Metronidazole and Ursodeoxycholic Acid for Primary Sclerosing Cholangitis: A Randomized Placebo-Controlled Trial

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No effective medical therapy is currently available for primary sclerosing cholangitis (PSC). Ursodeoxycholic acid (UDCA) improves liver enzymes, but its effect on liver histology is controversial. Metronidazole (MTZ) prevents PSC-like liver damage in animal models and reduces intestinal permeability. We recruited 80 patients with PSC into a randomized placebo-controlled study to evaluate the effect of UDCA and MTZ (UDCA/MTZ) compared with UDCA/placebo on the progression of PSC. Patients (41 UDCA/placebo and 39 UDCA/MTZ) were followed every third month. Assessment of liver function test, histological stage and grade, and cholangiography (via ERCP) at baseline showed no differences between the groups. After 36 months, serum aminotransferases γ-glutamyltransferase, and alkaline phosphatase (ALP) decreased markedly in both groups, serum ALP more significantly in the UDCA/MTZ group (−337 ± 54 U/L, $P < .05$) compared with the UDCA/placebo group. The New Mayo Risk Score decreased markedly only in the UDCA/MTZ group (−0.50 ± 0.13, $P < .01$). The number of patients with improvement of stage ($P < .05$) and grade ($P < .05$) was higher in the combination group. ERCP findings showed no progression or improvement in 77% and 68% of patients on UDCA/MTZ and UDCA/placebo, respectively. In conclusion, combining MTZ with UDCA in PSC improved serum ALP levels and New Mayo Risk Score, but no statistically significant effect on disease progression as assessed via liver histology or ERCP was seen. Long-term studies using a higher dose of UDCA combined with MTZ in larger patient populations are indicated. (HEPATOLOGY 2004;40:1379–1386.)

Primary sclerosing cholangitis (PSC) is a progressive cholestatic liver disease of unknown etiology characterized by inflammation and fibrosis of the intra- and extrahepatic bile ducts.[1] Approximately 80% of patients have concomitant inflammatory bowel disease, most commonly ulcerative colitis. The strong association between PSC and inflammatory bowel disease has been speculated to cause increased translocation of colonic bacteria and endotoxins, as well as enhanced absorption of toxic bile acids to the liver via the portal vein.[1,2] This may lead to activation of Kupffer cells in the liver, with consequent overproduction of tumor necrosis factor and immunoactivation of biliary epithelial cells in genetically susceptible patients.[2,3] A close genetic association of PSC with HLA-DR3, DQ2 has been reported[4] and is described in other autoimmune liver diseases, as well.[5] Moreover, in PSC biliary epithelial cells have demonstrated strong immunostaining for endotoxin.[6] In rats, overproduction of tumor necrosis factor has been associated with bile duct destruction and proliferation,[7,8] and

Abbreviations: PSC, primary sclerosing cholangitis; UDCA, ursodeoxycholic acid; MTZ, metronidazole; ERCP, endoscopic retrograde cholangio-pancreaticography; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase.

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the self-filling loops of the small bowel associated with bacterial overgrowth with *Bacteroides* species can lead to hepatobiliary injury resembling PSC in humans.[9]

Liver damage can be prevented with metronidazole (MTZ) or tetracycline.[9] In humans, metronidazole has been shown to induce significant inhibition of the endotoxin-stimulated tumor necrosis factor alpha production of human peripheral blood mononuclear cells at therapeutic levels.[10] Metronidazole also has beneficial effects in healing intestinal damage, reduces intestinal permeability, and has a direct protective effect on the uncoupling of mitochondrial oxidative phosphorylation caused by nonsteroidal anti-inflammatory drugs.[11]

At this time no effective medical therapy is available for treatment of PSC. Various treatment strategies have been evaluated in PSC and negative studies have been published on glucocorticoids, budesonide, azathioprine, colchicine, cyclosporine, D-penicillamine, methotrexate, tacrolimus, and pentoxifyllene[12] and in a small series even in combination with ursodeoxycholic acid (UDCA). In the largest controlled trial conducted so far, UDCA at 13 to 15 mg/kg was not associated with any significant changes in symptoms or in liver histology, whereas liver function tests were improved during UDCA therapy.[13] In this study, however, a significant proportion of the patients already had advanced disease at baseline, and 34% in the UDCA group had varices. In a recent study with 2 years' follow-up, a higher dose of UDCA (20 mg/kg/d) appears to have a beneficial effect on both liver histology and cholangiographic findings at ERCP.[14] Only one small, uncontrolled long-term study using antibiotics in the treatment of PSC has been published so far.[15] The study compared tetracycline, steroid therapy, and colectomy in the management of PSC, demonstrating that none of the strategies had a beneficial effect on the clinical course of the disease.

In the present study, we evaluated the combination of UDCA and MTZ in the treatment of PSC compared with UDCA and placebo in a randomized, controlled 3-year trial.

