BILE ACID THERAPY - STATUS IN 1993

MEDICAL GRAND ROUNDS

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The notion that bile acids may induce liver injury largely stems from the observations of Holsti who demonstrated that desiccated hog bile, when fed to rabbits, can induce cirrhosis (1). Subsequently he demonstrated that this effect could be induced by lithocholic acid (2), its glycine conjugate (glycolithocholate) and by chenodeoxycholic acid (3).

Subsequently, lithocholic acid was fed to a wide variety of experimental animals, and almost uniformly induced abnormal histological findings in liver. The alterations vary somewhat from species to species, but bile ductular and bile duct proliferation has been a common finding in all. This evidence has been exhaustively reviewed in the excellent publication of Palmer (4).

Infusion of lithocholate, its glycine or taurine conjugates, and the 3-sulfates of conjugated lithocholates has been shown to impair bile flow and bile acid excretion in intact rats and hamsters, and in their isolated perfused livers demonstrating a rapid dysfunctional aspect of these lithocholate compounds (5-7). Moreover, infusion of the dihydroxy bile acids, chenodeoxycholic and deoxycholic acids have also been shown to impair bile flow and to induce hepatocyte damage in the intact and perfused liver of the rat (6-8).

Morphologic changes occur rapidly in livers exposed to lithocholate (7,9) or chenodeoxycholate (9).

Lithocholate exerts its major effect on the canalicular and pericanalicular regions of the hepatocyte, whereas chenodeoxycholic acid induces more widespread ultrastructural changes. Prior to these observations with chenodeoxycholate in the perfused rat liver, it had been speculated that liver toxicity in rabbits fed chenodeoxycholate was due to lithocholate produced by dehydroxylation of chenodeoxycholate in the gastrointestinal tract. The ultrastructural changes reported by Miyai et al (7) clearly demonstrated that chenodeoxycholic acid *per se* can be hepatotoxic.

1. Holsti P. Experimental cirrhosis of the liver in rabbits induced by gastric instillation of desiccated whole bile. Acta Path Microbiol Scand Suppl 113:1-67, 1956.

2. Holsti P. Cirrhosis of the liver induced in rabbits by gastric installation of 3-monohydroxycholanic acid. Nature 186:250, 1960.

3. Holsti P. Bile acids as a cause of liver injury. Cirrhogenic effect of chenodeoxycholic acid in rabbits. Acta Path Microbiol Scand 54:479, 1962.

4. Palmer RH. Bile acids, liver injury, and liver disease. Arch Intern Med 130:606-617, 1972.

5. Javitt NB, Emerman S. Effect of sodium taurolithocholate on bile flow and bile acid excretion. J Clin Invest 47:1002-1014, 1968.

6. Fisher MM, Magnusson R, Miyai K. Bile acid metabolism in mammals. I. Bile acid-induced intrahepatic cholestasis. Laboratory Invest 25:88-91, 1971.

7. Miyai K, Price VM, Fisher MM. Bile acid metabolism in mammals. Ultrastructural studies on the intrahepatic cholestasis induced by lithocholic and chenodeoxycholic acids in the rat. Laboratory Invest 24:292-302, 1971.

8. Heuman DM, Mills AS, McCall J, et al. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. In vivo studies in the rat. Gastroenterology 100:203-211, 1991.

9. Schaffner F, Javitt NB. Morphologic changes in hamster liver during intrahepatic cholestasis induced by taurolithocholate. Laboratory Invest 15:1783-1792, 1966.

The mechanisms by which bile acids induce cell damage have been explored in various model systems *in vitro*. In general, these studies suggest that the damaging effects are related to the amphipathic structure of these compounds and involve interactions with membranes or more polar structures. Thus, bile acids can induce hemolysis, damage the membranes of isolated hepatocytes, isolated mitochondria and lipid vesicles (10-13).

In the various *in vitro* model systems including perfused livers and in intact animals, toxicity of bile acids appears to be inversely related to the degree of hydroxylation and to the orientation of the hydroxyl groups such that lithocholic acid is more toxic than chenodeoxycholic and deoxycholic acids which in turn are more toxic than cholic acid. Conjugated bile acids in general are less toxic than their unconjugated moieties. In general, then, toxicity is directly correlated with the degree of hydrophobicity of the bile acid compounds.

