an increase in BSP retention above the upper limit of normal 0.8 ± 0.3 (1 SD) in seven of eight control rats at 1 week and four of five rats at 2 weeks. In rats that received UDCA, BSP retention exceeded the upper limit of normal in two of five rats surviving 1 week and the two rats surviving 2 weeks (difference not significant).

ANIT Studies. In contrast to EE treatment, ANIT produced marked elevations in serum analytes indicative of cholestasis that peaked on the third day following administration of the drug (Fig. 2). However, there were

Table I. Influence of UDCA on Weight of Rats Treated with EE, ANIT, or Cyclosporine

Cholestatic agent	Day	*n*	Average Δ wt/day (g)
EE			
Control	7	8	−8.5 ± 2.5
	15	6	−3.0 ± 1.7
UDCA	7	6	−7.3 ± 1.9
ANIT			
Control	1	24	−37 ± 4.8
	3	16	−17 ± 3.3
	5	8	−13 ± 4.1
UDCA	1	22	−32 ± 8.5
	3	14	−18 ± 2.6
	5	6	−14 ± 2.2
Cyclosporine			
Control	7	8	−6.4 ± 1.5
UDCA	7	8	−8.6 ± 1.4

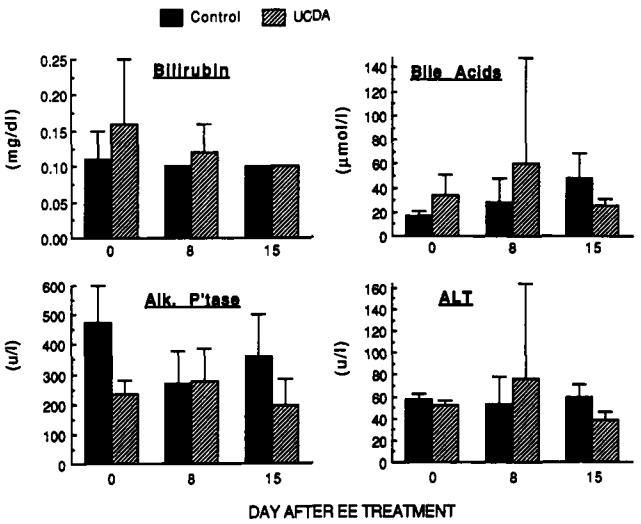

Figure 1. Serum analytes before and after 8 and 15 days of EE administration to control rats and rats treated with UDCA. None of the measurements changed significantly with EE therapy.

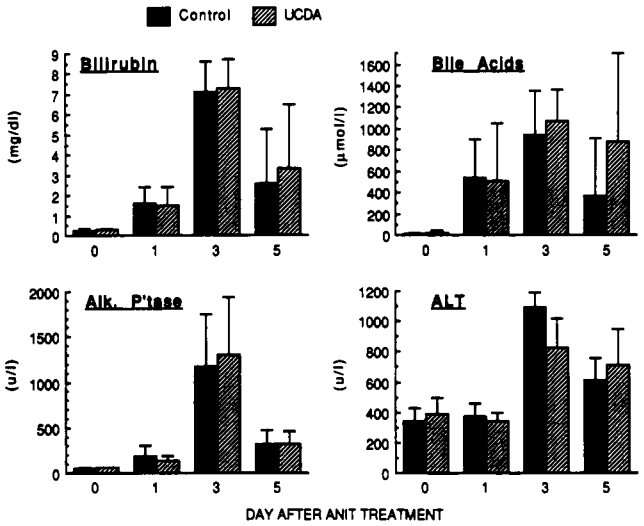

Figure 2. Serum analytes before and 1, 3, and 5 days after administration of ANIT to control rats and rats treated with UDCA. All four measurements increased significantly ($P < 0.05$) after ANIT administration, but no significant difference was observed between control and UDCA-treated rats.