Patients and Methods

Patient Selection. Patients with suspected or known PSC were screened for elevated liver enzymes (serum alanine aminotransferase [ALT] $>$ 50 U/L, normal range 10-50 U/L and/or serum ALP $>$ 275 U/L, normal range 60-275 U/L), and diagnosis was confirmed via both liver histology and ERCP (compatible with PSC) from Helsinki, Tampere, and Turku University Hospitals, Finland. All the participating hospitals were tertiary referral centers. Patients aged 16 to 65 years were included in the study if they had PSC documented via ERCP and histological analysis. Patients were excluded if they had (1) end-stage liver disease with decompensation, which was defined as ascites not easily controlled by diuretic therapy (Child-Turcotte-Pugh C), (2) other coexisting liver diseases, (3) suspected cholangiocarcinoma, (4) suspected or documented malignancy, (5) recurrent ascending cholangitis requiring antibiotic therapy, or (6) if they were pregnant. Previous use of UDCA was not an exclusion criterion, but use and duration of UDCA therapy before enrollment was noted and the patients on UDCA at baseline continued the therapy, except for 6 patients who stopped the medication 6 months before inclusion. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the Helsinki University Ethics Committee. All patients gave their written informed consent.

Study Design. In this multicenter, randomized, double-blind, placebo-controlled trial, the patients were randomized either to UDCA and placebo (n = 41) or UDCA and MTZ (n = 39). The sample size calculation was based on our previous experience on the combination therapy assuming that 30% improvement will be seen in serum ALP, thus requiring 80 patients. The duration of this pilot study of MTZ in PSC was planned for 36 months, because a shorter duration might not have demonstrated any changes other than effect on liver function tests. Randomization was done centrally with computer-generated blocks, and patients were stratified according to the type of inflammatory bowel disease, distribution of PSC (intrahepatic or intra- and extrahepatic disease), and possible previous colectomy.

UDCA at 15 mg/kg/d was administered in the form of 150-mg capsules (Adursal 150 mg, Leiras Finland, Helsinki, Finland). MTZ at 600 to 800 mg/d was given in the form of 200-mg tablets (Trikozol 200 mg, Orion Pharma, Espoo, Finland), and doses were adjusted according to body weight: 600 mg for patients weighing 75 kg or less and 800 mg for patients weighing over 75 kg, or the same number of placebo tablets.

Methods. A complete history and physical examination was performed upon entry into the study. ERCP was performed within 6 months and liver biopsy within 12 months prior to enrollment. Abdominal Doppler ultrasound, esophago-gastroduodenoscopy with duodenal bile sample, and colonoscopy were performed for every patient. Bile samples were obtained after stimulation with cholecystokinin (1-2 U/kg intravenously) before the duodenal biopsies to analyze bile acid composition. Patients on UDCA therapy before inclusion stopped UDCA before the duodenal bile sample was collected. Bile acids, cholesterol, and phospholipids were analyzed from duo-

denal bile aspirates via gas–liquid chromatography, and molar concentrations were calculated.[16] Serum levels for full blood count, ALT, AST (normal range, 15-35 U/L), ALP, bilirubin (<20 μmol/L), prothrombin time (70%-130%), bile acids (<8 mmol/L) albumin (36-48 g/L), procollagen III N-terminal peptide (1.7-4.2 μg/L), and serum immunoglobulin G (6.8-15.0 g/L) and M (0.36-2.59 g/L) were determined using routine laboratory methods. Serum perinuclear antineutrophil cytoplasmic antibody staining patterns and titers were determined via indirect immunofluorescence employing ethanol and formalin-fixed human granulocytes.

Patients were followed up every 3 months with a symptom questionnaire, clinical examination, and liver function tests. At the end of the study, ERCP, liver biopsy, and abdominal ultrasound were repeated in addition to the clinical evaluation, symptom questionnaire, and liver function tests (including AST). Liver biopsies were evaluated by a sole pathologist who was blinded to the clinical data and biopsy sequence. The liver biopsies were classified according to stage and grade, modified from Ludwig et al.[17] and Desmet et al.[18] Stage reflects disease progression and morphological changes; grade reflects necroinflammatory activity. Stage was scored as follows: normal = 0; portal edema = 1; periportal fibrosis = 2; septa formation and ductal loss = 3; cirrhosis = 4. Grade scores were as follows: no inflammation = 0; portal inflammation and pericholangitis < 20% of areas = 1; portal inflammation and pericholangitis > 20% of areas = 2; mild interface inflammation ("piecemeal necrosis") = 3; marked interface inflammation = 4. ERCP findings were analyzed by two radiologists independently, specialized in hepatobiliary diseases, and blinded to clinical data and the order of examinations. Cholangiograms were performed with balloon catheter occlusion whenever possible, to ensure adequate filling pressure in intrahepatic peripheral branches. ERCP findings were classified according to Craig et al.[19] and by global assessment, with findings graded as "improvement," "no change," or "worsening." The New Mayo Risk Score was calculated at entry and at the end of the treatment period using the revised PSC Mayo Risk Score[20]: risk = $0.03 \times$ age (yr) + $0.54 \times \log_e$(total bilirubin [mg/dL]) $- 0.84 \times$ albumin (g/dL) + $0.54 \times \log_e$(AST IU/L) + $1.24 \times$ variceal bleeding (yes = 1; no = 0).

