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Biliary Bile Acids in Primary Biliary Cirrhosis: Effect of Ursodeoxycholic Acid

Burton Combes¹, Robert L. Carithers Jr², Willis C. Maddrey^{1,3}, Santiago Munoz³, Guadalupe Garcia-Tsao⁴, Gregory F. Bonner⁴, James L. Boyer⁴, Velimir A. Luketic⁵, Mitchell L. Shiffman⁵, Marion G. Peters⁶, Heather White⁶, Rowen K. Zetterman⁷, Richard Risser¹, Stephen S. Rossi⁸, and Alan F. Hofmann⁸

¹University of Texas Southwestern Medical Center at Dallas, Dallas, TX

²University of Washington, Seattle, WA

³Thomas Jefferson Medical College, Philadelphia, PA

⁴Yale University School of Medicine, New Haven, CT

⁵Medical College of Virginia of Virginia Commonwealth University, Richmond, VA

⁶Washington University, St. Louis, MO

⁷University of Nebraska Medical Center, Omaha, NE

⁸University of California, San Diego, CA

Abstract

Bile acid composition in fasting duodenal bile was assessed at entry and at 2 years in patients with primary biliary cirrhosis (PBC) enrolled in a randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid (UDCA) (10–12 mg/kg/d) taken as a single bedtime dose. Specimens were analyzed by a high-pressure liquid chromatography method that had been validated against gas chromatography. Percent composition in bile (mean \pm SD) for 98 patients at entry for cholic (CA), chenodeoxycholic (CDCA), deoxycholic (DCA), lithocholic (LCA), and ursodeoxycholic (UDCA) acids, respectively, were 57.4 ± 18.6 , 31.5 ± 15.5 , 8.0 ± 9.3 , 0.3 ± 1.0 , and 0.6 ± 0.9 . Values for CA were increased, whereas those for CDCA, DCA, LCA, and UDCA were decreased when compared with values in normal persons. Bile acid composition of the major bile acids did not change after 2 years on placebo medication. By contrast, in patients receiving UDCA for 2 years, bile became enriched with UDCA on average to 40.1%, and significant decreases were noted for CA (to 32.2%) and CDCA (to 19.5%). No change in percent composition was observed for DCA and LCA. Percent composition at entry and changes in composition after 2 years on UDCA were similar in patients with varying severity of PBC. In patients whose bile was not enriched in UDCA (entry and placebo-treated specimens), CA, CDCA, DCA, and the small amount of UDCA found in some of these specimens were conjugated to a greater extent with

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Address reprint requests to: Burton Combes, M.D., UT Southwestern Medical Center at Dallas, 5323 Harry Hines Boulevard, Dallas, TX 75235-9151.

Dr. Munoz is now at the Albert Einstein Medical Center, Philadelphia, PA

glycine (52%–64%) than with taurine (36%–48%). Treatment with UDCA caused the proportion of all endogenous bile acids conjugated with glycine to increase to 69% to 78%, while the proportion conjugated with taurine (22%–31%) fell ($P < .05$). Administered UDCA was also conjugated predominantly with glycine (87%).

Despite extensive use of ursodeoxycholic acid (UDCA) in the therapy of primary biliary cirrhosis (PBC), relatively little information is available on the effect of UDCA on the composition of bile acids excreted in bile. Mazzella et al.¹ presented data for 9 patients with histological stages I to III after 4 weeks of 600 mg/d. Van de Meeberg et al.² in their report on cholestatic liver disease included data from 8 patients with PBC receiving 10 mg/kg/d for 3 months. Crosignani et al.³ provided their findings at 6 months for 10 patients (7 with stage IV histology, 1 each in stages I, II, and III) receiving 8 mg/kg/d (500 mg/d). Batta et al.⁴ reported their findings on 3 patients receiving 10 to 12 mg/kg/d (900 mg/d) for 6 months.

In the present report, data on bile acid composition in bile are provided for a much larger number of patients who were enrolled in a 2-year, randomized, double-blind, placebo-controlled trial of UDCA in PBC.⁵ Bile was analyzed in 98 patients obtained at the time of entry into the trial, and 2 years later from 51 patients who had been randomized to receive placebo medication and 55 patients receiving UDCA at bedtime at a dose of 10 to 12 mg/kg/d.