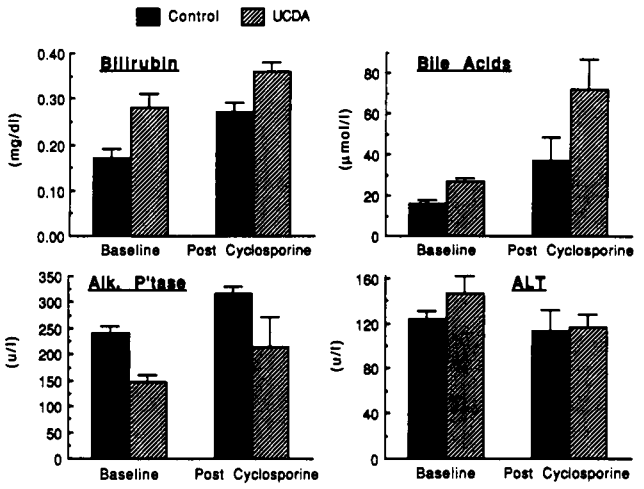

Figure 3. Serum analytes before and after 7 days of cyclosporine A administration to control rats and rats treated with UDCA. Cyclosporine A caused significant ($P < 0.05$) increases in serum bilirubin and bile acids in both groups of rats. After cyclosporine A, serum bile acids were significantly ($P < 0.05$) greater in UDCA-treated than in control rats.

no significant differences between the UDCA-treated group and controls with regard to the elevation of serum bile acids, bilirubin, alkaline phosphatase or ALT. Two of the 24 UDCA-treated animals died, whereas there were no deaths in the 24 control animals.

Cyclosporine A Studies. Cyclosporine A treatment caused small increases in serum bile acids ($P < 0.05$) and total bilirubin ($P < 0.05$) (Fig. 3). Alkaline phosphatase was not significantly influenced by cyclosporine A administration. When UDCA-treated and control animals were compared, UDCA caused a statistically significant ($P < 0.05$) increase in serum bile acids, whereas no significant differences ($P < 0.05$) were observed for total serum bilirubin, alkaline phosphatase, or ALT.

Discussion

Intrahepatic cholestasis may result from a decreased secretion of solutes (water is thought to follow passively) or an increased back diffusion of secreted solute into the blood stream (14). There are several mechanisms whereby substitution of UDCA for endogenous bile salts might be beneficial in cholestasis. UDCA is less damaging to membranes than are the native bile acids of humans and rats (15, 16), a property that appears to be attributable to the more hydrophilic nature of UDCA (17). In addition, UDCA infusion in rats induces a "hypercholeresis," i.e., a greater bile flow than would be expected for the quantity of bile acid administered (18). Such a hypercholeresis could benefit cholestasis by inducing a greater secretion of solute or by reducing the time available for back diffusion of solute.

While the exact mechanism whereby UDCA might improve cholestasis in patients with primary biliary cirrhosis is unknown, we postulated that UDCA could have a similar beneficial effect in drug-induced cholestasis. To test this hypothesis, we assessed the efficacy of UDCA in three different rat models. The animals received roughly 0.1 g/day of UDCA for 3 days prior to receiving the cholestatic drug and a similar dose daily during and/or following the administration of the drug. While this dose is much greater than that used in humans (15 mg/kg/day), UDCA represented roughly 66% of the total bile acids of treated rats, a value comparable to the 62% UDCA observed in the bile of primary biliary cirrhosis patients who responded favorably to UDCA (3). While measurements of bile UDCA concentrations were not obtained in the cholestatic animals, unconjugated UDCA is both passively and actively absorbed and appreciable malabsorption of this bile acid seems unlikely.

The most widely employed animal model of cholestasis is the rat treated with high doses of EE, a model that is thought to mimic a human form of cholestasis occurring in pregnancy or during the use of oral contraceptives. The mechanism of EE-induced cholestasis variably has been attributed to increased ductular permeability (17) or a decrease in bile acid-dependent (19) or -independent (20) flow.

