CLINICAL, THERAPEUTIC AND HISTO-MORPHOLOGICAL STUDIES IN PRIMARY BILIARY CIRRHOSIS AND PRIMARY SCLEROSING CHOLANGITIS

KLINISCHE, THERAPEUTISCHE EN HISTO-MORFOLOGISCHE STUDIES IN PRIMAIRE BILIAIRE CIRROSE EN PRIMAIRE SCLEROSERENDE CHOLANGITIS

ISBN 90-9013001-2

CLINICAL, THERAPEUTIC AND HISTO-MORPHOLOGICAL STUDIES IN PRIMARY BILIARY CIRRHOSIS AND PRIMARY SCLEROSING CHOLANGITIS

KLINISCHE, THERAPEUTISCHE EN HISTO-MORFOLOGISCHE STUDIES IN PRIMAIRE BILIAIRE CIRROSE EN PRIMAIRE SCLEROSERENDE CHOLANGITIS

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE
ERASMUS UNIVERSITEIT TE ROTTERDAM OP GEZAG VAN DE

rector magnificus

PROF. DR. P.W.C.AKKERMANS M.A.

EN VOLGENS BESLUIT VAN HET COLLEGE VOOR PROMOTIES
DE OPENBARE VERDEDIGING ZAL PLAATSVINDEN OP

WOENSDAG 29 SEPTEMBER 1999 OM 11.45 UUR

DOOR

HUBERT JULES FRANS VAN HOOGSTRATEN
GEBOREN TE ROTTERDAM

PROMOTIECOMMISSIE

PROMOTORES:	Prof. dr. S.W. Schalm Prof. dr. G.P. van Berge Henegouwen
OVERIGE LEDEN:	Prof. J.H.P. Wilson Prof. Dr. Th. Stijnen Prof. Dr. W. van Steenbergen

Voor Oma

CONTENTS

CHAPTER 1

TREATMENT OF CHOLESTATIC LIVER DISEASES: THE STATE OF THE ART IN 1994

Hubert JF van Hoogstraten, Solko W Schalm

1 Cholestatic liver diseases

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are chronic cholestatic liver diseases, biochemically characterised by an elevated serum bilirubin, alkaline phosphatase (APh) and γ-glutamyl transferase (γ-GT). Although PBC and PSC have their own distinctive clinical, immunological, radiological and histological features, both diseases show a necro-inflammatory process, predominantly confined to the bile ducts within the portal tracts. In most patients the disease gradually progresses and may lead to cirrhosis and liver failure. Transplantation may then be the only therapeutic option left.

1.1 Primary Biliary Cirrhosis

Introduction

In 1851, Addison and Gull described the first case of skin xanthomas in combination with severe jaundice (1). Since then, several reports have been published about this syndrome, which became known as xanthomateous biliary cirrhosis. In 1950, Ahrens et al proposed the term primary biliary cirrhosis (2), which is misleading because cirrhosis is only present in end-stage disease; primary immune-cholangitis might be a more appropriate name (3, 4).
The reported prevalence of PBC varies greatly between different populations, ranging from 5 to more than 300 cases per million people, and seems to be increasing (5-10). According to the European Liver Transplantation Registration, in 1998, PBC was the third leading indication for liver transplantation in Europe.

Clinical features and natural course

Patients, 70% of whom are middle aged women, typically present with pruritus and fatigue. Other complaints include upper right abdominal pain, arthralgia, dry eyes and mouth and the presence of xanthomas. Because of increased biochemical screening during treatment for other diseases and because of an increased awareness, more and more patients present in an asymptomatic state. Although disputed, PBC is frequently associated with autoimmune diseases such as keratoconjunctivitis sicca, polyarthritis, scleroderma and autoimmune thyroiditis (11, 12). IgM and cholesterol levels are often elevated (13, 14). However, an elevated serum cholesterol does not seem to lead to an increased mortality of cardiovascular disease (15). Pivotal in the diagnosis of PBC is the presence of antimitochondrial antibodies (AMA) in serum, which are present in 90%-95% of patients (16). AMA are directed against a series of intra-mitochondrial proteins, in particular the 2-oxo acid dehydrogenase complexes, including the dihydrolipoylacetyltransferase component of the pyruvate dehydrogenase complex E2, the branched-chain 2-oxo acid dehydrogenase complex E2, and the 2-oxoglutarate dehydrogenase complex E2. AMA

are found in serum and in bile (17-19). The role of AMA in the aetiology of PBC is still debated (see below).
The progression of PBC is slow. In patients without any symptoms, survival has been reported to be equal to the normal population (20). Others, however, have found a decreased survival in asymptomatic patients (21, 22).

Histomorphology

Histologically, PBC is characterised by a non-suppurative destructive cholangitis or a granulomatous cholangitis. Four histological stages have been identified by Scheuer et al., which were later modified by Ludwig et al., and are now generally used (4, 23). In stage I, florid bile duct lesions and necrotic bile ducts with shrunken and vacuolated epithelial cells are seen. Surrounding the bile ducts, a cellular infiltrate consisting of lymphocytes, plasma cells, histiocytes, eosinophils and giant cells is noticed. Non-caseating granulomas are commonly observed. In stage II, the lesions are not confined to the portal tracts but start to infiltrate in the surrounding parenchyma with destruction of the limiting plate and piecemeal necrosis. A gradual loss of bile ducts occurs with consequent ductular proliferation. Stage III shows progression of fibrosis with the formation of porto-portal septa. In stage IV the disease has progressed to cirrhosis.

Pathogenesis

Although direct proof is lacking, there is growing evidence that PBC is an immune mediated disease. Indirect proof lies in the presence of auto-antibodies, particularly AMA, which are present in 90%-95% of patients (24); an elevated IgM; a strong association with other autoimmune diseases (12); and numerous defects in cellular and humoral immunological regulation mechanisms (table 1) (25).
Genetic factors may also play a role. Although data are few, PBC has an estimated prevalence ranging from 2.4% to 14.9% in family members (26, 27). Furthermore, an association with HLA-DR2, 3,4 and 8 has been reported in various populations (28).

Treatment

At the end of 1994, no cure for PBC was available. The effects of a number of therapeutic agents were assessed, which will be discussed below.

Ursodeoxycholic acid

In 1981, Leuschner et al were the first to report about the beneficial effects of UDCA in patients with chronic active hepatitis and gallstones (29). This was followed in 1987 by Poupon et al reporting uncontrolled data about an improvement in liver function tests and pruritus in PBC patients treated with UDCA (30).

Table 1: Reported immunological abnormalities in PBC (25).
Presence of AMA and other antibodies
Skin test anergy
Increased serum IgG
Increased serum IgM
Increased complement activation and turn-over
Circulating-immune-complex-like activity
Suppresser T lymphocyte dysfunction
Decreased mixed lymphocyte response
Circulating activated B lymphocytes
Alteration of T cell subsets
Cytotoxic lymphocytes infiltrating bile ducts epithelium cells
Expression of class II MHC antigens by bile duct epithelium
Decreased Kupffer cell function

Table 2: Major clinical trials in PBC with UDCA mono-therapy until the end of 1994.

	year	design	follow-up	n	Results		
					biochemistry	histology	survival
Leuschner	1989	RCT	9 months	20	improved	trend	n.a.
Battezzati	1993	RCT	6 months	88	improved	n.a.	n.a.
Poupon	1994	RCT	4 years	145	improved	n.a.	improved
Lindor	1994	RCT	2 years	180	improved	n.s.	n.s.
Heathcote	1994	RCT	2 years	222	improved	trend	n.s.
Turner	1994	RCT	2 years	64	improved	n.s.	n.s.

RCT: randomised controlled trial; improved: significantly improved; trend: improvements were reported, which were not significant; n.a.: not assessed; n.s.: not significant.
UDCA did not clearly improve symptoms in any of the trials.

Subsequently, a number of randomised controlled trials with UDCA in PBC were performed (31-35)(table 2). In all studies significant improvements in serum liver function tests were found whereas effects of UDCA on histology were inconsistent. Effects on pruritus and fatigue were absent. In the French study, UDCA significantly, although modestly, improved transplantation free survival (32). At the end of 1994, it was generally accepted that UDCA was the treatment of choice for patients with PBC.

UDCA (3a, 7b-dihydroxy-5b cholan-24-oic acid) was firstly isolated from bile of the Chinese black bear and was named after this species (36). In healthy subjects, it is only present in small quantities of up to 5 mol% in the bile acid pool (37-39). Various mechanisms of action of UDCA in cholestatic liver diseases have been suggested: membrano-protection, induction of increased bile flow and immunomodulation (40).

Hydrophobicity and the membrano-disruptive properties of bile acids decrease in the following order: Deoxycholate > chenodeoxycholate > cholate > ursodeoxycholate > β-muricholate (41). In isolated rat (42) and human hepatocytes (43, 44) and in a rat model of cholestasis (45), it has been shown that UDCA protects against the toxic effects of more lipophilic bile acids. Furthermore, UDCA stabilises isolated red blood cell, hepatocyte and artificial membranes (46, 47). Thus, the disruption of the canalicular membrane by the retention of more lipophilic endogenous bile acids in cholestatic liver disease might be counteracted by UDCA.

There is growing evidence that conjugates of UDCA stimulate bile flow. It has been shown that tauro-ursodeoxycholate stimulates bile flow in isolated rat livers after bile duct ligation (48). In vivo, increased phospholipid and endogenous bile acid excretion has been observed in PSC patients treated with UDCA (49). UDCA may be excreted in bile in the non-conjugated state, which can quickly be converted into the protonated form in the small bile ducts. This is accompanied by secretion of bile that is rich in bicarbonate. Next, the protonated form of UDCA will be absorbed in the duct, transported back to the liver cell and again be excreted in bile. This process of "cholehepatic shunt" leads to a choleresis, rich in bicarbonate. Whether this process plays a part in man has not been demonstrated so far. It has clearly been established in animals (50).