10. Kappas A, Palmer RH. Selected aspects of steroid pharmacology. Pharmacol Rev 15:123-167, 1963.

11. Weissman G, Keiser H. Hemolysis and augmentation of hemolysis by neutral steroids and bile acids. Biochem Pharmacol 14:537-546, 1965.

12. Schoelmerich J, Becher M-S, Schmidt K, et al. 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.

13. Lee MJ, Whitehouse MW. Inhibition of electron transport and coupled phosphorylation in liver mitochondria by cholanic (bile) acids and their conjugates. Biochim Biophys Acta 100:317-328, 1965.

BILE ACID TOXICITY IN MAN

Evidence that bile acids may damage human liver has come from a number of sources:

1. Feeding bile acids

(a) Deoxycholic acid when given orally in a dose of 750 mg per day led to elevations of SGOT to 4 times the upper limit of normal in 2 of 7 healthy persons. These elevations returned to normal when deoxycholate ingestion was stopped (14).

14. LaRusso NF, Szczepanik PA, Hofmann AF. Effect of deoxycholic acid ingestion on bile acid metabolism and biliary lipid secretion in normal subjects. Gastroenterology 72:132-140, 1977.

(b) Chenodeoxycholic acid when fed in doses of 750-1000 mg per day to patients with cholesterol gallstones led to elevated transaminases in up to 30 percent (15-18). The elevations were usually transient, returned to normal with cessation of therapy, or even with continued treatment.

In the National Cooperative Gallstone Study (18), moderate changes were present in about 12 percent of liver biopsies while milder changes were seen more frequently (19). Electron microscopic changes, already present prior to initiation of therapy, increased in frequency and severity over the two years of the treatment trial. A drug effect was postulated by the lack of correlation of the abnormalities with the duration of cholelithiasis prior to CDCA treatment and their increasing prevalence after only 9 months of therapy (20). It was not known whether clinically significant hepatotoxicity would develop if therapy with chenodeoxycholic acid was continued beyond 24 months.

Since lithocholic acid is a product of the metabolism of chenodeoxycholic acid it was speculated that the markers of liver toxicity were caused by lithocholate. However, in a thorough analysis of biliary bile acids (21), no significant differences were seen between gallstone patients with and without evidence of liver injury for percent lithocholate amidates, or for percent sulfated or unsulfated lithocholate amidates. Sulfation is felt to make lithocholate less toxic. Lithocholate was partially sulfated in all of the bile specimens but the extent of sulfation varied widely during the course of therapy. Mean values in the healthy subjects were similar and also showed a comparable wide range in the extent of lithocholate sulfation. It was concluded that (1) accumulation of unsulfated lithocholate *per se* in circulating bile acids was not the cause of liver injury, and (2) liver injury was related to enrichment in circulating bile acids with chenodeoxycholic acid, alone or with lithocholate (21).

15. Gerolami A, Sarles H, Breete R. Controlled trial of chenodeoxycholate for radiolucent gallstones. A multicenter study. Digestion 16:299-307, 1977.

16. Mok HYI, Beel GD, Dowlery RA. Effect of different doses of chenodeoxycholic acid on bile lipid composition and on frequency of side effects in patients with gallstones. Lancet 2:253-257, 1974.

17. Menghini G, Palbotta B. Marked increase in serum levels of liver enzymes in patients receiving chenic acid and phenobarbital for gallstone dissolution. Digestion 14:163-169, 1976.

18. Schoenfield LJ, Lachin JM, The Steering Committee and the National Cooperative Gallstone Group. Chenodiol (chenodeoxycholic acid) for dissolution of gallstones: The national cooperative gallstone study. A controlled trial of efficacy and safety. Ann Intern Med 95:257-282, 1981.

19. Fisher RL, Anderson DW, Boyer JL, et al. A prospective morphologic evaluation of hepatic toxicity of chenodeoxycholic acid in patients with cholelithiasis: The National Cooperative Gallstone Study. Hepatology 2:187-201, 1982.