PATIENTS AND METHODS

A total of 151 patients with PBC defined by the criteria of: 1) a cholestatic liver disease of at least 6 months' duration; 2) a serum alkaline phosphatase value at least 1.5 times the upper limit of normal; 3) a positive antimitochondrial antibody test; 4) exclusion of biliary obstruction by ultrasonography, computed tomography, or by endoscopic cholangiography; and 5) a liver biopsy specimen compatible with the diagnosis of PBC, were enrolled in a 2-year randomized, double-blind, controlled treatment trial. Seventy-seven patients received UDCA in a dose of 10 to 12 mg/kg/d at bedtime, and 74 received placebo medication. Patients were stratified into four groups on the basis of: 1) a serum bilirubin of < 2 mg/dL or ≥ 2 mg/dL; and 2) liver histology of either stages I and II or stages III and IV, as defined by Ludwig.⁶ Patients in stratum 1 had a serum bilirubin < 2 and stage I or II histology, stratum 2, had a bilirubin of < 2 and stage III or IV histology; stratum 3 had a bilirubin of ≥ 2 and stage I or II histology; stratum 4 had a bilirubin of ≥ 2 and stage III or IV histology.

Fasting duodenal bile specimens were obtained in the morning at entry and at 2 years at the time of upper endoscopy and after stimulation with intravenously administered cholecystokinin. Trial medication, *i.e.*, UDCA or placebo, was not administered the evening before the 2-year endoscopy. Samples were stored frozen at -20°C until bile acid analysis. Bile acid composition was measured by a high-pressure liquid chromatography method,⁷ which had been validated against gas chromatography.⁸ For determination of the mode of conjugation of the four major biliary bile acids (cholic acid [CA], chenodeoxycholic acid [CDCA], deoxycholic acid [DCA], UDCA), chromatograms were selected that had excellent peak resolution of the corresponding glycine and taurine conjugates of those acids and in which unknown bile acids and non-bile acid components had distinctly different retention

times. There were 61 such chromatograms: 12 from patients before treatment, 22 from patients who received placebo, and 27 from patients who had received UDCA. The mode of conjugation was expressed as percent conjugation with glycine, this unit being preferable to the glycine/taurine ratio.⁹ For evaluation of the effect of UDCA on the mode of conjugation, samples were assigned to two groups based on biliary bile acid composition: UDCA < 5%, and UDCA > 30%. The first group consisted of patients entering the trial as well as patients who had completed 2 years of therapy with placebo. One patient with 13% UDCA in biliary bile acids was excluded.

Statistical Methods

Means for each bile acid component (CA, CDCA, DCA, UDCA, lithocholic acid [LCA], sulfolithocholic acid [SLCA], and nonidentified peaks [NIP]) for patients with PBC were calculated and compared with normal controls using two-tailed *t* tests.¹⁰ MANOVA¹¹ was performed to simultaneously examine the seven different acid components for the three strata of patients at entry; in addition, one-way ANOVA¹⁰ was also performed across the three strata for each acid component separately. To simultaneously examine the seven different acid components for the two drug groups at entry, a MANOVA was performed, followed by separate one-way ANOVAs for each acid component. Separate analyses for each type of bile acid component at 2 years versus entry bile acid was performed for the two drug groups (placebo and UDCA) using an independent sample *t* test (some of the patients did not have measurements for both entry and at 2 years). Finally, Pearson product-moment correlation coefficients¹⁰ were performed to examine the relationship between changes in the UDCA bile acid and the changes in the other six levels of bile acids from entry to 2 years for all patients treated with UDCA.

RESULTS

Patient Characteristics at Entry

Demographic, laboratory, and histological characteristics of the patients at entry were presented in detail in a previous publication.⁵ The patients were predominantly middle-aged, white women. The results of initial laboratory tests and histological staging were comparable in the patients randomized to receive either placebo or UDCA. Approximately 70% had an entry serum bilirubin of less than 2 mg/dL. One third demonstrated stage I,II histology; two thirds demonstrated stage III,IV histology.

Number of Bile Specimens Analyzed

Analyses were performed on 98 patients at entry, and on 106 at 2 years. The majority of the patients had paired entry and 2-year bile specimens (Table 1). The number of patients who had only an entry or 2-year specimen available for analysis are also indicated in Table 1. There were few patients in stratum 3. Bile acid data for patients in strata 3 and 4 were combined in the descriptions provided below.

Bile Acid Composition at Entry

Comparison With Normal Values—Mean values for individual bile acids at entry in 98 patients with PBC (all strata) are compared with those obtained previously in gallbladder

bile obtained from 14 normal control persons (see Rossi⁷) in Table 2. CA levels were higher than normal, whereas CDCA, DCA, UDCA, LCA, and bile acids in NIP were lower than normal. Values for SLCA were not significantly different from normal.

Mean values for the individual bile acids in the various strata (stratum 1, stratum 2, strata 3–4, data not shown), when compared with normal values yielded similar results, with the exception that LCA in stratum 1 was not significantly different from normal.