Bile salts, including UDCA, are strong suppressers of lymphocyte proliferation in vitro (51-54), probably by a direct toxic effect. *In vivo*, UDCA reduces the aberrant expression of class I major histocompatibility complex molecules on hepatocytes (55), which might be secondary to diminished cholestasis and immunological stimulation.

Other drugs

At the end of 1994, a variety of immuno-suppressive (56-66), cupruretic (67-72), anti-fibrotic (73-79) and other drugs (80-82) has been evaluated in PBC (table 2). Although improvements in liver function tests together with histological improvements were noted especially with immunosuppressive drugs, effects on symptoms and survival have been disappointing, which might be explained by insufficient patient numbers and a too short follow-up period.

Table 3: Major randomised clinical trials with other drugs than UDCA or in combination with UDCA in PBC until the end of 1994.

	design	duration	n	Results			
				biochemistry	histology	survival	symptoms
Prednis(ol)on							
Mitchison (56)	RCT	3 years	36	improved	stable	n.s.	improved
Colchicine							
Bodenheimer (73)	RCT	33 months	57	improved	n.s.	n.s.	n.s.
Kaplan (74)	RCT	2 years	60	improved	n.s.	trend	n.s.
Cyclosporin A							
Minuk (60)	RCT	1 years	12	improved	n.s.	n.a.	n.s
Wiesner (61)	RCT	1 years	29	improved	trend	trend	trend
Lombard (62)	RCT	2.5 years	349	improved	n.s.	improved	improved
Malotilate							
Multi centre (81)	RCT	28 months	101	improved	+/0	n.s.	n.s.
Chlorambucil							
Hoofnagle (63)	RCT	52 months	24	improved	+/0	n.s.	n.a.
Thalidomide							
McCormick (82)	RCT	6 months	18	n.s.	n.s.	n.a.	n.s.
D-Penicillamine							
Dickson (68)	RCT	5 years	227	n.s.	n.s.	n.s.	n.s.
Matloff (69)	RCT	2 years	52	n.s.	n.s	n.s	n.s.
Epstein (70)	RCT	33 months	87	improved	+/0	improved	n.a.
Taal (71)	RCT	1 year	24	n.s.	n.s.	n.a.	n.s.
Neuberger (72)	RCT	2 years	189	n.s.	n.s.	n.s.	n.a.
Azathioprine							
Christensen (156)	RCT	± 5 years	248	n.a.	n.a.	improved	n.a.
Heathcote (59)	RCT	± 2.5 years	55	n.s.	n.s.	n.s.	n.s.

RCT: randomised controlled trial; improved: significantly improved; n.s.: not significant; trend: improvements were reported, which were not significant; +/0: improvement in markers of inflammation but no change in level of fibrosis; n.a.: not assessed.

Biochemical remission

In 1994, studies became available showing that treatment with UDCA leads to biochemical remission in 15-20% of patients with PBC (83, 84). As in other autoimmune diseases, e.g. autoimmune hepatitis, biochemical remission of the disease could be indicative of a subsequently favourable long-term outcome. The prognostic significance of biochemical remission, however, was not determined. Furthermore, no data were available concerning the relative importance as a prognostic factor of each biochemical parameter comprising biochemical remission. The prognostic value of bilirubin in untreated PBC patients was already established (20, 21, 85). Whether serum bilirubin continued to be of prognostic value in PBC patients treated with UDCA was still to be assessed.

1.2 Primary Sclerosing cholangitis

A French surgeon described the first case of PSC in 1924 (86), followed in 1925 by similar observations of strongly icteric patients, complaining of severe pruritus, in whom an enlarged fibrotic and obliterated bile duct was found during operation (87, 88). Before the introduction of endoscopic retrograde cholangiography (ERC), which is pivotal in the diagnosis, only few cases were reported. Since this technique has become available, the reported prevalence is seen to be rising (89, 90); increased awareness may also play a role. The true prevalence of the disease is unknown, but estimates range from 20-70 cases per million based on a prevalence of PSC in 2.5%-7.5% of patients with ulcerative colitis. It is the fourth leading indication for liver transplantation in adults in the United States (91).

Clinical features and natural course

The majority of patients (approximately 65%) are men with a mean age at diagnosis of about 40 years. Patients may be asymptomatic at presentation. The majority of patients (80%), however, present with symptoms including fatigue, pruritus, jaundice and right upper quadrant abdominal pain. 70-80% of patients are diagnosed with concomitant inflammatory bowel disease (IBD) of which 90% is classified as ulcerative colitis (UC) (92-96). Its is still debated whether PSC is an independent risk factor for the development of colorectal neoplasm in patients with PSC and UC (97, 98).
Perinuclear antineutrophil cytoplasmic antibodies (pANCA), which are antibodies against myeloperoxidase and elastase located close to the cell nucleus of polymorphonuclear neutrophil leukocytes after ethanol fixation (99), are found in about 70% of patients with PSC (100-102) and in approximately 25% of their relatives (103).
Although characteristic histological lesions (see below) may be the only indication of PSC, leading to the diagnosis of "small-duct PSC" (104, 105); in about 90% of cases, typical cholangiographic lesions are found. Diffuse multifocal strictures involving both the intrahepatic and extrahepatic ducts are the most common findings. Strictures are usually short, with normal or dilated segments in-between, leading to a characteristic "beaded" appearance. Sometimes diverticula are seen. The pancreatic duct may be involved as well. With more advanced disease, long confluent strictures occur (106-108). Apart from diffuse irregularities, a so-called single "dominant stricture" may be present.
Transplantation free survival of PSC patients is around 12-17 years from the time of diagnosis (92, 94-96). In a-symptomatic patients, the prognosis is better, but still worse than in age matched controls (109). An important factor influencing the prognosis is the increased risk of cholangiocarcinoma, which has an incidence between 10 and 15% in PSC patients (91). The incidence of cholangiocarcinoma rises with increasing disease severity (110).

Histomorphology

Although only found in a minority of cases, the characteristic histological lesion in PSC is a fibrous-obliterative cholangitis, a combination of cholangitis with so-called "onion-skin" fibrosis, consisting of concentric periductal layers of collagen fibres (104). Furthermore, bile-duct proliferation, periductal inflammation, ductal obliteration and loss of bile ducts may be seen. The ductal changes are associated with portal oedema, mild portal and periportal inflammation, cholestasis and, consequently, periportal copper accumulation. Eventually fibrosis and cirrhosis occur. Four stages are identified: In stage 1, lesions are confined to the portal tracts; in stage 2, periportal fibrosis or inflammation are found in the portal and periportal areas; in stage 3, septal fibrosis, bridging necrosis or both occur and in stage 4 cirrhosis is found (111). The lesions are not equally distributed throughout the liver and sampling error might occur (112).

Aetiology

The aetiology of PSC is largely unknown, although a number of immunologic (113) and non-immunological (114) factors have been considered (91, 115). Because ulcerative colitis is present in the majority of patients, it has been suggested that portal bacteremia or absorption of various toxins from the inflamed colon may play a role in the pathogenesis of PSC. However, as PSC may well develop in the absence of ulcerative colitis or after proctocolectomy, it is now generally believed that bacterial products are not likely to be of major importance in the pathogenesis of PSC. As in other autoimmune diseases, it has been suggested that viral infection -especially CMV- may trigger an autoimmune process through cross-reactivity between viral and liver-derived antigens. Arterial insufficiency, e.g. after transplantation or after hepatic artery infusion of fluorodeoxyuridine as a treatment for liver metastases, may cause cholangiographic findings similar of PSC. However, in patients with PSC evidence of vascular abnormalities is generally absent.
PSC may be an immune mediated disease (113) and a number of humoral and cellular abnormalities have been observed. A close association has been found between PSC and HLA-B8, HLA-Dr3 or HLA-Drw52a phenotype (116-118). In the majority of PSC patients and in patients with ulcerative colitis (99, 119) or autoimmune hepatitis (120), autoantibodies, particularly pANCA, are present, which may imply a common autoimmune mediated pathogenesis. Furthermore, the simultaneous presence of AIH and PSC has been described in children (121) and in a number of adults (122-125).
Finally, a number of other immune abnormalities have been observed, such as an increased CD4/CD8 T-cell ratio (126), an enhanced expression of HLA class II antigens on biliary epithelial cells (127), and a significantly increased concentration of serum intercellular adhesion molecule-I (128). Whether these changes are primary or secondary remains to be elucidated.

Treatment

At the end of 1994, PSC was not curable. The effects of a number of therapeutic agents and of endoscopic interventions were assessed, which will be discussed below.

UDCA monotherapy

When, in 1994, the beneficial effects of UDCA on serum liver function tests in PBC were established, this drug was also evaluated in PSC (129-135). Results were identical: Significant improvements in serum liver function tests were observed without significant changes in symptoms. UDCA became the predominant bile salt in serum and in bile. In one study, improvements in histological findings were observed (132). The effects of UDCA on survival were unknown at the end of 1994.

Other medical treatment

Until the end of 1994, less than 5 randomised clinical trials have been performed in PSC. The effects of d-penicillamine have been evaluated in a 3-year randomised controlled trial in 70 PSC patients. The absence of any effects of the drug on liver function tests, histology, cholangiographic findings and histology in combination with serious adverse events in 21% of patients taking the drug, made the authors conclude that additional trials with d-penicillamine should be discouraged (136).
Several immunosuppressive drugs have been evaluated. After they had shown the beneficial effects of methotrexate in PSC in 2 series of case studies (137, 138), Kaplan et al reported the results of a 2-year randomised controlled trial assessing the effects of methotrexate in 24 PSC patients. No changes in serum liver tests, histology, cholangiographic findings and histology were found and the authors conclude that the use of methotrexate in PSC must not be recommended (137).
Although beneficial effects of colchicine in a single patient have been reported (139), results of a 3-year randomised controlled trial in 84 PSC patients did not show any effects of 1 mg colchicine per day (140). In 12 patients treated with a combination of prednisone and colchicine, there was only a trend towards improved survival after 2 years of treatment without concomitant improvements in liver function tests and histology (141).
Two series of case studies showed sustained clinical, biochemical and histological improvement in PSC patients treated with prednisone mono-therapy (142, 143).
The use of azathioprine mono-therapy has been reported in 2 case reports; one patient showed marked improvements (144), whereas the other patient died of complications (145).
No effects of cyclosporine on serum liver function tests or survival have been observed in a randomised controlled trial of 2 years in 34 patients (146).