While one report indicated that serum bilirubin and bile acids rose significantly in EE-induced cholestasis in rats (10), most studies have demonstrated only a decrease in the rate of bile flow with no detectable increase in serum markers of cholestasis (21). Although we employed the technique reported to produce cholestasis, (1) no significant rise was observed in serum bile acids, bilirubin, or alkaline phosphatase during 14 days

of EE administration. However, EE caused an appreciable increase in BSP retention in rats, a marked weight loss, and a high mortality. None of these untoward effects were influenced significantly by UDCA administration. Thus, to the extent that failure to clear BSP, weight loss, and mortality reflect the severity of the cholestasis, UDCA produced no beneficial effect.

Despite the widespread use of EE-induced cholestasis in rats as a model of the human form of cholestasis, it should be noted that the failure of serum bile acids and alkaline phosphatase to rise in the EE-treated rat contrasts with the findings commonly observed in human forms of presumed estrogen-induced cholestasis. Indeed, the high mortality in rats suggests that extrahepatic organs were suffering far greater toxicity than was the liver. Thus, the rat model may not adequately reflect the situation in patients and it may be inappropriate to extrapolate a negative result with UDCA in EE-treated rats to human forms of estrogen-induced cholestasis.

A second widely employed model of drug-induced cholestasis involves the administration of ANIT to rats. In contrast to the situation with EE, there is general agreement that ANIT cholestasis results from excessive permeability of the ductules and ducts with resultant back diffusion of solute (22–24). In our studies, ANIT caused marked elevation of serum bilirubin, bile acids, and alkaline phosphatase, but no significant difference between UDCA-treated and untreated animals was observed.

Lastly, we used an animal model of cholestasis in which large doses of cyclosporine A were administered to rats (6). As reported previously, we found that cyclosporine A caused small but significant increases in serum bile acids and serum bilirubin. Administration of UDCA had no apparent benefit in this form of cholestasis and, in fact, serum bile acids were significantly higher in UDCA-treated rats than in controls.

We conclude that UDCA was of no apparent benefit in three different rat models of drug-induced cholestasis. Although purely speculative, the ability of UDCA to reduce cholestasis in primary biliary cirrhosis may reflect the spotty injury to bile ducts that is characteristic of the early phases of this disease. UDCA may enhance bile flow in the remaining ducts, thus reducing cholestasis. In contrast, drugs may cause more diffuse injury, thus reducing the ability of the liver to respond to UDCA with choleresis.

Some forms of drug-related cholestasis in humans presumably result from pathological alterations that were not mimicked by the three drugs used in the present report. In addition, muricholic acid comprises about 50% of the bile acids of rats (16). Muricholic acid is relatively hydrophilic; thus, replacement of this bile acid with UDCA might not produce an appreciable increase in the overall hydrophilicity of bile. Thus, while our findings in rats provide no support for the use of UDCA in drug-induced cholestasis, the possibility remains that some forms of drug-induced cholestasis in humans might be responsive to UDCA therapy.