Comparison Among Patient Strata—A MANOVA comparing the three strata across the full set of bile acid components showed no significant difference between strata (Wilk's Lambda, $P = .15$). DCA appeared to decrease and CA appeared to increase with worsening stages of PBC. Univariate ANOVA results for each bile acid component separately showed a significant result for DCA (11.1% for stratum 1, 8.1% for stratum 2, 3.5% for strata 3–4; $P = .01$), and nearly significant results for CA (53.1% for stratum 1, 57.0% for stratum 2, 64.0% for strata 3–4; $P = .07$) and for LCA (0.6% for stratum 1, 0.1% for stratum 2, 0.2% for strata 3–4; $P = .08$). However, the multivariate analysis indicates that these results may be spurious considering the intercorrelation among the seven components.

Comparison by Drug Assignment—A MANOVA comparing values in patients assigned to receive either placebo or UDCA showed no significant differences across the full set of bile acid components (Wilk's Lambda, $P = .61$). The set of univariate ANOVA results for each bile acid component separately also showed no significant difference in results.

Bile Acid Composition at 2 Years; Comparison With Entry Values

Placebo—No significant changes in bile acid composition were noted for CA, CDCA, DCA, UDCA or LCA. SLCA decreased from 0.8% to 0.3% ($P = .03$), and NIP increased marginally from 1.4% to 2.7% ($P = .06$) (Table 3).

UDCA—UDCA became the major bile acid in bile, accounting for 40.1% of the bile acids detected. This was accompanied by statistically significant decreases in CA, CDCA, and NIP. No change in percent composition was noted for DCA, LCA, or SLCA (Table 3). Alterations in composition of the major bile acids in bile were comparable for the various strata (see Fig. 1).

Correlation of Changes in UDCA With Changes in Other Bile Acid Components

Significant negative correlations were found between UDCA and CA ($r = -.51$, $P = .001$) (Fig. 2A), and CDCA ($r = -.33$, $P = .04$) (Fig. 2B). Thus, as UDCA increased, CA and CDCA decreased. Correlations between UDCA with changes in other bile acid components were not significant statistically.

Mode of Conjugation

In patients whose bile was not enriched in UDCA, the three major endogenous bile acids (CA, CDCA, DCA) and UDCA were conjugated to a greater extent with glycine (52%–64%) than with taurine (36%–48%), but there was considerable between-patient variation.

Treatment with UDCA caused the proportion of all endogenous bile acids conjugated with glycine to increase to 69% to 78% with a reciprocal decrease in the proportion conjugated with taurine (22%–31%; $P < .05$). Administered UDCA was also conjugated predominantly with glycine (87%). The proportion of individual bile acids conjugated with glycine is illustrated in Fig. 3. In subjects whose bile was enriched in UDCA, the preferential conjugation with glycine of UDCA was not influenced by the degree of UDCA enrichment in biliary bile acids (data not shown). For all endogenous bile acids, the percent conjugation with glycine was fairly similar in a given patient (for CA vs. CDCA: $r = .78$; for CA vs. DCA: $r = .67$; and for CDCA vs. DCA: $r = .55$ [data not shown]). The correlation coefficients were significant ($P < .002$).

DISCUSSION

The bile acid pool becomes significantly enriched with UDCA in patients with PBC who take this compound. This is substantiated by measurements of the biliary composition of UDCA reported in the studies containing sufficient data for all of the major bile acids (see Table 4). UDCA comprised 32% to 40% of the bile acids in bile with doses up to 10 to 12 mg/kg/d and with treatment encompassing periods of 4 weeks to 2 years. The range of values was broad and was not accounted for by the different doses of UDCA administered. In the treatment trial of Lindor et al.,¹² patients with PBC receiving an even higher dose of 13 to 15 mg/kg/d in divided doses with meals and at bedtime achieved a mean UDCA level in bile at 2 years of 39.5%. The range of values varied from barely detectable to over 70%.

The factors responsible for the great intersubject variation in enrichment in biliary bile acids have yet to be elucidated. In each of the above reports, values were obtained from a single specimen of bile obtained after a period of UDCA. The extent to which biliary enrichment with UDCA varies from day to day in a given patient is unknown. Enrichment in biliary bile acids by an administered bile acid depends on multiple factors. These include compliance, fraction absorbed (bioavailability), conservation of the administered bile acid and its metabolites, and the effect of the administered bile acid on endogenous bile acid metabolism.¹³ In the present study, we identified several patients whose biliary bile became enriched with CDCA during therapy with UDCA (see Fig. 2), presumably because of bacterial conversion of administered UDCA to CDCA.^{14,15} Such enrichment in CDCA should decrease the enrichment in UDCA. Our own data, presented here (see Fig. 1) and elsewhere for our UDCA results alone,⁵ indicate that the extent of enrichment with UDCA is comparable in patients in the different strata. Thus, severity of liver disease does not appear to be a major determinant of the extent of enrichment of bile with UDCA.