Endoscopic intervention therapy

The strictures of the bile ducts, which causes back pressure and debris formation, are accessible for decompression during ERC. Endoscopic procedures could be an effective therapeutic strategy for extra-hepatic dominant strictures, because these procedures can be performed with relative ease and be repeated with limited discomfort and risk to the patient (147). Several case series are described applying different techniques to manage symptomatic disease, including endoscopic dilatation with bougies or balloons, removal of debris, stenting and biliary lavage. Results are identical: marked and sustained improvements are achieved in serum liver function tests, especially an improvement in bilirubin, and in clinical symptoms without serious risks to the patient (147-152). After an initial report describing favourable effects of biliary lavage with a combination of normal saline and prednisolone through a naso-biliary drain (153), this procedure has been evaluated in a randomised trial. In this study, comparing the effects of biliary lavage with normal saline versus lavage with a solution of prednisolone, biochemical values remained unchanged or worsened in all patients, while bacterial colonisation occurred in all patients within a few days after start of the lavage. No cholangiographic changes were seen. The authors conclude that biliary lavage is not beneficial in PSC (154).

1.3 Aims of this thesis

In PBC:

1. To evaluate the course of serum liver function tests during treatment with UDCA for a period of more than 4 years and to assess which factors predict survival in our patient cohort, in particular the prognostic value of biochemical remission and bilirubin during UDCA therapy.
2. To investigate whether treatment with a dose of 20 mg UDCA /kg/day is superior to a dose of 10 mg UDCA /kg/day regarding symptoms, liver biochemical values, biliary enrichment with UDCA and to describe possible side-effects of treatment with high dose UDCA.
3. To evaluate, in a randomised controlled design, the possible beneficial effects of azathioprine and prednisone added to UDCA on symptoms, histology and biochemical markers of cholestasis, inflammation and fibrogenesis.

In PSC:

4. To study in a cohort of patients treated with UDCA: its effects on symptoms, histology, cholangiographical findings and serum liver function tests. To study whether a multiple daily dose scheme of UDCA is superior to a single dose scheme and to compare predicted survival with the observed survival after 2 years of treatment.
5. To assess the effects of treatment with a combination of UDCA and a corticosteroid (budesonide or prednisone) on symptoms, liver biochemistry and to compare the anti-proliferative properties in bile before and after this treatment in a lymphocyte stimulation test.
6. To study the overlap syndrome of PSC and AIH.

References

1. Addison T, Gull W. On a certain affection of the skin, vitiligoidea-α. plana, β. tuberosa. Guy's Hosp. Rep. 1851;7:265-77.
2. Ahrens EH, Payne MA, Kunkel HG, Eisenmenger WJ, Blondheim SH. Primary biliary cirrhosis. Medicine 1950;29:299-364.
3. Schalm SW, van Buuren HR. Early cirrhosis - or primary cholangitis? Lancet 1997;349:285.
4. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 978;379:103-12.
5. Triger DR, Berg PA, Rodes J. Epidemiology of primary biliary cirrhosis. Liver 1984;4:195-200.
6. Watson RG, Angus PW, Dewar M, Goss B, Sewell RB, Smallwood RA. Low prevalence of primary biliary cirrhosis in Victoria, Australia. Melbourne Liver Group. Gut 1995;36:927-30.
7. Metcalf JV, Bhopal RS, Scott LRMD, Gray J, Howel D, James OFW. Temporal and geographical variations in the prevalence of primary biliary cirrhosis in north east England. Hepatology 1995;22:384A.
8. Danielsson A, Boqvist L, Uddenfeldt P. Epidemiology of primary biliary cirrhosis in a defined rural population in the northern part of Sweden. Hepatology 1990;11:458-64.
9. Metcalf JV, Howel D, James OF, Bhopal R. Primary biliary cirrhosis: epidemiology helping the clinician. BMJ 1996;312:1181-2.
10. Almdal TP, Sorensen TI. Incidence of parenchymal liver diseases in Denmark, 1981 to 1985: analysis of hospitalization registry data. The Danish Association for the Study of the Liver. Hepatology 1991;13:650-5.
11. Christensen E, Crowe J, Doniach D, et al. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. Gastroenterology 1980;78:236-46.
12. Culp KS, Fleming CR, Duffy J, Baldus WP, Dickson ER. Autoimmune associations in primary biliary cirrhosis. Mayo Clin Proc 1982;57:365-70.
13. Poupon RE, Ouguerram K, Chretien Y, et al. Cholesterol-lowering effect of ursodeoxycholic acid in patients with primary biliary cirrhosis. Hepatology 1993;17:577-82.
14. Balan V, Dickson ER, Jorgensen RA, Lindor KD. Effect of ursodeoxycholic acid on serum lipids of patients with primary biliary cirrhosis. Mayo Clin Proc 1994;69:923-9.
15. Crippin JS, Lindor KD, Jorgensen R, et al. Hypercholesterolemia and atherosclerosis in primary biliary cirrhosis: what is the risk? Hepatology 1992;15:858-62.
16. Berg PA, Klein R, Lindenborn-Fotinos J. Antimitochondrial antibodies in primary biliary cirrhosis. J Hepatol 1986;2:123-31.
17. Manns M. Autoantibodies and antigens in liver diseases—updated. J Hepatol 1989;9:272-80.
18. Nishio A, Van de Water J, Leung PS, et al. Comparative studies of antimitochondrial autoantibodies in sera and bile in primary biliary cirrhosis. Hepatology 1997;25:1085-9.
19. Fussey SP, Guest JR, James OF, Bassendine MF, Yeaman SJ. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. Proc Natl Acad Sci U S A 1988;85:8654-8.
20. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308:1-7.

21. Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. J Hepatol 1994;20:707-13.
22. Balasubramaniam K, Grambsch PM, Wiesner RH, Lindor KD, Dickson ER. Diminished survival in asymptomatic primary biliary cirrhosis. A prospective study. Gastroenterology 1990;98:1567-71.
23. Scheuer PJ. Primary Biliary Cirrhosis. Proc R Soc Med 1967;60:1257-60.
24. Invernizzi P, Crosignani A, Battezzati PM, et al. Comparison of the clinical features and clinical course of antimitochondrial antibody-positive and -negative primary biliary cirrhosis. Hepatology 1997;25:1090-5.
25. Laurin JM, Lindor KD. Primary biliary cirrhosis. Dig Dis 1994;12:331-50.
26. Bach N, Schaffner F. Familial primary biliary cirrhosis. J Hepatol 1994;20:698-701.
27. Brind AM, Bray GP, Portmann BC, Williams R. Prevalence and pattern of familial disease in primary biliary cirrhosis. Gut 1995;36:615-7.
28. Gregory WL, Bassendine MF. Genetic factors in primary biliary cirrhosis. J Hepatol 1994;20:689-92.
29. Leuschner U, Leuschner M, Sieratzki J, Kurtz W, Hubner K. Gallstone dissolution with ursodeoxycholic acid in patients with chronic active hepatitis and two years follow-up. A pilot study. Dig Dis Sci 1985;30:642-9.
30. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet 1987;1:834-6.
31. Battezzati PM, Podda M, Bianchi FB, et al. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis. Preliminary analysis of a double-blind multicenter trial. Italian Multicenter Group for the Study of UDCA in PBC. J Hepatol 1993;17:332-8.
32. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med 1994;330:1342-7.
33. Lindor KD, Dickson ER, Baldus WP, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
34. Heathcote EJ, Cauch-Dudek K, Walker V, et al. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
35. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OF. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroenterol Hepatol 1994;9:162-8.
36. Poupon RE, Poupon R. Ursodeoxycholic acid for the treatment of cholestatic diseases. Prog Liver Dis 1992;10:219-38.
37. Ward A, Brogden RN, Heel RC, Speight TM, Avery GS. Ursodeoxycholic acid: a review of its pharmacological properties and therapeutic efficacy. Drugs 1984;27:95-131.
38. Batta AK, Salen G, Mirchandani R, et al. Effect of long-term treatment with ursodiol on clinical and biochemical features and biliary bile acid metabolism in patients with primary biliary cirrhosis. Am J Gastroenterol 1993;88:691-700.
39. Hofmann AF. Pharmacology of ursodeoxycholic acid, an enterohepatic drug. Scand J Gastroenterol Suppl 1994;204:1-15.
40. Levy S, Capron JP, Erlinger S. Mecanisme d'action de l'acide ursodesoxycholique dans les maladies du foie cholestatiques. Gastroenterol Clin Biol 1996;20:549-59.
41. Queneau PE, Montet JC. Hepatoprotection by hydrophilic bile salts. J Hepatol 1994;21:260-8.
42. Pazzi P, Puviani AC, Libera MD, et al. Bile salt-induced cytotoxicity and ursodeoxycho-