The patients were treated for 36 months unless they were referred for liver transplantation, developed a malignancy or a decompensated liver cirrhosis, or died. Treatment response was followed by: (1) clinical symptoms and cholangitis episodes; (2) liver function tests (ALP, ALT, AST, bilirubin, and albumin); (3) New Mayo Risk Score; (4) liver histology; and (5) ERCP findings. Clinical symptoms: fatigue, fever, pruritus, and right upper quadrant pain were scored as follows: 0 = absent; 1 = mild; 2 = moderate; 3 = severe. After 36 months, MTZ/placebo was stopped and patients were followed up for at least 12 weeks afterward.

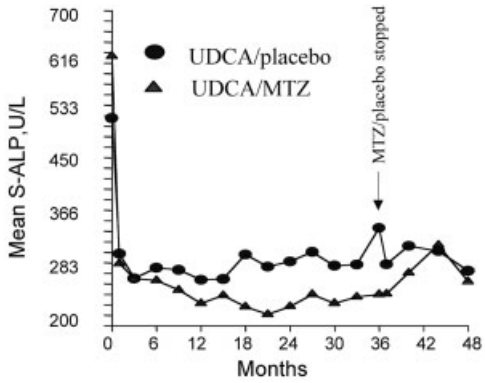

Fig. 1. The effect of UDCA/placebo or UDCA/MTZ on mean serum ALP levels during 36 months. After 36 months, all patients were placed on UDCA monotherapy (15 mg/kg/d). S-ALP, serum alkaline phosphatase; UDCA, ursodeoxycholic acid; MTZ, metronidazole.

Stastitical Analysis. Statistical analyses were conducted with NCSS 2000 software (Kaysville, UT). Results between groups are expressed as the mean ± SE. Two-sample *t* tests or Wilcoxon rank sum tests served to examine differences between groups. ANOVA determined the effect of therapy on liver histology and ERCP findings.

Results

Clinical Data. Eighty patients fulfilling the entry criteria were randomized to either UDCA and placebo (n = 41) or UDCA and MTZ (n = 39) (Fig. 1). Baseline clinical data are presented in Table 1. No significant differences appeared between treatment arms. One patient had small-duct PSC with typical histology but normal ERCP. In total, half of the patients had no or only mild symptoms (sum score of symptoms: fatigue, fever, pruritus, right upper quadrant pain ≤ 1) at entry. Of the study population, 81% had concomitant inflammatory bowel disease, most frequently ulcerative colitis (80%). At the end of the study, 75% of UDCA/placebo patients and 79% of those on UDCA/MTZ were asymptomatic; the difference was considered insignificant. Only 2 patients in both groups had varices at baseline endoscopy. Three patients had overlapping syndromes of autoimmune hepatitis and PSC diagnosed by positive smooth muscle antibodies, elevation of serum immunoglobulin G, and typical histology with interface inflammation in addition to typical ERCP findings. Two of these pa-

Table 1. Clinical Characteristics of the Study Patients at Baseline

Characteristics	Total	UDCA + Placebo	UDCA + MTZ
No.	80	41	39
Females, %	48	42	51
Age, yr	39 ± 2	41 ± 2	37 ± 2
Age at diagnosis, yr	36 ± 2	37 ± 2	34 ± 2
Duration of PSC, yr	3.7 ± 0.7	3.7 ± 0.7	3.7 ± 0.6
Distribution of PSC			
Intrahepatic only, %	51	52	48
Varices	4	2	2
S-pANCA positive, %	58	58	57
Concomitant IBD, %	81	87	79
Ulcerative colitis, %	80	84	76
Crohn's disease, %	15	13	17
Indeterminate colitis, %	5	3	7
Previous use of UDCA			
No., %	22 (28)	8 (20)	14 (36)
Range, mo	1–60	7–60	1–41
Concomitant medication			
5-aminosalicylates	52	28	24
Azathioprine	4	2	2

NOTE. Data are presented as the mean ± SE. No statistical differences were noted between the treatment groups.

Abbreviations: S-pANCA, serum perinuclear anti-neutrophil cytoplasmic antibodies; IBD, inflammatory bowel disease.

tients had a flare of autoimmune hepatitis with significant elevation of serum ALT levels and were treated with prednisone. Both patients were in the UDCA/placebo group. During the treatment period, 3 patients in the UDCA/placebo group underwent liver transplantation: 1 at 6 months, and 2 at 16 months. In the UDCA/MTZ group, 2 patients developed cholangiocarcinoma diagnosed at 15 and 34 months, and 1 patient discontinued the study because of liver transplantation.