Relatively little information is available about the effects of divided doses taken with meals versus a single daily bedtime dose of UDCA on the extent of biliary enrichment. In a group of 27 patients with cholestatic liver disease (19 primary sclerosing cholangitis and 8 PBC) who received 10 mg/kg/d for 3 months, mean enrichment of UDCA in bile was 40.1% in 13 patients receiving a single bedtime dose, and 40.8% in 14 patients receiving three divided doses with meals.² In our PBC patients, a single bedtime dose of 10 to 12 mg/kg/d for 2 years yielded a mean UDCA value in bile of approximately 40%. This compares with a mean value of 39.5% for the PBC patients of Lindor et al.,¹² who ingested 13 to 15 mg/kg/d

in divided doses with meals and at bedtime for 2 years. Thus, it appears that a single bedtime dose of UDCA yields similar biliary enrichment with UDCA as multiple divided doses. Intuitively, a single-dose regimen should improve compliance with intake of UDCA.

The relative proportions of the other major bile acids in bile also changed with ingestion of UDCA. Since submission of our manuscript, Lindor et al.¹⁶ have also reported on the effect of UDCA on biliary bile acid composition, and their findings are incorporated in Table 4. CA fell significantly in all four studies. CDCA fell in three of the four. DCA decreased in one, and LCA increased in two of the four. Although LCA increased in our patients, the change was not statistically significant. Our data obtained at 2 years and presented in Fig. 2 indicate that there is an inverse relationship between the degree of enrichment of UDCA in bile and the fall in CA and CDCA.

Assessments of bile acid composition in serum by others^{1,3,16-19} also revealed decreases in CA (all 6 reports), in CDCA (5 of the 6 reports), and lesser falls in DCA (5 of the 6 reports) during treatment with UDCA. In serum as in bile, the relative changes in bile acid composition were comparable in patients with milder and more severe forms of PBC.

Our study also indicates that in PBC patients, treatment with UDCA caused the proportion of endogenous bile acids conjugated with glycine to increase with the result that endogenous bile acids became conjugated mostly with glycine. Administered UDCA was also conjugated predominantly with glycine. This finding was also observed by Crosignani et al. in their analysis of biliary bile acids in 10 PBC patients, but not commented upon.³ We explain this finding as follows: The steady-state mode of conjugation depends on the partition of bile acid conjugation between glycine and taurine in the hepatocyte, and the relative susceptibility of glycine or taurine conjugates to bacterial deconjugation during enterohepatic cycling.²⁰ In healthy subjects, the majority of bile acid conjugation involves reconjugation of unconjugated bile acids formed in the small intestine by bacterial deconjugation of previously secreted bile acids.²¹ Such reconjugation should occur in the periportal hepatocytes, whereas bile acid synthesis is considered to occur largely in pericentral hepatocytes.^{22,23} Administered UDCA also is likely to be conjugated predominantly in the periportal hepatocytes, and there is evidence that UDCA conjugates are extensively deconjugated during enterohepatic cycling.^{24,25} Therefore, periportal hepatocytes must conjugate not only newly ingested UDCA, but also unconjugated UDCA as well as unconjugated endogenous bile acids originating from bacterial deconjugation of secreted bile acids and subsequent absorption of the unconjugated steroid moiety. We postulate that the large flux of unconjugated UDCA through the periportal hepatocyte induces transient depletion of its taurine stores, which, in turn, should result in preferential conjugation of UDCA as well as endogenous bile acids with glycine.²⁶ A similar mode of conjugation of primary bile acids was not unanticipated, because such has been observed previously on a small number of bile samples from healthy subjects⁷ as well as bile samples from many vertebrates.²⁷

A large body of evidence indicates that certain bile acids can induce cholestasis and liver cell necrosis when administered intravenously or intraduodenally to intact animals,²⁸⁻³³ and can decrease bile flow in the isolated perfused rat liver.^{34,35} Toxicity has been shown to

correlate with increasing hydrophobicity of the bile acids as measured by reverse-phase high-pressure liquid chromatography.^{36,37} UDCA in its conjugated forms usually is able to prevent or minimize both cholestasis and liver cell injury in these experimental models,^{30–33} even when the absolute load of the toxic bile acid presented to the liver is not lowered. This hepatoprotective effect can occur very rapidly and need not require changes in pool size of the toxic bile acid. Hepatoprotection may involve inhibition of transport of endogenous hepatotoxic bile acids such as CDCA (as its glycine or taurine conjugate) into hepatocyte organelles.³⁸

CDCA and DCA feeding to humans can induce liver injury, and toxicity has been attributed to the fed bile acid.^{8,39,40} Little information is available about the effects of feeding CA to PBC patients. When Guldutuna et al.⁴¹ administered CA to their patients with PBC, worsening of liver test results was observed in the second and third months of treatment; but CA administration to PBC patients or healthy subjects causes biliary bile acids to become enriched in DCA, its 7-deoxy metabolite.