late cytoprotection: in- vitro study in perifused rat hepatocytes. Eur J Gastroenterol Hepatol 1997;9:703-9.
43. Marschall HU, Roeb E, Yildiz Y, et al. Study of human isoursodeoxycholic acid metabolism. J Hepatol 1997;26:863-70.
44. Galle PR, Theilmann L, Raedsch R, Otto G, Stiehl A. Ursodeoxycholate reduces hepatotoxicity of bile salts in primary human hepatocytes. Hepatology 1990;12:486-91.
45. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. In vivo studies in the rat. Gastroenterology 1991;100:203-11.
46. Heuman DM, Bajaj R. Ursodeoxycholate conjugates protect against disruption of cholesterol-rich membranes by bile salts. Gastroenterology 1994;106:1333-41.
47. Guldutuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U. Molecular aspects of membrane stabilization by ursodeoxycholate. Gastroenterology 1993;104:1736-44.
48. Beuers U, Nathanson MH, Isales CM, Boyer JL. Tauroursodeoxycholic acid stimulates hepatocellular exocytosis and mobilizes extracellular Ca++ mechanisms defective in cholestasis. J Clin Invest 1993;92:2984-93.
49. Stiehl A, Rudolph G, Sauer P, Theilmann L. Biliary secretion of bile acids and lipids in primary sclerosing cholangitis. Influence of cholestasis and effect of ursodeoxycholic acid treatment. J Hepatol 1995;23:283-9.
50. Erlinger S. Hypercholeretic bile acids: a clue to the mechanism? Hepatology 1990;11:888-90.
51. Calmus Y, Weill B, Ozier Y, Chereau C, Houssin D, Poupon R. Immunosuppressive properties of chenodeoxycholic and ursodeoxycholic acids in the mouse. Gastroenterology 1992;103:617-21.
52. Lacaille F, Paradis K. The immunosuppressive effect of ursodeoxycholic acid: a comparative in vitro study on human peripheral blood mononuclear cells. Hepatology 1993;18:165-72.
53. Keane RM, Gadacz TR, Munster AM, Birmingham W, Winchurch RA. Impairment of human lymphocyte function by bile salts. Surgery 1984;95:439-43.
54. Yoshikawa M, Tsujii T, Matsumura K, et al. Immunomodulatory effects of ursodeoxycholic acid on immune responses. Hepatology 1992;16:358-64.
55. Calmus Y, Gane P, Rouger P, Poupon R. Hepatic expression of class I and class II major histocompatibility complex molecules in primary biliary cirrhosis: effect of ursodeoxycholic acid. Hepatology 1990;11:12-5.
56. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 1992;15:336-44.
57. Kaplan MM, Knox TA. Treatment of primary biliary cirrhosis with low-dose weekly methotrexate. Gastroenterology 1991;101:1332-8.
58. Christensen E, Neuberger J, Crowe J, et al. Azathioprine and prognosis in primary biliary cirrhosis. Gastroenterology 1986;90:508-9.
59. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.
60. Minuk GY, Bohme CE, Burgess E, et al. Pilot study of cyclosporin A in patients with symptomatic primary biliary cirrhosis. Gastroenterology 1988;95:1356-63.
61. Wiesner RH, Ludwig J, Lindor KD, et al. A controlled trial of cyclosporine in the treatment of primary biliary cirrhosis. N Engl J Med 1990;322:1419-24.
62. Lombard M, Portmann B, Neuberger J, et al. Cyclosporin A treatment in primary bilia-

ry cirrhosis: results of a long-term placebo controlled trial. Gastroenterology 1993;104:519-26.
63. Hoofnagle JH, Davis GL, Schafer DF, et al. Randomized trial of chlorambucil for primary biliary cirrhosis. Gastroenterology 1986;91:1327-34.
64. Lindgren S, Danielsson A, Olsson R, Prytz H, Eriksson S. Prednimustin treatment in primary biliary cirrhosis: a preliminary study. J Intern Med 1992;231:139-41.
65. Kaplan MM. The therapeutic effects of ursodiol and methotrexate are additive and well tolerated in primary biliary cirrhosis (PBC). Hepatology 1992;16:92A.
66. Buscher HP, Zietzschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
67. Bodenheimer H, Jr., Schaffner F, Sternlieb I, Klion FM, Vernace S, Pezzullo J. A prospective clinical trial of D-penicillamine in the treatment of primary biliary cirrhosis. Hepatology 1985;5:1139-42.
68. Dickson ER, Fleming TR, Wiesner RH, et al. Trial of penicillamine in advanced primary biliary cirrhosis. N Engl J Med 1985;312:1011-5.
69. Matloff DS, Alpert E, Resnick RH, Kaplan MM. A prospective trial of D-penicillamine in primary biliary cirrhosis. N Engl J Med 1982;306:319-26.
70. Epstein O, Jain S, Lee RG, et al. D-penicillamine treatment improves survival in primary biliary cirrhosis. Lancet 1981;1:1275-7.
71. Taal BG, Schalm SW, Ten Kate FW, Van Berge Henegouwen GP, Brandt KH. Low therapeutic value of D-penicillamine in a short-term prospective trial in primary biliary cirrhosis. Liver 1983;3:345-52.
72. Neuberger J, Christensen E, Portmann B, et al. Double blind controlled trial of d-penicillamine in patients with primary biliary cirrhosis. Gut 1985;26:114-9.
73. Bodenheimer H, Jr., Schaffner F, Pezzullo J. Evaluation of colchicine therapy in primary biliary cirrhosis. Gastroenterology 1988;95:124-9.
74. Kaplan MM, Alling DW, Zimmerman HJ, et al. A prospective trial of colchicine for primary biliary cirrhosis. N Engl J Med 1986;315:1448-54.
75. Shibata J, Fujiyama S, Honda Y, Sato T. Combination therapy with ursodeoxycholic acid and colchicine for primary biliary cirrhosis. J Gastroenterol Hepatol 1992;7:277-82.
76. Raedsch R, Stiehl A, Walker S, Scherrmann JM, Kommerell B. Kombinierte Ursodeoxycholsaure plus Colchizin—Behandlung bei primar biliarer Zirrhose: Ergebnisse einer Placebo-kontrollierten Doppelblindstudie. Z Gastroenterol 1992;1:55-7.
77. Bobadilla J, Vargas F, Dehesa M, et al. Colchicine and ursodiol in the treatment of primary biliary cirrhoss. Hepatology 1994;20:332A.
78. Almassio P, Provenzano G, Battezzatti P, et al. The Italian multi-centre randomized controlled trial of ursodeoxycholic acid versus ursodeoxycholic acid plus colchicine in symptomatic primary biliary cirrhosis. Hepatology 1994;20:267A.
79. Goddard C, Hunt L, Smith A, Fallowfield G, Rowan B, Warnes T. A trial of ursodeoxycholic acid (UDCA) and colchicine in primary biliary cirrhosis (PBC). Hepatology 1994;20:151A.
80. Hishon S, Tobin G, Ciclitira PJ. A clinical trial of levamisole in primary biliary cirrhosis. Postgrad Med J 1982;58:701-3.
81. Anonymous. The results of a randomized double blind controlled trial evaluating malotilate in primary biliary cirrhosis. A European multicentre study group. J Hepatol 1993;17:227-35.
82. McCormick PA, Scott F, Epstein O, Burroughs AK, Scheuer PJ, McIntyre N. Thalid-

omide as therapy for primary biliary cirrhosis: a double-blind placebo controlled pilot study. J Hepatol 1994;21:496-9.
83. Jorgensen RA, Dickson ER, Hofmann AF, Rossi SS, Lindor KD. Characterisation of patients with a complete biochemical response to ursodeoxycholic acid. Gut 1995;36:935-8.
84. Wolfhagen FH, van Buuren HR, Schalm SW, et al. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis? The Dutch Multicentre PBC Study Group. J Hepatol 1995;22:381.
85. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10:1-7.
86. Delbet MP. Retriecissement du choledoque. Cholecysto-duodenostomie. Bull et Memoirs de la Soc Nat de Chir 1924;1:1144-1146.
87. Lafourcade MJ. Deux observations d'obliteration cicatricielle du choledoque. Anastomose laterale entre le choledoque et le duodenum dans le premier cas. Reconstitution par prothese avec tube de caoutchouc dans le second. Bull et Memoirs de la Soc Nat de Chir 1925;2:828-831.
88. Miller RT. Benign stricture of the bile ducts. Ann Surg 1927;86:296-303.
89. Helzberg JH, Petersen JM, Boyer JL. Improved survival with primary sclerosing cholangitis. A review of clinicopathologic features and comparison of symptomatic and asymptomatic patients. Gastroenterology 1987;92:1869-75.
90. Escorsell A, Pares A, Rodes J, Solis-Herruzo JA, Miras M, de la Morena E. Epidemiology of primary sclerosing cholangitis in Spain. Spanish Association for the Study of the Liver. J Hepatol 1994;21:787-91.
91. Harnois DM, Lindor KD. Primary sclerosing cholangitis: evolving concepts in diagnosis and treatment. Dig Dis 1997;15:23-41.
92. Farrant JM, Hayllar KM, Wilkinson ML, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology 1991;100:1710-7.
93. Chapman RW, Arborgh BA, Rhodes JM, et al. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. Gut 1980;21:870-7.
94. Wiesner RH, Grambsch PM, Dickson ER, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989;10:430-6.
95. Broome U, Olsson R, Loof L, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38:610-5.
96. Aadland E, Schrumpf E, Fausa O, et al. Primary sclerosing cholangitis: a long-term follow-up study. Scand J Gastroenterol 1987;22:655-64.
97. Brentnall TA, Haggitt RC, Rabinovitch PS, et al. Risk and natural history of colonic neoplasia in patients with primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1996;110:331-8.
98. Loftus E, Jr., Sandborn WJ, Tremaine WJ, et al. Risk of colorectal neoplasia in patients with primary sclerosing cholangitis. Gastroenterology 1996;110:432-40.
99. Peen E, Almer S, Bodemar G, et al. Anti-lactoferrin antibodies and other types of ANCA in ulcerative colitis, primary sclerosing cholangitis, and Crohn's disease. Gut 1993;34:56-62.
100. Lo SK, Fleming KA, Chapman RW. Prevalence of anti-neutrophil antibody in primary sclerosing cholangitis and ulcerative colitis using an alkaline phosphatase technique. Gut 1992;33:1370-5.
101. Mulder AH, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver

diseases. Hepatology 1993;17:411-7.
102. Bansi DS, Bauducci M, Bergqvist A, et al. Detection of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis: a comparison of the alkaline phosphatase and immunofluorescent techniques. Eur J Gastroenterol Hepatol 1997;9:575-80.
103. Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1994;107:532-6.
104. Ludwig J. Small-duct primary sclerosing cholangitis. Semin Liver Dis 1991;11:11-7.
105. Wee A, Ludwig J. Pericholangitis in chronic ulcerative colitis: primary sclerosing cholangitis of the small bile ducts? Ann Intern Med 1985;102:581-7.
106. Krieger J, Seaman WB, Porter MR. The roentgenologic appearance of sclerosing cholangitis. Radiology 1970;95:369-75.
107. Chen LY, Goldberg HI. Sclerosing cholangitis: broad spectrum of radiographic features. Gastrointest Radiol 1984;9:39-47.
108. MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary sclerosing cholangitis: findings on cholangiography and pancreatography. Radiology 1983;149:39-44.
109. Porayko MK, Wiesner RH, LaRusso NF, et al. Patients with asymptomatic primary sclerosing cholangitis frequently have progressive disease. Gastroenterology 1990;98:1594-602.
110. Nashan B, Schlitt HJ, Tusch G, et al. Biliary malignancies in primary sclerosing cholangitis: timing for liver transplantation. Hepatology 1996;23:1105-11.
111. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1:632-40.
112. Olsson R, Hagerstrand I, Broome U, et al. Sampling variability of percutaneous liver biopsy in primary sclerosing cholangitis. J Clin Pathol 1995;48:933-5.
113. Chapman RW. Role of immune factors in the pathogenesis of primary sclerosing cholangitis. Semin Liver Dis 1991;11:1-4.
114. Sherlock S. Pathogenesis of sclerosing cholangitis: the role of nonimmune factors. Sem Liver Dis 1991;11:5-10.
115. Boberg KM, Lundin KE, Schrumpf E. Etiology and pathogenesis in primary sclerosing cholangitis. Scand J Gastroenterol Suppl 1994;204:47-58.
116. Prochazka EJ, Terasaki PI, Park MS, Goldstein LI, Busuttil RW. Association of primary sclerosing cholangitis with HLA-DRw52a. N Engl J Med 1990;322:1842-4.
117. Zetterquist H, Broome U, Einarsson K, Olerup O. HLA class II genes in primary sclerosing cholangitis and chronic inflammatory bowel disease: no HLA-DRw52a association in Swedish patients with sclerosing cholangitis. Gut 1992;33:942-6.
118. Mehal WZ, Lo YM, Wordsworth BP, et al. HLA DR4 is a marker for rapid disease progression in primary sclerosing cholangitis. Gastroenterology 1994;106:160-7.
119. Oudkerk Pool M, Ellerbroek PM, Ridwan BU, et al. Serum antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease are mainly associated with ulcerative colitis. A correlation study between perinuclear antineutrophil cytoplasmic autoantibodies and clinical parameters, medical, and surgical treatment. Gut 1993;34:46-50.
120. Targan SR, Landers C, Vidrich A, Czaja AJ. High-titer antineutrophil cytoplasmic antibodies in type-1 autoimmune hepatitis. Gastroenterology 1995;108:1159-66.
121. el-Shabrawi M, Wilkinson ML, Portmann B, et al. Primary sclerosing cholangitis in childhood. Gastroenterology 1987;92:1226-35.
122. Gohlke F, Lohse AW, Dienes HP, et al. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. J Hepatol 1996;24:699-705.

123. Rabinovitz M, Demetris AJ, Bou-Abboud CF, Van Thiel DH. Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis. Dig Dis Sci 1992;37:1606-11.
124. Minuk GY, Sutherland LR, Pappas SC, Kelly JK, Martin SE. Autoimmune chronic active hepatitis (lupoid hepatitis) and primary sclerosing cholangitis in two young adult females. Can J Gastroenterol 1988;2:22-27.
125. Lawrence SP, Sherman KE, Lawson JM, Goodman ZD. A 39 year old man with chronic hepatitis. Semin Liver Dis 1994;14:97-105.
126. Lindor KD, Wiesner RH, Katzmann JA, LaRusso NF, Beaver SJ. Lymphocyte subsets in primary sclerosing cholangitis. Dig Dis Sci 1987;32:720-5.
127. Chapman RW, Kelly PM, Heryet A, Jewell DP, Fleming KA. Expression of HLA-DR antigens on bile duct epithelium in primary sclerosing cholangitis. Gut 1988;29:422-7.
128. Adams DH, Mainolfi E, Burra P, et al. Detection of circulating intercellular adhesion molecule-1 in chronic liver diseases. Hepatology 1992;16:810-4.
129. Hayashi H, Higuchi T, Ichimiya H, Hishida N, Sakamoto N. Asymptomatic primary sclerosing cholangitis treated with ursodeoxycholic acid. Gastroenterology 1990;99:533-5.
130. O'Brien CB, Senior JR, Arora-Mirchandani R, Batta AK, Salen G. Ursodeoxycholic acid for the treatment of primary sclerosing cholangitis: a 30-month pilot study. Hepatology 1991;14:838-47.
131. Chazouilleres O, Poupon R, Capron JP, et al. Ursodeoxycholic acid for primary sclerosing cholangitis. J Hepatol 1990;11:120-3.
132. Beuers U, Spengler U, Kruis W, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatology 1992;16:707-14.
133. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Theilmann 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.
134. De Maria N, Colantoni A, Rosenbloom E, Van Thiel DH. Ursodeoxycholic acid does not improve the clinical course of primary sclerosing cholangitis over a 2-year period. Hepatogastroenterology 1996;43:1472-9.
135. Mitchell S, Bansi D, Hunt N, Christie J, Fleming K, Chapman R. High dose ursodeoxycholic acid (UDCA) in primary sclerosing cholangitis (PSC): Results after two years of a randomised double-blind, placebo-controlled trial. Gastroenterology 1997;112 (suppl):A1335.
136. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. Gastroenterology 1988;95:1036-42.
137. Knox TA, Kaplan MM. Treatment of primary sclerosing cholangitis with oral methotrexate. Am J Gastroenterol 1991;86:546-52.
138. Kaplan MM, Arora S, Pincus SH. Primary sclerosing cholangitis and low-dose oral pulse methotrexate therapy. Clinical and histologic response. Ann Intern Med 1987;106:231-5.
139. Leiser A, Kadish U. Beneficial effect of colchicine in a case of sclerosing cholangitis. Am J Med Sci 1986;291:416-8.
140. Olsson R, Broome U, Danielsson A, et al. Colchicine treatment of primary sclerosing cholangitis. Gastroenterology 1995;108:1199-203.
141. Lindor KD, Wiesner RH, Colwell LJ, Steiner B, Beaver S, LaRusso NF. The combination of prednisone and colchicine in patients with primary sclerosing cholangitis. Am J Gastroenterol 1991;86:57-61.

142. Myers RN, Cooper JH, Padis N. Primary sclerosing cholangitis. Complete gross and histologic reversal after long-term steroid therapy. Am J Gastroenterol 1970;53:527-38.
143. Burgert SL, Brown BP, Kirkpatrick RB, LaBrecque DR. Positive corticosteroid response in early sclerosing cholangitis. Gastroenterology 1984;86:1037.
144. Javett SL. Azathioprine in primary sclerosing cholangitis. Lancet 1971;1:810.
145. Wagner A. Azathioprine treatment in primary sclerosing cholangitis. Lancet 1971;2:663-4.
146. Wiesner RH, Steiner B, LaRusso NF, Lindor KD, Baldus WP. A controlled trial evaluating cyclosporine in the treatment of primary sclerosing cholangitis. Hepatology 1991;14:63A.
147. Gaing AA, Geders JM, Cohen SA, Siegel JH. Endoscopic management of primary sclerosing cholangitis: review, and report of an open series. Am J Gastroenterol 1993;88:2000-8.
148. Hamilton I, Soutar JS, Bouchier IA, Cuschieri A. Short-term biliary dilatation and stenting in primary sclerosing cholangitis. J Clin Gastroenterol 1987;9:70-5.
149. Johnson GK, Geenen JE, Venu RP, Schmalz MJ, Hogan WJ. Endoscopic treatment of biliary tract strictures in sclerosing cholangitis: a larger series and recommendations for treatment. Gastrointest Endosc 1991;37:38-43.
150. Springer DJ, Gaing AA, Siegel JH. Radiologic regression of primary sclerosing cholangitis following combination therapy with an endoprosthesis and ursodeoxycholic acid. Am J Gastroenterol 1993;88:1957-9.
151. Lombard M, Farrant M, Karani J, Westaby D, Williams R. Improving biliary-enteric drainage in primary sclerosing cholangitis: experience with endoscopic methods. Gut 1991;32:1364-8.
152. Lee JG, Schutz SM, England RE, Leung JW, Cotton PB. Endoscopic therapy of sclerosing cholangitis. Hepatology 1995;21:661-7.
153. Grijm R, Huibregtse K, Bartelsman J, Mathus-Vliegen EM, Dekker W, Tytgat GN. Therapeutic investigations in primary sclerosing cholangitis. Dig Dis Sci 1986;31:792-8.
154. Allison MC, Burroughs AK, Noone P, Summerfield JA. Biliary lavage with corticosteroids in primary sclerosing cholangitis. A clinical, cholangiographic and bacteriological study. J Hepatol 1986;3:118-22.
155. Leuschner U, Fischer H, Kurtz W, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
156. Christensen E, Neuberger J, Crowe J, et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology 1985;89:1084-91.