20. Phillips J, Fisher RL, Anderson DW, et al. Ultrastructural evidence of intrahepatic cholestasis before and after chenodeoxycholic acid therapy in patients with cholelithiasis: The National Cooperative Gallstone Study. Hepatology 3:209-220, 1983.

21. Fisher RL, Hofmann AF, Converse JL, et al. The lack of relationship between hepatotoxicity and lithocholic-acid sulfation in biliary bile acids during chenodiol therapy in the National Cooperative Gallstone Study. Hepatology 14:454-463, 1991.

2. Bile Acid Concentrations in Serum and Liver

Total serum bile acids rise in patients with a wide variety of liver diseases. Dihydroxy and trihydroxy bile acids increase (22,23). Values may be elevated up to 100 times the normal concentration. The increase is accounted for largely by conjugated bile acids. Bile acid concentrations also increase in liver tissue. Elevations in chenodeoxycholic and cholic acids account for most of the rise (24-26).

22. Makino I, Nakagawa S, Nashimo K. Conjugated and unconjugated serum bile acid levels in patients with hepatobiliary diseases. Gastroenterology 56:1033-1039, 1969.

23. Lewis NB, Weaver V, Panveliwalla D. Serum bile acids in liver disease. Gut 12:145-152, 1971.

24. Greim H, Truelzsch D, Czygan P, et al. Mechanism of cholestasis. 6. Bile acids in human livers with or without biliary obstruction. Gastroenterology 63:846-850, 1972.

25. Akashi Y, Miyazaki H, Yanagisawa J, et al. Bile acid metabolism in cirrhotic liver tissue - altered synthesis and impaired hepatic secretion. Clinical Chimica Acta 168:199-206, 1987.

26. Kurtz W, Guelduetana S, Leuschner U. Elevated liver tissue bile acids in steatosis and chronic hepatitis. Abstract from the X International Bile Acid Meeting. In: Trends in Bile Acid Research. Freiburg, West Germany, June 9-11, 1988, p.192.

3. Effects of Cholestyramine

Cholestyramine resin, an insoluble chloride salt of a quaternary ammonium anion exchange resin, has a strong affinity for bile salts. When taken by mouth, the resin exchanges chloride for bile salts in the intestinal tract forming an insoluble complex that is excreted in the feces. The level of fecal cholate rises several fold (27).

Serum bile acid concentrations may be lowered and although the number of observations is limited when this occurred, striking improvement in liver tests, including elevated levels of transaminases, and improved growth were documented in some patients (28-30). Such results support the notion that bile salts may contribute to some of the chemical abnormalities noted in liver disease.

27. Thompson WG. Cholestyramine. Canadian Med Assoc J 104:305-309, 1971.

28. Visintine RE, Michaels GD, Fukayama G, et al. Xanthomatous biliary cirrhosis treated with cholestyramine. A bile-acid-adsorbing resin. Lancet 2:341-343, 1961.

29. Sharp HL, Carey JB Jr., White JG, et al. Cholestyramine therapy in patients with a paucity of intrahepatic bile ducts. J Pediatrics 71:723-736, 1967.

30. Nelson R, Murphy GM, Elkins S, et al. Cholestyramine therapy in cholestatic liver disease of children. Gut 15:825, 1974 (Abstract).

4. Ursodeoxycholic Acid (UDCA) Therapy in Primary Biliary Cirrhosis (PBC)

UDCA has been used extensively in attempts to dissolve cholesterol gallstones (31,32). Whatever the merits of this therapy may be, David et al (33), in an abstract in 1985, reported improvement in GOT, GPT, GGT and serum alkaline phosphatase (SAP) in 6 patients with PBC and in bile acid clearance in 3, while on 10 mg UDCA/kg body weight/day. Soon thereafter, Fisher and Paradine in another preliminary report (34) found convincing improvement in alkaline phosphatase (decrease 54%), AST (decrease 52%), ALT (decrease 43%) and GGT (decrease 32%) in 10 anicteric women while on 10-15 mg/kg/day for up to 6 months. Poupon and associates in 1987 reported on responses of 15 patients treated with 13-15 mg/kg/day for 2 years (35). Four were in stage I, 6 in stage II, 1 in stage III and 3 in stage IV of their disease. Serum bilirubin, SAP, AST, ALT and GGT fell significantly in all patients. Pruritus improved. tests worsened in the 3 patients in whom therapy was stopped for 3 months, then improved again when