The changes in biliary bile acid composition^{1,3,16} (also presented in this article) observed during treatment of patients with PBC with UDCA support the hepatoprotective mechanism proposed for UDCA. Thus, the composition of biliary bile acids becomes less hydrophobic, and presumably, the intrahepatic composition does as well. Moreover, the ratio of UDCA to the other bile acids increases significantly. While considerable attention has been directed toward the potential hepatotoxicity of the dihydroxy bile acids, CDCA and DCA, the impressive decreases in CA observed in the present and other reports in bile and in serum^{1,3,16–19} that accompany improvement in laboratory tests during UDCA therapy raise the possibility that CA also contributes to ongoing hepatic injury in PBC. While CA is much less toxic than CDCA and DCA, CA can induce cholestasis and liver cell injury^{29–33,35} that is protected against by UDCA.

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Abbreviations

UDCA	ursodeoxycholic acid
PBC	primary biliary cirrhosis
CA	cholic acid
CDCA	chenodeoxycholic acid
DCA	deoxycholic acid

LCA	lithocholic acid
SLCA	sulfolithocholic acid
NIP	nonidentified peaks

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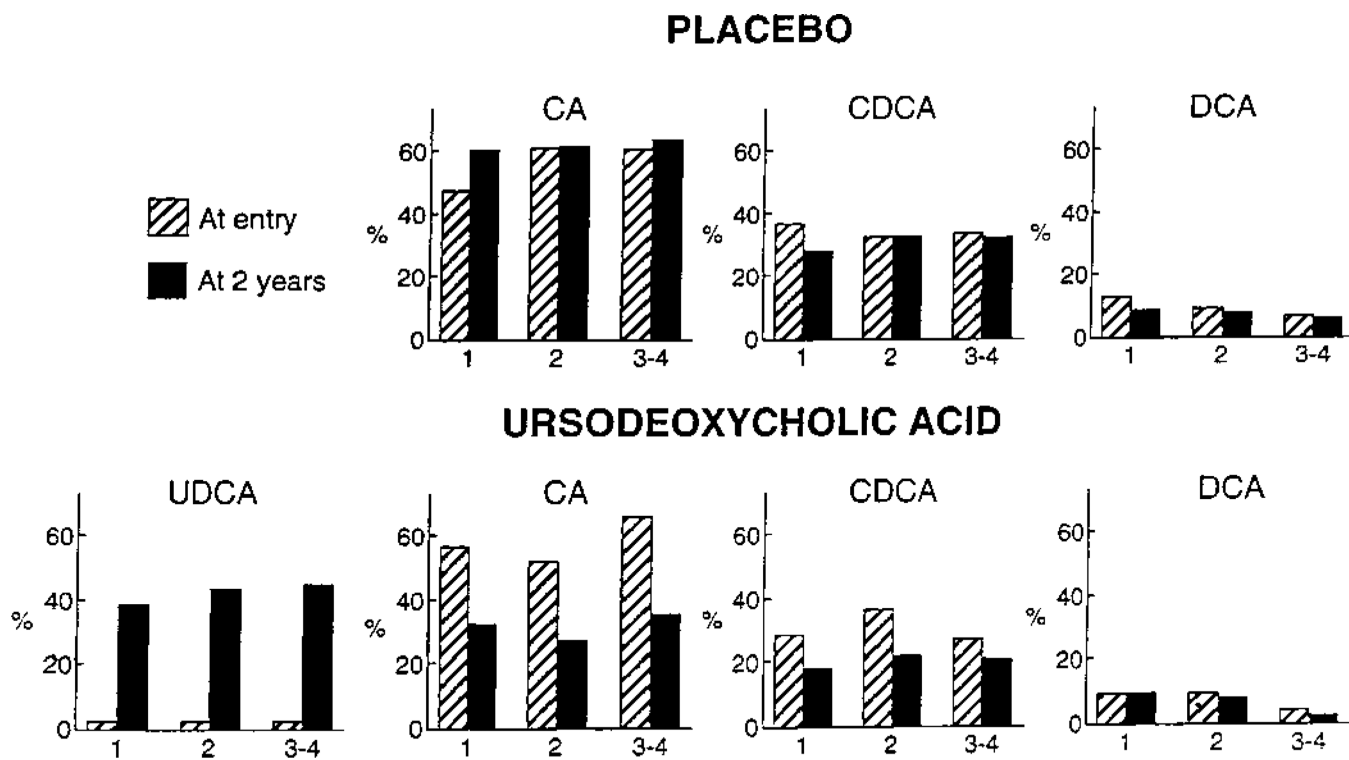


Fig. 1.