Laboratory Values. Baseline levels and changes in liver biochemistry and the Mayo Risk Score after 3 years of treatment are summarized in Table 2. At entry, the treatment groups were comparable with regard to liver function tests and risk score. Serum immunoglobulin levels were similar at baseline, and no differences were found during the treatment period. During therapy, ALP levels dropped in both groups—more significantly in the UDCA/MTZ group—and remained lower throughout the study period (Fig. 1). After 36 months of therapy, when MTZ or placebo was stopped and patients continued only on UDCA monotherapy, the difference between study groups disappeared. ALT and AST were significantly improved in both groups, and bilirubin values (which were within normal range at entry) remained unchanged. Serum procollagen III N-terminal peptide levels were similar in both groups and did not change markedly during therapy. In patients treated with UDCA/MTZ, however, both the New Mayo Risk Score and serum ALP levels decreased ($P < .05$) compared with the UDCA/placebo group, and the mean ALP value even normalized (see Table 2). The molar percentage of UDCA rose markedly from 2.6% to 51.3% in the UDCA/placebo group and from 1.1% to 48.0% in the UDCA/MTZ group, though this change demonstrated no differences between groups.

Histology. Adequate liver biopsies at entry and at 36 months were available for comparative analysis from 36 patients in the UDCA/placebo group and from 32 patients in the UDCA/MTZ group. The results are presented in Tables 3 and 4. In biopsies at baseline, no significant differences existed between the treatment arms for stage or grade (see Table 3). Improvement of stage was

Table 2. Laboratory Data for Study Patients

	UDCA + Placebo			UDCA + MTZ		
Variable	At Baseline (n = 41)	At 36 Months (n = 37)	Change from Baseline	At Baseline (n = 39)	At 36 Months (n = 34)	Change From Baseline
ALP (U/L)	567 ± 63	313 ± 33††	−214 ± 50	643 ± 65	253 ± 34††	−337 ± 54*
AST (U/L)	81.8 ± 13.2	48.0 ± 5.2††	−28.0 ± 10.4	79.0 ± 7.1	45.6 ± 4.8††	−32.4 ± 7.4
ALT (U/L)	126 ± 18	59 ± 9††	−71 ± 15	140 ± 19	49 ± 9††	−95 ± 17
GGT (U/L)	519 ± 91	168 ± 49††	−358 ± 71	584 ± 92	214 ± 53††	−394 ± 82
Total bilirubin (μmol/L)	15.8 ± 1.5	15.0 ± 1.2	−0.8 ± 1.3	16.1 ± 1.6	13.5 ± 1.1	−1.0 ± 1.0
Bile acids (mmol/L)	26.5 ± 8.5	21.1 ± 5.2	10.5 ± 5.9	11.6 ± 1.9	29.2 ± 7.9	17.4 ± 6.4
Albumin (mg/L)	40.5 ± 0.7	41.0 ± 0.8	−0.2 ± 0.7	40.3 ± 0.7	42.0 ± 0.8	1.4 ± 0.9
Prothrombin time (s)	104 ± 5	107 ± 6	5 ± 5	112 ± 5	98 ± 7	−12 ± 6**
B platelets × 10^9/L	247 ± 12	241 ± 11	−0.2 ± 0.7	273 ± 16	247 ± 15	1.4 ± 0.9
PIIINP (μg/L)	4.6 ± 0.3	4.0 ± 0.3	−0.6 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	−0.1 ± 0.4
Biliary UDCA (%)	2.6 ± 0.5	51.3 ± 2.1††	48.5 ± 2.0	1.1 ± 0.3	48.0 ± 2.6††	46.2 ± 3.1
New Mayo Risk Score	−0.15 ± 0.11	−0.21 ± 0.10	−0.06 ± 0.08	−0.18 ± 0.11	−0.50 ± 0.13††	−0.32 ± 0.10*

NOTE. Data are presented as the mean ± SE.

Abbreviations: GGT, γ-glutamyltransferase; PIIINP, procollagen III N-terminal peptide.

*$P < .05$, **$P < .01$; differences between treatment groups.

††$P < .01$; differences from baseline.

Table 3. Liver Histology at Baseline

Histology	At Baseline	
	UDCA + Placebo (n = 37)	UDCA + MTZ (n = 34)
Stage, n (%)		
0 (normal)	10 (27)	7 (21)
1 (portal edema)	7 (19)	5 (15)
2 (periportal fibrosis)	15 (41)	13 (38)
3 (septal formation and ductal loss)	3 (8)	9 (26)
4 (cirrhosis)	2 (5)	0 (0)
Grade, n (%)		
0 (no inflammation)	5 (14)	10 (29)
1 (portal inflammation > 20% of areas)	13 (36)	6 (18)
2 (portal inflammation < 20% of areas)	7 (36)	5 (15)
3 (mild interface inflammation)	6 (17)	8 (23)
4 (marked interface inflammation)	6 (14)	5 (15)

NOTE. No statistically significant differences were noted between groups.

seen in 34% of patients in the UDCA/MTZ group, significantly more often than in the UDCA/placebo group ($P = .047$) (see Table 4). Either no change or improvement of stage was seen in 69% of patients treated with UDCA/placebo and in 62% of patients on UDCA/MTZ, showing no difference between groups. Progression in stage of liver histology was seen in 31% to 38% of patients in the UDCA/placebo and UDCA/MTZ groups, respectively. Grade showed improvement during UDCA/MTZ therapy in 34.4% of study subjects, whereas only 14% of those undergoing UDCA monotherapy demonstrated improvement, significantly less than in the combination group ($P = .014$). Improvement or no sign of progression in grade was evident in 72% of patients on UDCA/MTZ therapy but only in 58% on UDCA/placebo therapy. The mean grade decreased more significantly in the combination group (-0.18 ± 0.18) compared with patients treated with UDCA alone (0.39 ± 0.15) ($P = .009$).