therapy was reinstituted. Neither histological improvement nor progression was noted in the 6 patients undergoing repeat biopsy at the end of two years. However, after a mean of 31 months of therapy, Poupon and associates (36) found a statistically significant trend to improvement in fibrosis, periportal and lobular necrosis, lobular inflammation and histologic stage in 12 of their originally treated patients. None of these studies was controlled or randomized.

Leuschner and associates (37) recently reported on their double-blind, randomized, controlled trial of UDCA (10 mg/kg/day) versus placebo for 9 months in 20 patients with stage I to III disease. The study was prompted by this group's earlier favorable results noted above (33). Two patients on placebo withdrew from the study. Mean values of glutamate dehydrogenase, AST, ALT alkaline phosphatase and GGT fell by 48-79% by 18-24 weeks. No improvement was observed in the placebo group. Liver histology improved in 6 of the UDCA group and deteriorated in 4 patients on placebo. Tests returned to pretreatment levels within 14 weeks when UDCA was discontinued. There were no deaths. Similar findings have been reported by others (Podda et al (38), Matsuzaki et al (39)) in studies with varying designs.

A number of additional trials are in progress or have just been completed. Results are available primarily in abstracts (40-48). The composite information can be summarized as follows: Most investigators find an improvement in pruritus. Liver tests uniformly improve, particularly in patients with stage I-III disease. Stage IV patients (cirrhosis) with a serum bilirubin of 4 mg% or greater may not improve or even worsen. Hadziyannis (45) reports initial improvements, but a tendency for the bilirubin to worsen after the first year of treatment. Variable experiences are reported with liver histology from improvement in inflammation, periportal necrosis; in histologic stage, and extent of fibrosis (36,37), to no improvement or to worsening (41,45). Information is available on development of complications of liver disease, need for transplantation, and survival. No beneficial effect is reported by Hadziyannis in 50 patients (25 on UDCA) whereas Poupon et al (42) report a favorable effect for UDCA after 2 years in 146 patients (73 on UDCA) that is even more impressive after an additional 2 years when all patients had been placed on open label UDCA (46).

There is evidence that UDCA might slow down progression of portal hypertension (44). Both Heathcote et al in a multicenter trial in Canada (47) and Lindor et al at the Mayo Clinic (48) find a lower incidence of liver transplantation and of deaths at 2 years in patients receiving UDCA rather than a placebo. The improvements with UDCA therapy were not statistically significant in these latter two reports, however.

Similar improvements in liver tests are being reported in patients with sclerosing cholangitis. There are only a few publications and abstracts at this time.

31. Bachrach WH, Hofmann AF. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. Part 1. Dig Dis Sci 27:737-761, 1982.

32. Bachrach WH, Hofmann AF. Ursodeoxycholic acid in the treatment of cholesterol cholelithiasis. Part II. Dig Dis Sci 27:833-856, 1982.

33. David R, Kurtz W, Strohm WD, et al. Die Wirkung von Ursodeoxycholsaeure bei chronischen Lebererkrankungen. Eine Pilotstudie. Z Gastroenterologie 23:240, 1985.

34. Fisher MM, and Paradine ME. Influence of ursodeoxycholic acid (UDCA) on biochemical parameters in cholestatic liver disease. Gastroenterology 90:1725, 1986 (Abstract).

35. Poupon R, Chretien Y, Poupon RE, et al. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet i:834-836, 1987.

36. Poupon R, Blakau B, Legendre C, et al. Ursodeoxycholic acid improves histologic features and progression of primary biliary cirrhosis. Hepatology 10:637, 1989 (Abstract).

37. Leuschner U, Fischer H, Kurtz W, et al. Ursodeoxycholic acid in primary biliary cirrhosis: Results of a controlled double-blind trial. Gastroenterology 97:1268-1274, 1989.