1. Frezza M, Tritapepe R, Pozzato, DiPadova C. Prevention by S-adenosylmethionine of estrogen-induced hepatobiliary toxicity in susceptible women. Am J Gastroenterol **83**:1098–1102, 1988.
2. Stramentinoli G, DiPadova C, Gulano M, Rovagnati P, Galli-Kienle M. Ethynylestradiol-induced impairment of bile secretion in the rat: Protective effects of *S*-adenosyl-L-methionine and its implication in estrogen metabolism. Gastroenterology **80**:154–158, 1981.
3. Poupon R, Calmus Y, Cretien Y, Ballet F, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet **1**:834–836, 1987.
4. Podda M, Battezati PM, Bertolini E, Zuin M, Crosignani A, Petroni ML, Rizzoli R, Roda E. Effects of ursodeoxycholic acid (UDCA) on liver function tests and bile acid metabolism in anicteric primary biliary cirrhosis (PBC): A dose-response study. Hepatology **8**:1266, 1988.
5. Batta AK, Salen G, Arora R, Shefer S, Tint GS, Abron J, Eskreis D, Katz S. Effect of ursodeoxycholic acid on bile acid metabolism in primary biliary cirrhosis. Hepatology **10**:414–419, 1989.
6. Hadziyannis S, Hadziyannis E. A randomized controlled trial of ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC). Hepatology **8**:1421, 1988.
7. Leuschner U, Fischer H, Kurtz W, Güldütuna S, Hübner, Hellstern A, Gatzen M, Leuschner M. Ursodeoxycholic acid in primary biliary cirrhosis: Results of a controlled double-blind trial. Gastroenterology **97**:1268–1274, 1989.
8. Chazouill'eres O, Poupon R, Capron JP, Metman EH, Dhumeaux D, Amouretti M, Couzigou P, Labayle D, Trinchet JC. Ursodeoxycholic acid for primary sclerosing cholangitis. J Hepatol **11**:120–123, 1990.
9. Hayashi H, Higuchi T, Ichimiya H, Hishida N, Sakamoto N. Asymptomatic primary sclerosing cholangitis treated with ursodeoxycholic acid. Gastroenterology **99**:533–535, 1990.
10. Elias E, Iqbal S, Knutton S, Hickey A, Coleman R. Increased tight junction permeability: A possible mechanism of estrogen cholestasis. Eur J Clin Invest **13**:383–390, 1983.
11. Stone BG, Udani M, Sanghvi A, Warty V, Plocki K, Bedetti CD, Van Thiel DH. Cyclosporine A-induced cholestasis: The mechanism in a rat model. Gastroenterology **93**:344–351, 1987.
12. Duane WC, Ginsberg RL, Bennion LJ. Effects of fasting on bile acid metabolism and biliary lipid composition in man. J Lipid Res **17**:211–219, 1976.
13. Hanson NQ, Frier EF. Enzyme inactivation in serum before determination of total bile acids. Clin Chem **29**:1073–1075, 1983.
14. Scharschmidt BF. Bile formation and cholestasis, metabolism and enterohepatic circulation of bile acids, and gallstone formation. In: Zakin D, Boyer TD, Eds. Hepatology: A Textbook of Liver Disease. Philadelphia: WB Saunders, pp297–351, 1982.
15. Scholmerich J, Becher MS, Schmidt K, Schubert R, Kremer B, Feldhaus S, Gerok W. Influence of hydroxylation and conjugation of bile salts on their membrane-damaging properties—Studies on isolated hepatocytes and lipid membrane vesicles. Hepatology **4**:661–666, 1984.
16. Heuman DM, Vlahcevic ZR, Bailey ML, Hylemon PB. Regulation of bile acid synthesis. II: Effect of bile acid feeding on enzymes regulating hepatic cholesterol and bile acid synthesis in the rat. Hepatology **8**:892–897, 1986.
17. Iqbal S, Mills CO, Elias E. Biliary permeability during ethinyl estradiol-induced cholestasis studied by segmented retrograde intrabiliary infections in rats. J Hepatol **1**:211–219, 1985.

18. Dumont M, Erlinger S, Uchman S. Hypercholeresis induced by ursodeoxycholic acid and 7-ketolithocholic acid in the rat: Possible role of bicarbonate transport. Gastroenterology **79**:82–89, 1980.
19. David R, Kern F. Effects of ethinyl estradiol and phenobarbital on bile acid synthesis and biliary bile acid and cholesterol excretion. Gastroenterology **70**:1130–1135, 1976.
20. Gumucio JJ, Valdivieso VD. Studies on the mechanism of the ethynyl estradiol impairment of bile flow and bile salt excretion in the rat. Gastroenterology **61**:339–344, 1971.
21. Simon FR, Arias IM. Alteration of bile canalicular enzymes in cholestasis. A possible cause of bile secretory failure. J Clin Invest **52**:765–775, 1973.
22. Woolley J, Mullock BM, Hinton RH. Reflux of biliary components into blood in experimental intrahepatic cholestasis induced in rats by the treatment with a-naphthylisothiocyanate. Clin Chim Acta **92**:381–386, 1979.
23. Krell H, Hoke H, Pfaff E. Development of intrahepatic cholestasis by a-naphthylisothiocyanate in rats. Gastroenterology **82**:507–514, 1982.
24. Kan KS, Coleman R. 1-Naphthylisothiocyanate-induced permeability of hepatic tight junctions to proteins. Biochem J **238**: 323–328, 1986.