ERCP Findings. ERCP findings with adequate filling at baseline and at 36 months were available for comparative analysis for 48 patients, 23 of whom were in the UDCA/MTZ group. In a global assessment, improvement or no change was seen in 68% of patients in the UDCA/placebo group and in 83% of patients in the combination group. Worsening of cholangiographic findings were seen in 32% of the UDCA/placebo patients and 17% of the UDCA/MTZ patients. Differences between groups did not reach significance.

Table 4. Effect of Therapy on Liver Histology (Stage and Grade) During 3 Years Follow-up

Change in Histology	UDCA + Placebo (n = 36)	UDCA + MTZ (n = 32)	*P* Value
Stage, n (%)			
Improvement	5 (14.0)	11 (34.4)	.047
No change	20 (55.5)	9 (28.1)	.022
Worsening	11 (30.5)	12 (37.5)	NS
Grade, n (%)			
Improvement	6 (16.6)	14 (43.8)	.014
No change	15 (41.7)	9 (28.1)	NS
Worsening	15 (41.7)	9 (28.1)	NS

Abbreviation: NS, not significant.

Table 5. Cumulative Number of Side Effects During 36-Months Treatment Period

Side Effects	UDCA + Placebo (n = 37)	UDCA + MTZ (n = 34)
Dryness of mouth	1	
Discoloration of tongue		1
Metallic taste		1
Nausea		4
Dyspepsia		2
Bloating		1
Diarrhea/loose stools	1	5
Dizziness		1
Weakness/numbness in extremities	3	2
Headache		1
Depression	1	
Arthralgia	1	
Total sum	7	18*
Action taken: dose reduction/temporary discontinuation	1/0	5/1

*$P < .05$.

Side Effects. Both UDCA and MTZ therapy were well tolerated with no serious side effects (side effects are summarized in Table 5). Seven patients in the UDCA/placebo group reported side effects, as did 18 patients in the UDCA/MTZ group ($P < .05$). Although the dose of study medication was reduced temporarily in 5 cases, none of the patients discontinued the study medication because of side effects.

Discussion

In this randomized, placebo-controlled trial of antibiotics—in addition to UDCA—in the treatment of PSC, a significant decrease in serum ALP, ALT, AST, and γ-glutamyltransferase activity was seen in both treatment arms. Combining MTZ with UDCA improved serum ALP values significantly and the New Mayo Risk Score even further. In the UDCA/MTZ group, more patients had an improvement of both stage and grade of liver histology, and the decrease of the mean grade was greater ($P = .009$). However, no differences between groups in patients with either improvement or no change in stage or grade were seen. In ERCP findings, the proportion of patients with either improvement or no progression was 83% of those on UDCA/MTZ therapy compared with 68% of those on monotherapy, showing a trend favoring combination therapy.

Because the etiopathogenesis of PSC is unknown, no specific therapy is currently available. Moreover, there are

no tools for screening and diagnosing the disease early enough (*i.e.*, when it is amenable to treatment), well before irreversible strictures, fibrosis, and cirrhosis have developed. Most previous studies have evaluated the effect of immunosuppressive agents in PSC (*e.g.*, prednisone, budesonide, azathioprine, methotrexate, and penicillamine) with completely negative or very limited effects,[21–30] suggesting that immunological factors probably play little or no role in the pathogenesis of PSC by the time marked strictures or cirrhosis have developed. PSC is a progressive disease leading to obstruction of bile ducts and cholestasis, which in turn leads to increased endotoxin levels in the portal space and activation of Kupffer cells and macrophages. UDCA has been shown to displace hydrophobic endogenous bile acids associated with increased cell membrane fluidity and permeability and with increased secretion of bile acids and other organic compounds.[31] Moreover, UDCA has been shown to be immunomodulatory,[32] and in primary biliary cirrhosis it reduces the overexpression of hepatocellular and biliary expression of major histocompatibility complex Class I and II molecules,[33,34] unlike cyclosporin or corticosteroids.[35]