Changes in bile acid composition for the major bile acids in bile according to stratum (1, 2, 3-4) after 2 years of treatment with placebo or UDCA.

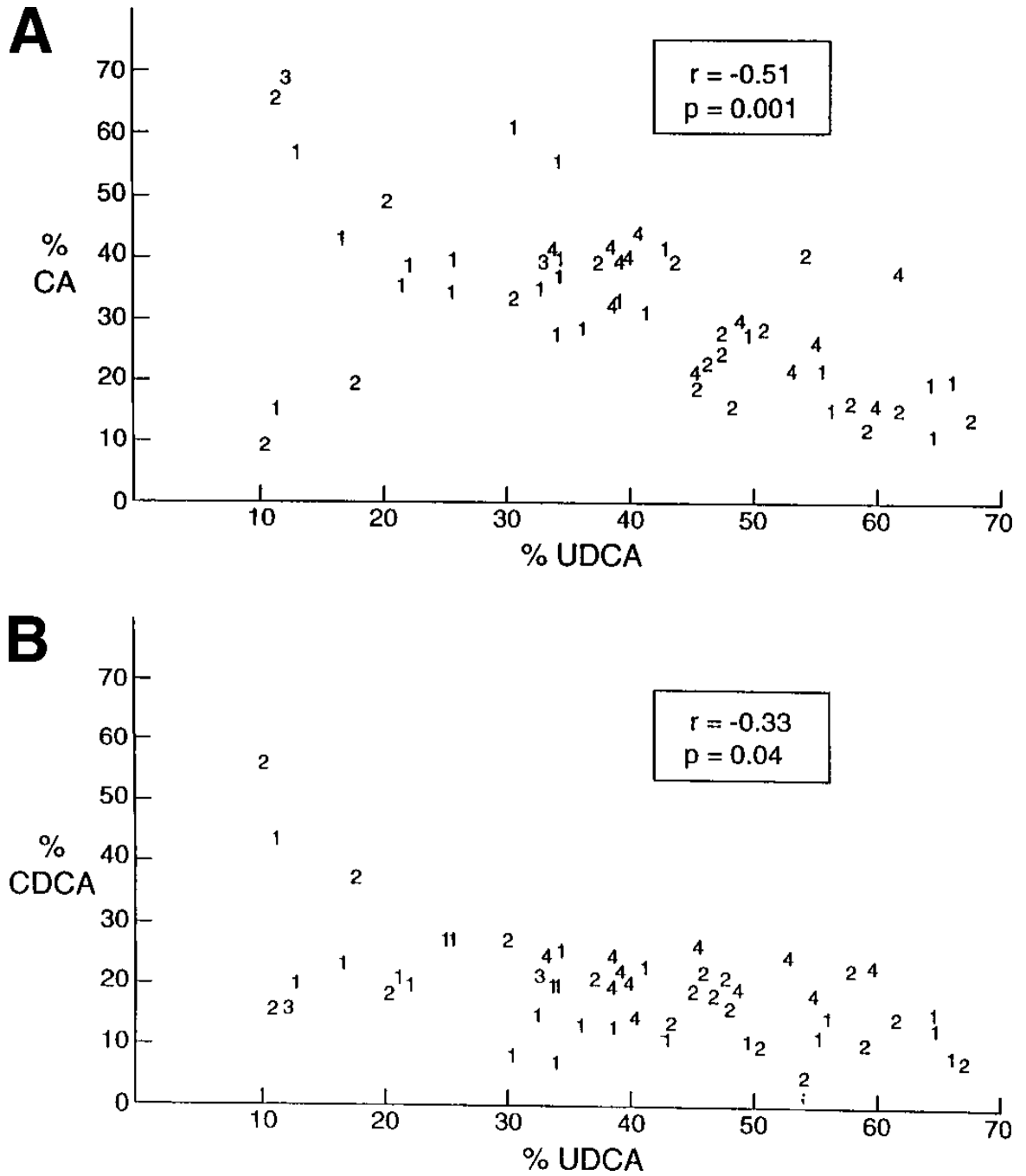


Fig. 2.