CHAPTER 2

PROGNOSTIC FACTORS AND LONG-TERM EFFECTS OF URSODEOXYCHOLIC ACID ON LIVER BIOCHEMICAL PARAMETERS IN PATIENTS WITH PRIMARY BILIARY CIRRHOSIS

Hubert JF van Hoogstraten[1], Bettina E Hansen[2], Henk R van Buuren[1], Fiebo JW ten Kate[3], Gerard P van Berge-Henegouwen[4], Solko W Schalm[1] for the Dutch Multi-Centre PBC Study Group.

[1]Dept. of Hepatogastroenterology, University Hospital Rotterdam; [2]Dept. of Biostatistics, Erasmus University Rotterdam; [3]Dept. of Pathology, Academic Medical Centre, Amsterdam; [4]Dept. of Gastroenterology, University Hospital Utrecht.

J Hepatol, 1999;31:256-262

Summary

Background: Serum bilirubin is a prognostic factor in untreated primary biliary cirrhosis (PBC), but this has been less extensively documented for patients treated with ursodeoxycholic acid (UDCA). Aims of this study were to define the effects of UDCA on serum liver tests and to assess prognostic factors in patients on prolonged UDCA treatment.

Methods: Analysis of laboratory parameters obtained before and during treatment with UDCA of 203 PBC patients who were followed for a mean of 48 months. Univariate and multivariate analyses were performed to assess the prognostic value of pre-entry and follow-up variables with respect to treatment failure and survival.

Results: Actuarial 5-year incidence of treatment failure and transplantation-free survival were 27% and 79%, respectively. According to the univariate analysis the following variables were significantly associated with prognosis: pre-entry presence of cirrhosis and pre-treatment levels of serum bilirubin and albumin, bilirubin levels during follow-up, the occurrence of biochemical remission and normalisation of serum bilirubin. Multivariate analysis revealed that bilirubin during follow-up proved to be the best predictor. Alkaline phosphatase (APh), aspartate aminotransaminase (AST) and immunoglobulin M (IgM) decreased significantly during the first 6 months of treatment and subsequently remained at this lower level. Serum bilirubin showed the same initial pattern but a significant increase was observed after 4 years of treatment.

Conclusions: Serum bilirubin in both UDCA-treated and untreated patients is the most powerful predictor of prognosis for PBC. The partial therapeutic efficacy of UDCA is illustrated by the finding that serum bilirubin, in contrast to APh and the transaminases, appears to increase after 4 years of treatment.

Introduction

Ursodeoxycholic acid (UDCA) has been established as the treatment of choice for primary biliary cirrhosis (PBC) since it is safe, virtually free of adverse effects and prolongs (transplantation-free) survival (1-5). Within 3 months of treatment with UDCA, pronounced beneficial effects are observed for liver biochemical parameters, especially liver tests indicative of cholestasis and inflammation, and serum immunoglobulin M (IgM), which is characteristically elevated in PBC (3, 5-10). The effects of UDCA on biochemical parameters beyond 2-3 years have not been well documented. In the reports of 4 major placebo-controlled trials to assess the effects of UDCA in PBC (1-4), these data were provided for a maximum follow-up period of 3 years. Leuschner et al. reported sustained improvement in glutamate dehydrogenase, alanine aminotransaminase (ALT), alkaline phosphatase (APh) and IgM in 22 patients who were followed for 4 to 12 years (11).

PBC has many features indicative of an autoimmune-mediated pathogenesis (12-14). As in other autoimmune diseases, e.g. autoimmune hepatitis, biochemical remission of the disease could be indicative of a subsequently favourable long-term outcome. For PBC, the significance of biochemical remission has not been determined.

The importance of bilirubin as a prognostic factor has been established in untreated PBC patients (15-18). In UDCA-treated PBC patients, serum bilirubin has been reported to remain a useful prognostic marker (19).

The present study was conducted to evaluate prognostic factors, including the occurrence of biochemical remission, and to define the long-term effects of UDCA on biochemical parameters in a cohort of patients with compensated PBC.

Patients and methods

A prospective follow-up study of a cohort of PBC patients, who were treated with UDCA according to a pre-defined protocol, was performed. The diagnosis of PBC was established on the basis of previously published criteria (20). Liver biopsy at entry was optional; however, a requirement at entry was that a biopsy specimen had to be available for histological review that showed features compatible with the diagnosis of PBC. The study was started in May, 1990, and follow-up data until 29 February, 1996, were used for the analyses. Patients were recruited and followed in 44 centres in The Netherlands. Exclusion criteria were age > 75 years, (risk of) pregnancy, evidence of extra-hepatic bile duct disease, treatment with UDCA in the 3 months before entry, concomitant serious disease limiting life expectancy and decompensated PBC, defined as cirrhosis with Child-Pugh class B or C disease. UDCA (Ursochol®, Zambon Nederland BV, Amersfoort, The Netherlands) was administered in a single dose of 10 mg per kg bodyweight per day at bedtime. Follow-up data were collected at 3-months intervals during the first year and every half year thereafter. At each visit a general clinical examination and blood studies including serum total bilirubin, APh, aspartate aminotransaminase (AST), albumin and IgM, were performed. Liver biopsy specimens were reviewed by the trial pathologist (FJWtK) and staged according to Ludwig et al. (21).

Statistical analysis

Laboratory parameters were expressed as multiples of the upper limit of normal (ULN), except for albumin which was expressed as multiples of the lower limit of normal (LLN). The course of laboratory parameters was analysed using a repeated measurement model (SAS for windows® version 6.11: proc mixed) after logarithmic transformation. Treatment failure was defined as death, liver transplantation, twofold increase in serum bilirubin, variceal bleeding or *de novo* ascites. Biochemical remission was defined as the simultaneous normalisation of serum bilirubin and AST with APh ≤1.5 x ULN. Transplantation-free survival, time to treatment failure and time to biochemical remission were computed by the Kaplan-Meier method and compared between groups by means of the log-rank test. Multivariate Cox analyses with backward elimination procedures were used to examine prognostic factors at entry and during follow-up (with time-dependent factors). Analysis of the predictive value of cirrhosis versus non-cirrhosis was performed for patients who had undergone liver biopsy within the year before entry or who had had cirrhosis in a previous biopsy. A p-value ≤0.05 was considered statistically significant.

Results

The study population consisted of 203 patients; 179 (88%) were female. Five patients tested negative for antimitochondrial antibodies. In all cases histology was compatible with the diagnosis of PBC. A liver biopsy had been obtained in 99 patients within the year before entry; for 16 of the remaining 104 patients cirrhosis had already been documented histologically (table 1).

Median follow-up was 47.3 (10-90 percentile: 10-60) months. Twelve patients (5.9%) who were lost to follow-up, were censored at the moment of their last visit. UDCA was discontinued in 5 cases because of gastro-intestinal complaints (n=3), deteriorating biochemical values (n=1) and the patient's wish to stop therapy (n=1).

Treatment failure/death

The group treatment failure consisted of 34 patients (table 2). Fourteen patients died: 2 from decompensation of the disease, 2 from hepatocellular carcinoma, 1 from hepatorenal syndrome, 1 from variceal bleeding and 8 from non-hepatological causes.

Five-year transplantation-free survival was 79% and treatment failure occurred in 27% of cases (figure 1). Patients with cirrhosis at entry had a significantly decreased five year transplantation-free survival (65% vs. 94%; p<0.01) and experienced more treatment failure (72% vs. 21%; p<0.01) compared to non-cirrhotic patients. No differences in the incidence of treatment failure or death were observed among Ludwig's histological stages I, II and III. In an univariate model, bilirubin, albumin, AST, normalisation of bilirubin, biochemical remission and bilirubin during follow-up were found to be of predictive significance for both treatment failure and death (table 3).

Three multivariate models were used (tables 4 and 5). All models included the variables given in table 3, but biochemical remission (model A), normalisation of

bilirubin (model B) and bilirubin during follow-up (model C) were included separately to avoid an excess of parameters. From the backward elimination procedure it appeared that bilirubin at entry or during follow-up was the most significant prognostic factor. In all models, age was also a predictor of death and transplantation, while the presence of cirrhosis predicted treatment failure.

Biochemistry

Within the first 6 months of follow-up, serum bilirubin (-6.5%; standard deviation (sd) 2.3; $p<0.01$)(figure 2), APh (-38.7%; sd 1.6; $p<0.01$), AST (-39.9%; sd 1.9; $p<0.01$), IgM (-18.9%; sd 2.8; $p<0.01$) and albumin (+2.2%; sd 0.9; $p=0.02$) (figure 3) gradually improved. APh, AST and IgM remained stable during further follow-up. Bilirubin, however, started to rise after 4 years ($p<0.05$).

Biochemical remission

The actuarial percentage of patients who exhibited biochemical remission at any time during follow-up was 42% (95% confidence interval (95 CI) 35%-49%) at 1 year and 54% (95 CI 46%-62%) at 5 years. In 50% of patients biochemical remission was maintained for 21 months (95 CI 11%-31%); the actuarial percentage of patients in sustained remission after 5 years

Table 1: Patient characteristics at entry.

	Total group n=203
Age (years; meanSD)	56.3 (±10.7)
Sex (male/female)	M 24 / F 179
Alkaline phosphatase (ULN)	3.0 (1.2-8.3)
AST (ULN)	1.7 (0.8-3.8)
Bilirubin (ULN)	0.8 (0.3-2.9)
- patients (no) with	
bilirubin > 1 X ULN	37 (19%)
bilirubin > 2 x ULN	20 (10%)
Albumin (LLN)	1.1 (0.8-1.3)
IgM (ULN)	2.0 (0.7-6.7)
Histological stage	
I	16
II	40
III	30
IV	29

Biochemical data are shown as geometric means with percentiles (5%; 95%) in parentheses. Histological stage provided for patients with previously established cirrhosis or sampled within the year before entry.

Table 2: Reason for first treatment failure.