In PSC, UDCA therapy at 13 to 15 mg/kg/d has been associated with significant improvement in biochemical test results[13,36] but has not been shown to have any effect on symptoms, liver histology, or time to transplantation.[13] A dosage of 20 mg/kg/d UDCA[14] used in 13 patients for 2 years did not affect symptoms compared with placebo, but 2 patients (15%) showed marked improvement in cholangiographic appearance and 3 patients (27%) showed similar improvement in histological inflammatory score. Another pilot study[37] of 30 patients treated with 25 to 30 mg/kg/d UDCA for 1 year improved the New Mayo Risk Score and AST and ALP levels significantly compared with a standard dose but did not have any influence on bilirubin. In primary biliary cirrhosis, doubling the dosage of UDCA to 28 to 32 mg/kg/d did not benefit most patients not responding to a dosage of 13 to 15 mg/kg/d.[38] We used a standard dosage of 15 mg/kg/d UDCA, because at the time of the study only small studies using UDCA 10 to 15 mg/kg/d had been published, some of them suggesting beneficial effects even on histology.[36,39]

In our study, 20% of patients in total were already on UDCA before the study, and the aim was to evaluate the possible additional effect of MTZ on the treatment response of UDCA, so we did not have a placebo group. A recent Scandinavian study demonstrated that patients with PSC who were treated with UDCA while on a waiting list for liver transplantation had significantly fewer cholangiocarcinomas than patients who did not receive UDCA therapy, suggesting that even a standard dose of UDCA may have a positive effect on the clinical outcome of the disease.[40] Very few studies have actually evaluated the effect of UDCA on ERCP changes,[3,14,41] one of them demonstrating that it does not prevent major bile duct occlusion even during therapy lasting up to 13 years,[41] making it questionable that a higher dose can really improve cholangiographic appearance within 2 years.[14] In the present study, we excluded patients with end-stage liver disease, and half of the patients were actually asymptomatic and had intrahepatic disease of only 3.4 years' mean duration (range, 0-18 years). In liver histology, stage was normal in 28% of patients in both groups, whereas only 2 patients in the UDCA/placebo group and 3 in the UDCA/MTZ group had cirrhosis at liver biopsy. Our patient population represents a more favorable opportunity to evaluate medical therapy for PSC than in most previous studies, where up to 23% to 25% of patients had cirrhosis.[13,36] UDCA therapy significantly improved liver biochemistry in both groups in accordance with previous findings.[13,14,36] Marked inflammation (grades 3-4), seen in 31% of patients in the UDCA/placebo group and in 38% of patients in the UDCA/MTZ group, suggests a chronically active disease process. The slight improvement in liver histology both in stage and in grade seen in UDCA/MTZ therapy versus UDCA alone would suggest an anti-inflammatory effect of MTZ. However, the effect of MTZ on liver histology during 3 years' therapy was only marginal regarding the number of patients with improvement or no progression, because no difference was found between groups. Unfortunately, we only had adequate comparable biopsies from 32 patients in the UDCA/MTZ group, and the difference between groups did not reach statistical significance. PSC is a segmental disease, and a single liver biopsy may be associated with a significant sampling error.[42] The role of liver biopsy in PSC has recently been challenged,[43] suggesting that it does not affect management. The present study demonstrates, however, that marked inflammation is present in a significant proportion of patients, which can be affected by the combination of MTZ and UDCA.

The follow-up of disease progression in PSC is problematic because there is poor correlation between ERCP findings, liver histology, and liver biochemistry.[14] We included the control ERCP in the study protocol because classification and staging of cholangiographic abnormalities has been shown to have prognostic value.[44] In those patients in whom comparable examinations with adequate filling were available, a trend showing either improvement or no progression of the disease was seen in the UDCA/MTZ group compared with those on UDCA monotherapy. However, no differences were evident dur-

ing 3 years of therapy between the groups regarding referrals for liver transplantation, progression to decompensated liver cirrhosis, development of malignancy, or death. Follow-up for 3 years is too short a period to expect differences in such parameters, especially in a patient population with a disease that is not associated with cirrhosis.

What is the rationale behind MTZ therapy in PSC? MTZ has a beneficial effect on intestinal inflammation and reduces colonic permeability[11] to bacterial endotoxins. It may inhibit endotoxin-induced tumor necrosis factor alpha production, activation of Kupffer cells and portal tract macrophages, and secretion of chemokines and cytokines by biliary epithelial cells. This leads to attenuation of inflammation in the liver, which is reflected by improvement in histology and normalization of liver function tests. MTZ therapy as such seems to have no effect on biliary secretion of UDCA, so the effects of MTZ on PSC are apparently not caused by changes in the metabolism of UDCA, because no differences were seen between UDCA contents in duodenal bile. Patients on UDCA/MTZ had significantly more side effects (53%) than patients on UDCA monotherapy; however, they were mostly mild, and none of them was severe enough to require cessation of therapy.

In conclusion, the addition of MTZ to UDCA therapy significantly improved liver function tests and reduced the New Mayo Risk Score. For both stage and grade, the number of patients with improved histology was higher in the combination group, with a significant reduction of mean grade ($P = .009$), and there was also a trend toward inhibition of progression of ERCP changes. To improve treatment results in PSC, we must know more about the pathogenic mechanisms and must aim for diagnosis at earlier stages, before irreversible strictures and fibrosis have developed. Meanwhile, the treatment target is to have UDCA attenuate the inflammatory process and prevent both further damage by endogenous toxic bile acids to the biliary tree and further development of cholangiocarcinoma. Longer follow-up studies using the combination of MTZ and a higher dose of UDCA are needed to demonstrate their effect on major end points such as cirrhosis, liver transplantation, development of cholangiocarcinoma, and death.