Comparison of percent CA (A), and percent CDCA (B) with percent UDCA in bile after 2 years of treatment with UDCA. The numbers indicate the stratum from which each bile specimen was obtained.

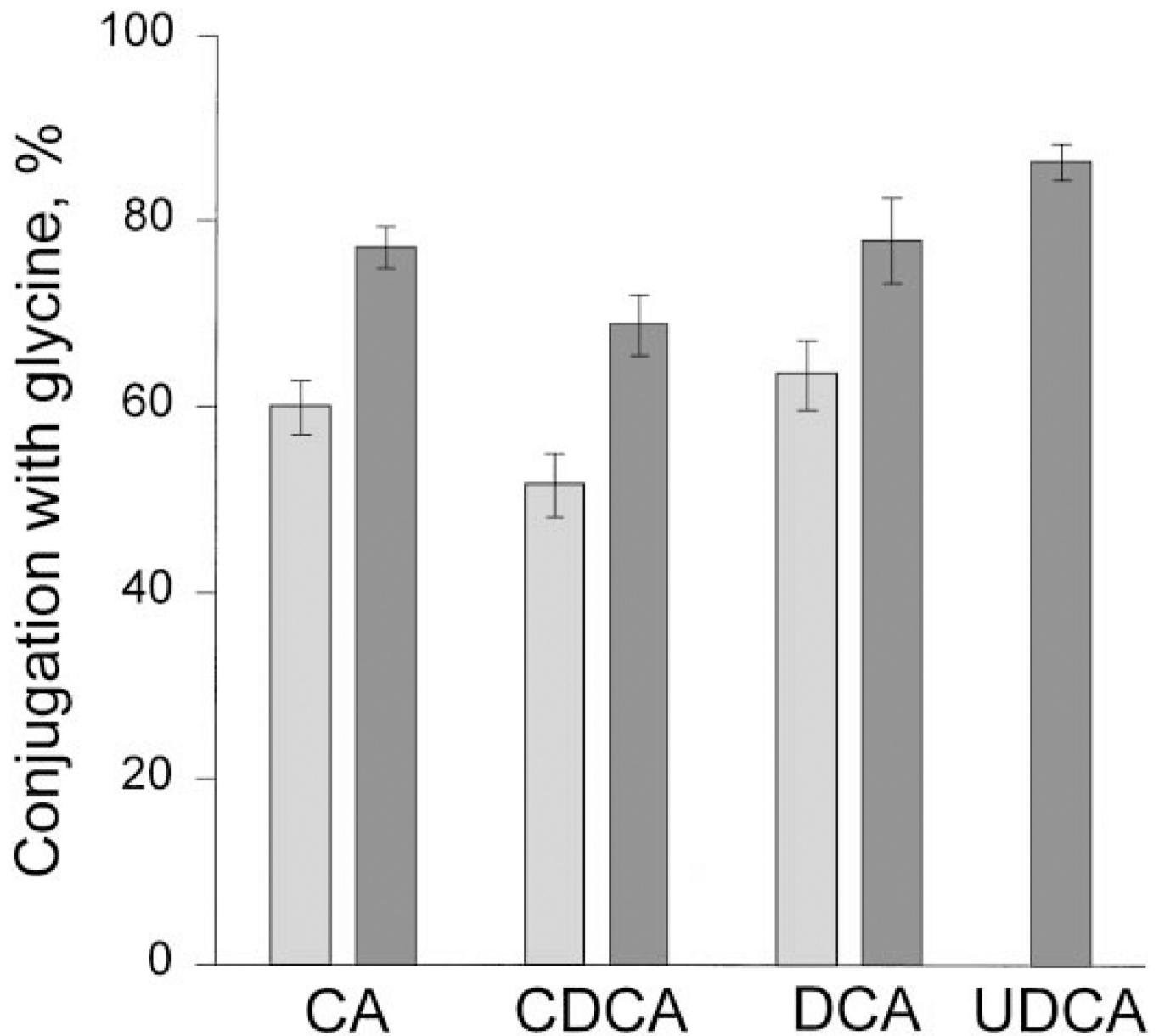


Fig. 3.

Mode of conjugation, expressed as percent conjugation with glycine (mean \pm SE), for endogenous bile acids (CA, CDCA, DCA), and UDCA in biliary bile acids of PBC patients on entry or after treatment with placebo (*light stippling*) or after treatment with UDCA (*dark stippling*). Patients who were pretreatment or who had been treated with placebo had < 5% UDCA in biliary bile acids; those treated with UDCA had > 30% UDCA in biliary bile acids. Treatment with UDCA caused the proportion of bile acids conjugated with glycine to increase ($P < .05$). (□), UDCA < 5%; (■), UDCA > 30%.