Event	n
Death	13 (14)
Transplantation	4 (10)
Doubling bilirubin	12
Variceal bleeding	1
De novo ascites	4

Total number of events in parentheses

Table 3: Univariate analysis of possible predictors of prognosis.

	Death - transplantation			Treatment failure		
Covariate	β	RR	95% CI	β	RR	95% CI
Age	0.03	1.03	0.99-1.08	0.01	1.01	0.97-1.04
Cirrhosis	2.1	8.3	2.2-32.0**	1.9	6.6	2.5-17.9**
Bilirubin at entry	2.8	17.0	4.9-59.1**	2.5	12.5	4.3-36.6**
AST at entry	1.7	5.7	0.9-34.6	1.5	4.5	1.0-21.0*
APh at entry	0.4	1.4	0.3-7.0	1.1	2.9	0.7-11.4
IgM at entry	-0.6	0.5	0.1-2.9	-0.3	0.8	0.2-2.9
Albumin at entry	-3.5	0.03	0.002-0.4**	-3.5	0.03	0.002-0.3**
Biochemical remission	-1.4	0.3	0.1-1.1	-1.8	0.2	0.04-0.7*
Normalisation bilirubin	-2.0	0.1	0.1-0.3**	-1.8	0.2	0.1-0.4**
Bilirubin during follow-up	3.1	21.0	7.7-57.7**	2.7	14.9	6.2-35.7**

*$p \leq 0.05$; **$p \leq 0.01$

Table 4: Multi-variate analysis of possible predictors of death or transplantation.

		Death – transplantation	
	Covariate	β	RR (95% CI)
Final model A	Age	0.05	1.1 (1.0-1.1)*
	Bilirubin at entry	3.2	25.3 (6.6-97.7)**
Final model B	Age	0.05	1.1 (1-1.1)*
	Bilirubin at entry	3.2	25.3 (6.6-97.7)**
Final model C	Age	0.07	1.1 (1.0-1.1)*
	Bilirubin during follow-up	3.6	36.3 (11.9-110.5)**

*$p \leq 0.05$; **$p \leq 0.005$
Full model included all variables listed in table 3.

was 34% (95 CI 21%-47%). Univariate analysis showed that low age, absence of cirrhosis and low values of AST, ALT, APh, bilirubin and IgG at entry significantly predicted biochemical remission. Low AST (relative risk (RR)=0.14; 95 CI 0.05-0.5; p=0.002), APh (RR=0.08; 95 CI 0.03-0.23; p<0.0001) and bilirubin (RR=0.21; 95 CI 0.08-0.55; p=0.001) were all predictive of biochemical remission in a multivariate model.

Table 5: Multi-variate analysis of possible predictors of treatment failure.

		Treatment failure	
	Covariate	β	RR (95% CI)
Final model A	Cirrhosis	1.1	2.9 (1.0-9.1)**
	Bilirubin at entry	2.3	10.1 (2.8-6.2)***
Final model B	Cirrhosis	1.3	3.7 (1.3-10.6)**
	Normalisation bilirubin		-1.6 0.2 (0.1-0.5)***
Final model C	Cirrhosis	1.1	3.0 (1.0-8.5)*
	Bilirubin during follow-up	2.6	13.0 (4.9-33.8)***

*$p \leq 0.1$; **$p \leq 0.05$; *** $p \leq 0.005$
Full model included all variables listed in table 3

Discussion

In the past, serum bilirubin has been demonstrated to be one of the most important prognostic factors in PBC. This study not only confirms that treatment with UDCA decreases serum levels of bilirubin but also shows that pre-treatment bilirubin levels remain of significant prognostic value for patients treated with this agent. Most importantly, our results indicate that during the treatment serum bilirubin also provides the most powerful prognostic information. This implies that in clinical practice serum bilirubin values do not have to be "corrected" for the fact that a patient is receiving UDCA. We confirm previous observations that for UDCA-treated patients serum bilirubin (4, 19) and normalisation of serum bilirubin during therapy (22) are of important prognostic significance.

The definition of biochemical remission used in this study was a modification of the definition proposed for autoimmune hepatitis (23, 24). Consistent with another recent study (25), the achievement of biochemical remission was significantly associated with a better prognosis, suggesting that biochemical remission might be an important short-term therapeutic goal in PBC. In the multivariate analysis, however, the prognostic significance of biochemical remission was not apparent.

Combined analysis of the French (3), Mayo Clinic (1) and Canadian (6) randomised controlled trials to evaluate the effects of UDCA in PBC showed a significantly longer survival free of transplantation in UDCA-treated patients (4). In these studies the 4-year transplantation-free survival rate was approximately 80%, which is comparable with the 88% 4-year survival rate observed in the present study. The slightly better value may be explained by variations in patient selection since our patients had less advanced disease.

This study further suggests that the effect of UDCA therapy on serum bilirubin is temporary, in contrast to the effects on serum transaminases and APh, which were maintained for a period of at least 5 years. This observation illustrates that UDCA has a limited potential to cure the disease and that long-term treatment does not prevent the ultimate development of progressive cholestasis and liver failure.
When this study was initiated in 1990 a UDCA dose of 10 mg/kg/day was considered adequate and was normal in our country. Two recent studies clearly indicate that higher doses have more pronounced effects, especially on APh and transaminases (26, 27) and in retrospect the dose we used was not optimal. However, the quantitative differences in biochemical response between doses of 10 and 20 mg/kg/day were rather small (26). In particular, no significant difference in serum bilirubin was found for patients treated with 10 mg/kg/day compared with patients treated with 20 mg/kg/day (26). It seems therefore unlikely that the main results of this study have been influenced in a major way by use of the 10 mg/kg/day dose. However, the possibility that higher UDCA doses would have yielded other results, especially for the biochemical parameters, must be considered. It seems unlikely that our choice for a single-dose regimen has influenced the results of the present study, since single- and multiple-dose regimens lead to similar improvements in serum liver function test and UDCA enrichment in bile in patients with cholestatic liver diseases (28, 29).
In conclusion, serum bilirubin, in particular during treatment with UDCA, is the factor that provides the most powerful prognostic information in PBC. The beneficial effects of UDCA on laboratory liver tests were sustained for at least 4 years; then serum bilirubin levels tended to rise. The latter observation can be considered another illustration of the partial therapeutic efficacy of UDCA in PBC, as shown in the present and other studies.

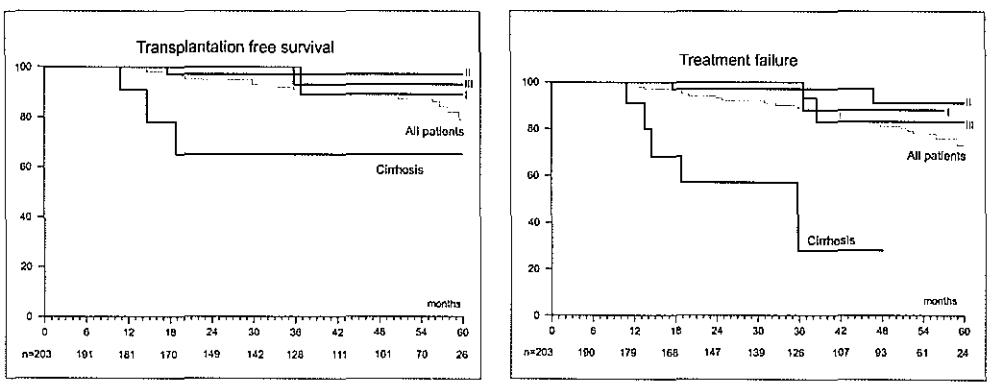
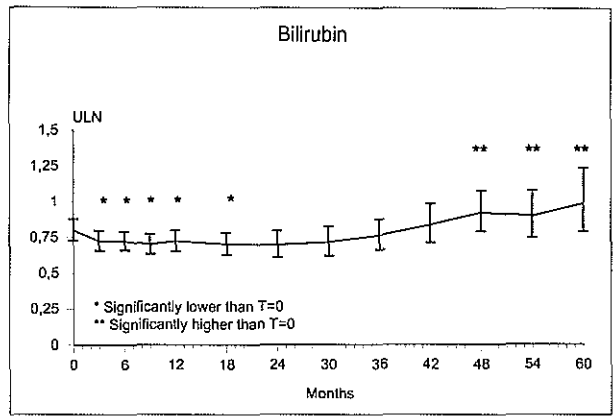
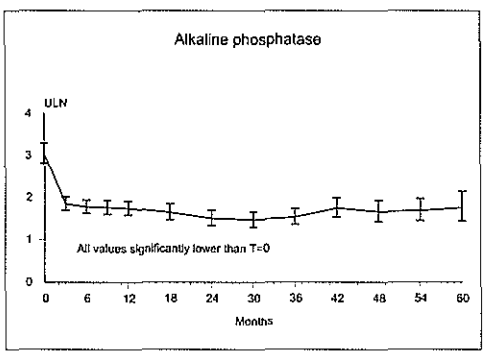
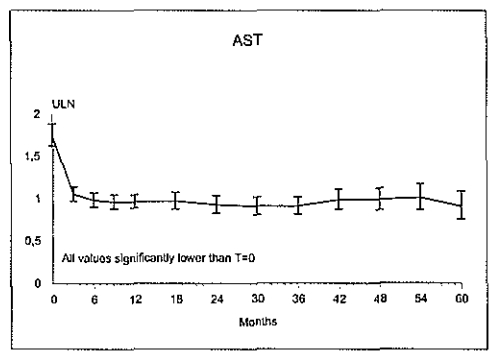


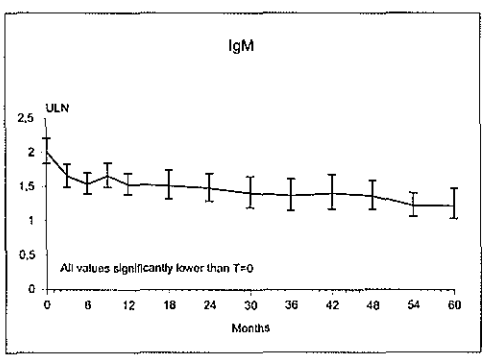
Figure 1: Proportion of patients who survived without transplantation and without treatment failure for the complete cohort or according to histological stage.