References

1. Angulo P, Lindor K. Primary sclerosing cholangitis. HEPATOLOGY 1999; 30:325–332.
2. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med 1995; 332:924–933.
3. Vierling JM. Hepatobiliary complications of ulcerative colitis and Crohn's disease. In: Zakim D, Boyer TD, eds. Hepatology. A Textbook of Liver Disease. Volume 2. 4th ed. Philadelphia, PA: Saunders, 2003;1221–1272.
4. Spurkland A, Saarinen S, Boberg KM, Mitchell S, Broome U, Caballeria L, et al. HLA class II haplotypes in primary sclerosing cholangitis patients from five European populations. Tissue Antigens 1999;53:459–469.
5. Donaldson PT, Albertini RJ, Krawitt EL. Immunogenetic studies in autoimmune hepatitis and primary sclerosing cholangitis. In: Krawitt EL, Wiesner RH, Nishioka, eds. Autoimmune Liver Diseases. 2nd ed. Amsterdam, the Netherlands: Elsevier Science; 1998:141–165.
6. Sasatomi K, Noguchi K, Sakisaka S, Sata M, Tanikawa K. Abnormal accumulation of endotoxin in biliary epithelial cells in primary biliary cirrhosis and primary sclerosing cholangitis. J Hepatol 1998;29:409–416.
7. Lichtman SN, Keku J, Clark RL, Schwab JH, Sartor RB. Biliary tract disease in rats with experimental small bowel bacterial overgrowth. HEPATOLOGY 1991;13:766–772.
8. Lichtman SN, Okoruwa EE, Keku J, Schwab JH, Sartor RB. Degradation of endogenous bacterial cell wall polymers by muralytic enzyme mutanolysin prevents hepatobiliary injury in genetically susceptible rats with experimental intestinal bacterial overgrowth. J Clin Invest 1992;90:1313–1322.
9. Lichtman SN, Keku J, Schwab JH, Sartor RB. Hepatic injury associated with small bowel bacterial overgrowth in rats is prevented by metronidazole and tetracycline. Gastroenterology 1991;100:513–519.
10. Krehmeier U, Bardenheuer M, Voggenreiter G, Obertacke U, Schade FU, Majetschak M. Effects of antimicrobial agents on spontaneous and endotoxin-induced cytokine release of human peripheral blood mononuclear cells. J Infect Chemother 2002;8:194–197.
11. Leite AZ, Sipah AM, Damiao AO, Coelho AM, Garcez AT, Machado MV, et al. Protective effect of metronidazole on uncoupling mitochondrial oxidative phosphorylation induced by NSAID: a new mechanism. Gut 2001; 48:163–167.
12. Mahadevan U, Bass NM. Sclerosing cholangitis and recurrent pyogenic cholangitis. In: Feldman M, Friedman LS, Sleisenger MH, eds. Sleisenger & Fordtran's Gastrointestinal and Liver Disease. Volume 1. 7th ed. Philadelphia, PA: Saunders, 2002:1131–1152.
13. Lindor KD. Ursodiol for primary sclerosing cholangitis. N Eng J Med 1997;336:691–695.
14. Mitchell SA, Bansi DS, Hunt N, Von Bergmann K, Fleming KA, Chapman RW. A preliminary trial of high-dose ursodeoxycholic acid in primary sclerosing cholangitis. Gastroenterology 2001;121:900–907.
15. Mistilis SP, Skyring AP, Goulston SJM. Effect of long term tetracycline therapy, steroid therapy and colectomy in pericholangitis associated with ulcerative colitis. Australas Ann Med 1965;14:286–294.
16. Grundy SM, Ahrens EH Jr, Miettinen TA. Quantitative isolation and gas-liquid chromatographic analysis of fecal bile acids. J Lipid Res 1965;6: 397–410.
17. Ludwig J, LaRusso NF, Wiesner RH. Primary sclerosing cholangitis. In: Peters R, Craig JR, eds. Liver Pathology. Contemporary Issues in Surgical Pathology. New York, NY: Churchill Livingstone, 1986:193–213.
18. Desmet VJ, Gerber M, Hoofnagle JH, Manns M, Scheuer P. Classification of chronic hepatitis: diagnosis, grading, and staging. HEPATOLOGY 1994; 6:1513–1520.
19. Craig DA, MacCarty RL, Wiesner RH, Grambsch PM, LaRusso NF. Primary sclerosing cholangitis: value of cholangiography in determining the prognosis. AJR Am J Roentgenol 1991;157:959–964.
20. Kim WR, Poterucha JJ, Wiesner RH, LaRusso NF, Lindor KD, Petz J, et al. The relative role of Child-Pugh classification and the Mayo natural history model in the assessment of survival in patients with primary sclerosing cholangitis. HEPATOLOGY 1999;29:1643–1648.
21. Lindor KD, Wiesner RH, Colwell LJ, Steiner B, Beaver S, LaRusso NF. The combination of prednisone and colcicine in patients with primary sclerosing cholangitis. Am J Gastroenterol 1991;86:57–61.
22. Allison MC, Burroughs AK, Noone P, Summerfield JA. Biliary lavage with corticosteroids in primary sclerosing cholangitis: A clinical, cholangiographic and bacteriological study. HEPATOLOGY 1986;3:118–122.
23. van Hoogstraaten HJF, Vleggaar FP, Boland GJ, van Steenbergen, Griffioen P, Hop WCJ, et al. Budesonide or prednisone in combination with ursodeoxycholic acid in primary sclerosing cholangitis: a randomized double-blind pilot study. Am J Gastroenterol 2000;95:2015–2022.