Table 1

Number of Bile Specimens Analyzed

	At Entry		At 2 Years	
	Placebo (n)	UDCA (n)	Placebo (n)	UDCA (n)
Stratum 1				
Paired	11	15	11	15
Unpaired	4	4	4	8
Total	15	19	15	23
Stratum 2				
Paired	18	14	18	14
Unpaired	3	4	9	4
Total	21	18	27	18
Strata 3-4				
Paired	2 (1)*	9 (2)	2 (1)	9 (2)
Unpaired	6	8 (3)	7 (1)	5
Total	8 (1)	17 (5)	9 (2)	14 (2)
All strata				
Paired	31	38	31	38
Unpaired	13	16	20	17
Total	44	54	51	55

* Number of patients in stratum 3 indicated in parentheses.

Table 2

Bile Acid Composition in Bile at Entry % of Total Bile Acids

Bile Acid	PBC All Strata										Difference of Mean from Normal $P < .05$	
	Normal Persons ⁷		95% Confidence Limits				Upper	Lower	SD	Mean		SD
	Mean	SD	Upper	Lower	Upper	Lower						
CA	36.5	8.3	57.4	18.6	53.7	61.1						Increased
CDCA	38.5	8.0	31.5	15.5	28.4	34.6						Decreased
DCA	15.8	8.4	8.0	9.3	6.2	9.8						Decreased
UDCA	2.1	1.4	0.6	0.9	0.4	0.8						Decreased
LCA	0.7	0.4	0.3	1.0	0.1	0.5						Decreased
SLCA	0.6	0.3	0.6	1.1	0.4	0.8						Not significant
NIP	5.8	2.5	1.6	2.3	1.1	2.1						Decreased

Table 3

Comparison of Bile Acid Composition at Baseline and After 2 Years on Placebo or UDCA

Bile Acid	Time	% of Bile Acids in Bile	
		Placebo	UDCA
CA	Entry		
	Mean	56.0	58.6
	SD	19.1	18.2
	2 years		
	Mean	61.4	32.2
	SD	16.5	13.8
	<i>P</i>	NS	<.001
CDCA	Entry		
	Mean	32.6	30.6
	SD	15.5	15.7
	2 years		
	Mean	28.9	19.5
	SD	12.6	9.3
	<i>P</i>	NS	<.001
DCA	Entry		
	Mean	8.1	7.9
	SD	9.0	9.6
	2 years		
	Mean	6.0	6.7
	SD	7.0	6.6
	<i>P</i>	NS	NS
UDCA	Entry		
	Mean	0.6	0.6
	SD	0.9	1.0
	2 years		
	Mean	0.4	40.1
	SD	0.6	15.8
	<i>P</i>	NS	<.001
LCA	Entry		
	Mean	0.4	0.2
	SD	1.3	0.7
	2 years		
	Mean	0.2	0.3
	SD	0.6	1.0
	<i>P</i>	NS	NS
SLCA	Entry		
	Mean	0.8	0.4
	SD	1.3	0.9

Bile Acid	Time	% of Bile Acids in Bile	
		Placebo	UDCA
	2 years		
	Mean	0.3	0.6
	SD	0.9	1.1
	<i>P</i>	.03	NS
NIP	Entry		
	Mean	1.4	1.8
	SD	2.5	2.2
	2 years		
	Mean	2.7	0.9
	SD	3.8	1.5
	<i>P</i>	.06	.012

Table 4

Effect of UDCA on Bile Acid Composition in Bile

	Mazzella et al. ¹	Crosignani et al. ²	Current Study	Lindor et al. ¹⁶
UDCA dose	600 mg/d	500 mg/d 8 mg/kg/d	10–12 mg/kg/d bedtime dose	13–15 mg/kg/d, divided dose with meals and at bedtime
Duration	4 wk	6 mo	2 yr	2 yr
n	9	10	55	55

	Bile Acid Composition in Bile (percent of total)											
	Entry	2 Years	P	Entry	2 Years	P						
UDCA	Trace	34.2	<.001	4.5	31.9	<.001	0.6	40.1	<.001	1.0	44.0	<.0001
CA	48.4	35.1	<.001	57.9	30.8	<.005	58.6	32.2	<.001	54.0	28.0	<.0001
CDCA	31.2	21.3	<.001	29.5	26.2	NS	30.6	19.5	<.001	32.0	18.0	<.002
DCA	16.5	6.8	<.001	8.1	11.1	NS	7.9	6.7	NS	10.0	10.0	NS
LCA	2.5	2.5	NS	0.8	3.0	<.05	0.2	0.3	NS	0.2	0.8	.009