38. Podda M, Ghezzi C, Battezzati PM, et al. Ursodeoxycholic acid for chronic liver disease. J Clin Gastroenterol 10(Suppl.2):S25-S31, 1988.

39. Mazusaki Y, Tanaka N, Osuga T, et al. Improvement of biliary enzyme levels and itching as a result of long-term administration of ursodeoxycholic acid in primary biliary cirrhosis. Am J Gastroenterol 85:15-23, 1990.

40. Raedsch R, Stiehl A, Theilman L, et al. Influence of ursodeoxycholic acid on primary biliary cirrhosis depending on stage of the disease. Gastroenterology 94:647, 1989 (Abstract).

41. Myszor M, Turner I, Mitchison H, et al. No symptomatic or histologic benefits from ursodeoxycholic acid treatment in PBC after 1 year. Controlled pilot study. Hepatology 12:415, 1990 (Abstract).

42. Poupon RE, Balkau B, Eschwege E, Poupon R, and the UDCA-PBC Study Group. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. New Engl J Med 324:1548-1554, 1991.

43. Wiesner RJ, Jorgensen R, and Perdigoto R. Ursodeoxycholic acid therapy for primary biliary cirrhosis: progression of disease despite near normalization of biochemical liver tests. Hepatology 12:843, 1990 (Abstract).

44. Huet PM, Williams B, Huet J, and Poupon R. Effects of ursodeoxycholic acid (UDCA) on hepatic function and portal hypertension in primary biliary cirrhosis (PBC). Hepatology 12:907, 1990 (Abstract).

45. Hadziyannis SJ. Presentation at XI International Bile Acid Meeting, Freiburg, Germany, October 1990.

46. Poupon RE, Chretien Y, Balkau B, et al. Ursodeoxycholic acid therapy for primary biliary cirrhosis: a four year controlled study. Hepatology 16:91A, 1992 (Abstract).

47. Heathcote EJL, Couch K, Walker V, et al. The Canadian multi-centre double blind randomized controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 16:91A, 1992 (Abstract).

48. Lindor DK, Baldus WP, Jorgensen RA, et al. Ursodeoxycholic acid (UDCA) is beneficial therapy for patients with primary biliary cirrhosis. Hepatology 16:91A, 1992 (Abstract).

Mechanism(s) of Ursodeoxycholic Acid (UDCA) Effectiveness

UDCA has now been shown to protect the liver against the cholestatic and hepatocellular necrotic effects of more hydrophobic bile salts. This has been demonstrated in intact animals (8,49) and in *in vitro* systems involving isolated hepatocytes and hepatocyte organelles (50-53).

49. Krol T, Kitamura T, Miyai K, et al. Tauroursodeoxycholate (TUDC) reduces ductular proliferation and portal inflammation in bile-duct-ligated hamsters. Hepatology 3:881, 1983 (Abstract).

50. Miyazaki K, Nakayama F, Koga A. Effect of chenodeoxycholic and ursodeoxycholic acids on isolated adult human hepatocytes. Dig Dis Sci 29:1123-1130, 1984.

51. Galle PR, Theilmann L, Raedsch R, et al. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human hepatocytes. Hepatology 12:486-491, 1990.

52. Spivey JR, Bronk SF, Gores GJ. Tauroursodeoxycholate prevents glycochenodeoxycholate induced nonlysosomal proteolysis and cytotoxicity in isolated rat hepatocytes. Hepatology 16:156A, 1992 (Abstract).

53. Kraehenbuehl S, Talos C, Reichen J. Mitochondrial toxicity of hydrophobic bile acids and partial reversal by ursodeoxycholate. Hepatology 16:156A, 1992 (Abstract).

Favorable results in the treatment of PBC and sclerosing cholangitis have now been extended to other hepatic diseases. These include:

Chronic Hepatitis

The seminal observations are those of Leuschner et al (54). Six patients with chronic active hepatitis (not autoimmune, not HBsAg positive) and cholesterol gallstones were treated with UDCA (8-11 mg/kg/day). SGOT and SGPT decreased on therapy. Gallstones dissolved in 5. Liver tests relapsed initially off therapy but tended to fall again. No improvement in tests or gallstones was observed in 4 comparable patients who were not given UDCA and served as controls.