24. Angulo P, Batts KP, Jorgensen RA, LaRusso NA, Lindor KD. Oral budesonide in the treatment of primary sclerosing cholangitis. Am J Gastroenterol 2000;95:2333–2337.
25. Jawett SL. Azathioprine treatment in primary sclerosing cholangitis. Lancet 1971;i:810–811.
26. Wagner A. Azathioprine treatment in primary sclerosing cholangitis. Lancet 1971;ii:663–664.
27. Knox TA, Kaplan MM. Treatment of primary sclerosing cholangitis with oral methotrexate. Am J Gastroenterol 1991;86:546–542.
28. Knox TA, Kaplan MM. A double-blind controlled trial of oral-pulse methotrexate therapy in the treatment of primary sclerosing cholangitis. Gastroenterology 1994;106:494–499.
29. Lindor KD, Jorgensen RA, Andersson ML, Gores GJ, Hofmannn AF, LaRusso NF. Ursodeoxycholic acid and methotrexate for primary sclerosing cholangitis: a pilot study. Am J Gastroenterol 1996;91:511–515.
30. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. Gastroenterology 1988;95:1036–1042.
31. Lazaridis KN, Gores GJ, Lindor KD. Ursodeoxycholic acid "mechanism of action and clinical use in hepatobiliary disorders." J Hepatol 2001;35:134–146.
32. Yoshikawa M, Tsujii T, Matsumura K, Yamao J, Matsumura Y, Kubo R, et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. HEPATOLOGY 1992;16:358–364.
33. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effects of ursodeoxycholic acid. HEPATOLOGY 1990;11:12–15.
34. Terasaki S, Nakanuma Y, Ogino H, Unoura M, Kabayashi K. Hepatocellular and biliary expression of HLA antigens in primary biliary cirrhosis. Before and after ursodeoxycholic acid therapy. Am J Gastroenterol 1991;86:1194–1199.
35. Calmus Y, Arvieux C, Gane P, Boucher E, Nordlinger B, Rouger P, et al. Cholestasis induces major histocompatibility complex class I expression in hepatocytes. Gastroenterology 1992;102:1371–1377.
36. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Thielmann L. Effect of ursodeoxycholic acid on liver and bile duct disease in primary sclerosing cholangitis. A 3-year pilot study with a placebo-controlled study period. J Hepatol 1994;20:57–64.
37. Harnois DM, Angulo P, Jorgensen RA, LaRusso NF, Lindor KD. High-dose ursodeoxycholic acid as a therapy for patients with primary sclerosing cholangitis. Am J Gastroenterol 2001;96:1558–1562.
38. Angulo P, Jorgensen RA, Lindor KD. Incomplete response to ursodeoxycholic acid in biliary cirrhosis: is a double dosage worthwhile? Am J Gastroenterol 2001;96:3152–3157.
39. Beuers U, Spengler U, Kruis W, Aydemir U, Wiebecke B, Heldwein W, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. HEPATOLOGY 1992;16:707–714.
40. Brandsaeter B, Isoniemi H, Broomé U, Olausson M, Backman L, Hansen B, et al. Liver transplantation for primary sclerosing cholangitis; predictors and consequences of hepatobiliary malignancy. J Hepatol 2004;40:815–822.
41. Stiehl A, Rudolph G, Kloters-Plachky P, Sauer P, Walker S. Development of dominant bile duct stenoses in patients with primary sclerosing cholangitis treated with ursodeoxycholic acid: outcome after endoscopic treatment. J Hepatol 2002;36:151–156.
42. Olsson R, Hägerstrand I, Broomé U, Danielsson Å, Järnerot G, Lööf L, et al. Sampling variability of percutaneous liver biopsy in primary sclerosing cholangitis. J Clin Pathol 1995;48:993–935.
43. Burak KW, Angulo P, Lindor KD. Is there a role for liver biopsy in primary sclerosing cholangitis? Am J Gastroenterol 2003;98:1155–1158.
44. Ponsioen CY, Vrouenraets SM, Prawirodirdjo W, Rajaram R, Rauws EA, Mulder CJ, et al. Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population. Gut 2002;51:562–566.