Subsequently, a number of studies have confirmed improvement in liver tests, mainly transaminases, GGT and alkaline phosphatase in patients with chronic hepatitis largely due to hepatitis B or C. Patients with chronic active hepatitis alone or with bridging necrosis or cirrhosis responded in a similar fashion (55-57).

Improvement in tests occurred with doses of UDCA as low as 250 mg per day (3.8 mg/kg/day). Additional improvements in test results were obtained with higher doses (up to 750 mg per day), but these were not striking in the short intervals that they were used.

Similar improvements in tests have been observed in a series of blood donors found to have elevated transaminases. The etiology of their hepatic disease was not reported (58).

54. Leuschner U, Leuschner M, Sieratzki J, et al. Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot study. Dig Dis Sci 30:642-649, 1985.

55. Podda M, Ghezzi C, Battezzati M, et al. Effects of ursodeoxycholic acid and taurine on serum liver enzymes and bile acids in chronic hepatitis. Gastroenterology 98:1044-1050, 1990.

56. Crosignani A, Battezzati PM, Setchell KDR, et al. Effects of ursodeoxycholic acid on serum liver enzymes and bile acid metabolism in chronic active hepatitis: A dose-response study. Hepatology 13:339-344, 1991.

57. Rolandi E, Franceschini R, Cataldi A, et al. Effects of ursodeoxycholic acid (UDCA) on serum liver damage indices in patients with chronic active hepatitis. A double-blind controlled study. Eur J Clin Pharmacol 40:473-476, 1991.

58. Bellentani S, Tabarroni G, Barchi T, et al. Effect of ursodeoxycholic acid treatment on alanine aminotransferase and gamma-glutamyltranspeptidase serum levels in patients with hypertransaminasemia. Results from a double-blind controlled trial. J Hepatol 8:7-12, 1989.

Benign Recurrent Intrahepatic Cholestasis

Ursodeoxycholic acid has been used to treat and to prevent episodes of recurrent cholestasis with variable success.

59. Bircher J. Treatment of patients with benign recurrent intrahepatic cholestasis. Hepatology 10:1030-1033, 1989.

60. Maggiore G, DeGiacomo C. Efficacy of ursodeoxycholic acid in preventing cholestatic episodes in a patient with benign recurrent intrahepatic cholestasis. Hepatology 16:504, 1992.

61. Crosignani A, Podda M, Bertolini E, et al. Failure of ursodeoxycholic acid to prevent a cholestatic episode in a patient with benign recurrent intrahepatic cholestasis: A study of bile acid metabolism. Hepatology 13:1076-1083, 1991.

62. Bijleveld CMA, Vonk RJ, Kuipers F. Reply (to Bircher J, Hepatology 10:1030-1033, 1989). Hepatology 10:1031-1032, 1989.

Cholestasis of Pregnancy

Patients with cholestasis of pregnancy, treated with UDCA (after the 25th week of pregnancy) have shown improvements in pruritus and liver tests while taking UDCA. Treatment was well tolerated (63,64).

63. Mazzella G, Rizzo N, Salzetta A, et al. Management of intrahepatic cholestasis in pregnancy. Lancet 338:1594-1595, 1991.

64. Palma J, Reyes H, Ribalta J, et al. Effects of ursodeoxycholic acid in patients with intrahepatic cholestasis of pregnancy. Hepatology 15:1043-1047, 1992.

References cited above are not intended to be exhaustive of the experiences gained in treating various liver diseases. They focus primarily on cholestatic diseases in adults. Comparable observations have been reported in various cholestatic hepatic diseases in children.

It is important to stress that clinical experiences with ursodeoxycholic acid thus far have largely shown improvement in the results of laboratory tests. Evidence for impressive beneficial effects on the progression of liver disease are still lacking.

An inference that can be drawn from these clinical observations, particularly in cholestatic liver diseases, is that increased concentrations of bile acids in liver may contribute significantly to liver dysfunction and that a hepatocytoprotective agent such as UDCA might play a useful role in the therapeutics of liver illness. Controlled clinical trials will be required to prove this, however.