*Figure 2: Geometric means (upper limit of normal) of serum bilirubin during 5 years of treatment with UDCA. Error bars indicate standard error of the mean. *Significantly lower than baseline. **Significantly higher than baseline.*









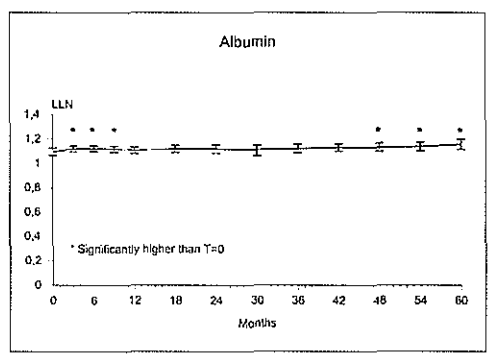


Figure 3: Geometric means (upper limit of normal) of alkaline phosphatase, aspartate minotransaminase (AST), immunoglobulin M (IgM) and albumin during 5 years of treatment with UDCA. Error bars indicate standard error of the mean.

Acknowledgement
The other members of the Dutch Multi-Centre PBC Study Group who participated in this study were: R Adang, University Hospital Maastricht; PL Batenburg, Zuiderziekenhuis Rotterdam; J van Hattum, University Hospital Utrecht; P Biemond and LRKW Lie, Ziekenhuis St. Franciscus, Roosendaal; PM Blom van Assendelft, Sophia Ziekenhuis, Zwolle; JGS Breed, St. Jans Gasthuis, Weert; ThJM van Ditzhuijsen and IP van Munster, Bosch Medicentrum, Den Bosch; LGJB Engels, Maasland Ziekenhuis, Sittard; J Ferwerda, Kennemer Gasthuis, Haarlem; JN Groen, Ziekenhuis St. Jansdal, Harderwijk; KJ Heering, Groene Hart Ziekenhuis, Gouda; PAM van Hees, St. Antonius Ziekenhuis, Nieuwegein; EW van der Hoek and AM Smit, Carolus Ziekenhuis, Den Bosch; JW Kappelle and P Spoelstra, Medisch Centrum Leeuwarden; MJ Kerbert- Dreteler and JH van Lijf, Medisch Spectrum Twente, Enschede; IJ Klompmaker and EB Haagsma, University Hospital Groningen; W Lesterhuis, ACM van Vliet and R Beukers, Albert Schweitzer Ziekenhuis, Dordrecht; BJ Looij, Maasland Ziekenhuis, Geleen; AWM van Milligen de Wit and RM Valentijn, Rode Kruis Ziekenhuis, Den Haag; CMJ van Nieuwkerk, University Hospital of the Free University, Amsterdam; JW den Ouden, St. Franciscus Gasthuis, Rotterdam; SHA Peters and PL Tjepkema, IJsselmeerziekenhuizen, Lelystad; GJ Ras, Ignatius Ziekenhuis, Breda; MCM Rijk, Ziekenhuis De Baronie, Breda; RJ Robijn, Ziekenhuiscentrum Apeldoorn; J Scherpenisse, Reinier de Graaf Gasthuis, Delft; P Stadhouder, Canisius-Wilhelmina Ziekenhuis, Nijmegen; TG Tan, St. Streekziekenhuis Midden-Twente, Hengelo; JC Thijs, Ziekenhuis Bethesda, Hoogeveen; K te Velde, St. Deventer Ziekenhuizen, Deventer; VMC Verstappen, St. Maartens Gasthuis, Venlo; MM Vidakovic, St. Lucas Andreas Ziekenhuis, Amsterdam; RA de Vries and JE Thies, Ziekenhuis Rijnstate, Arnhem; SDJ van der Werf, Westeinde Ziekenhuis, Den Haag; RAA van Zanten, Twenteborg Ziekenhuis, Almelo; RNM Zeijen and JJ Braun, Schieland Ziekenhuis, Schiedam; R Zwertbroek, Westfries Gasthuis, Hoorn; AW Saleh, Elisabeth Hospitaal, Willemstad, Curaçao.

References

1. Lindor KD, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology 1996;110:1515-8.
2. Kaplan MM. Primary biliary cirrhosis-a first step in prolonging survival. N Engl J Med 1994;330:1386-7.
3. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med 1994;330:1342-7.
4. Poupon RE, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1997;113:884-90.
5. Lim AG, Jazrawi RP, Northfield TC. The ursodeoxycholic acid story in primary biliary cirrhosis. Gut 1995;37:301-4.
6. Heathcote EJ, Cauch-Dudek K, Walker V, Bailey RJ, Blendis LM, Ghent CN, Michieletti P, et al. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
7. Lindor KD, Dickson ER, Baldus WP, Jorgensen RA, Ludwig J, Murtaugh PA, Harrison JM, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
8. Leuschner U, Fischer H, Kurtz W, Guldutuna S, Hubner K, Hellstern A, Gatzen M, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
9. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OF. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroenterol Hepatol 1994;9:162-8.
10. Combes B, Carithers R Jr., Maddrey WC, Lin D, McDonald MF, Wheeler DE, Eigenbrodt EH, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1995;22:759-66.
11. Leuschner U, Guldutuna S, Imhof M, Hubner K, Benjaminov A, Leuschner M. Effects of ursodeoxycholic acid after 4 to 12 years of therapy in early and late stages of primary biliary cirrhosis. Journal of Hepatology 1994;21:624-633.
12. Bjorkland A, Totterman TH. Is primary biliary cirrhosis an autoimmune disease? Scand J Gastroenterol Suppl 1994;204:32-9.
13. James SP, Hoofnagle JH, Strober W, Jones EA. NIH conference: Primary biliary cirrhosis: a model autoimmune disease. Ann Intern Med 1983;99:500-12.
14. Mackay IR, Gershwin ME. Primary biliary cirrhosis: considerations on pathogenesis based on identification of the M2 autoantigens. Springer Semin Immunopathol 1990;12:101-19.
15. Shapiro JM, Smith H, Schaffner F. Serum bilirubin: a prognostic factor in primary biliary cirrhosis. Gut 1979;20:137-40.
16. Roll J, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. N Engl J Med 1983;308:1-7.
17. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10:1-7.
18. Mahl TC, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. J Hepatol 1994;20:707-13.

19. Kilmurry MR, Heathcote EJ, Cauch-Dudek K, O'Rourke K, Bailey RJ, Blendis LM, Ghent CN, et al. Is the Mayo model for predicting survival useful after the introduction of ursodeoxycholic acid treatment for primary biliary cirrhosis? Hepatology 1996;23:1148-53.
20. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology 1983;30:178-82.
21. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379:103-12.
22. Bonnand A-M, Heathcote EJ, Lindor KD, Poupon RE. Clinical significance of serum bilirubin levels under ursodeoxycholic acid therapy in patients with primary biliary cirrhosis. J Hepatol 1997;26(suppl no.1):164.
23. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology 1993;18:998-1005.
24. Johnson PJ, McFarlane IG, Williams R. Azathioprine for long-term maintenance of remission in autoimmune hepatitis. N Engl J Med 1995;333:958-63.
25. Poupon R, Chretien Y, Chazouilleres O, Bonnand AM, Poupon RE. Biochemical response to ursodeoxycholic acid (UDCA) predicts progression of primary biliary cirrhosis. Hepatology 1997;26(suppl):400A.
26. van Hoogstraten HJF, de Smet MBM, Hop WCJ, van Buuren HR, vanBerge-Henegouwen GP, Schalm SW. A randomized controlled trial evaluating therapy with ursodeoxycholic acid in daily doses of 10 mg/kg versus 20 mg/kg in primary biliary cirrhosis. Gastroenterology 1998;114 (part2):A1358.
27. Lindor KD, Jorgensen R, Therneau TM, Smith C, Mahoney DW, Dickson ER. Comparison of three different doses of ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a randomized controlled trial. Hepatology 1997;26(suppl):438A.
28. van de Meeberg PC, Wolfhagen FHJ, van Berge Henegouwen GP, Salemans JMJI, Tangerman A, van Buuren HR, van Hattum J, et al. Single or multiple dose ursodeoxycholic acid for cholestatic liver disease: relation with biliary enrichment and biochemical response. J Hepatol 1996;25:887-894.
29. Van Hoogstraten HJ, De Smet MB, Renooij W, Breed JG, Engels LG, Den Ouden-Muller JW, Rijk MC, et al. A randomized trial in primary biliary cirrhosis comparing ursodeoxycholic acid in daily doses of either 10 mg/kg or 20 mg/kg. Dutch Multicentre PBC Study Group. Aliment Pharmacol Ther 1998;12:965-71.

CHAPTER 3

A RANDOMISED CONTROLLED TRIAL EVALUATING THERAPY WITH URSODEOXYCHOLIC ACID IN DAILY DOSES OF 10 MG/KG VERSUS 20 MG/KG IN PRIMARY BILIARY CIRRHOSIS

Hubert JF van Hoogstraten[1], Martin BM de Smet[2], Willem Renooij[2], Johannes GS Breed[3], Leopold GJB Engels[4], Janny W den Ouden-Muller[5], Marco CM Rijk[6], Adelbert M Smit[7], Rolf Zwertbroek[8], Wim CJ Hop[1], Gerard P van Berge Henegouwen[2], Solko W Schalm[1], Henk R van Buuren[1], representing the Dutch Multi-Centre PBC Study Group.

[1]University Hospital Rotterdam, [2]University Hospital Utrecht, [3]St Jans Gasthuis Weert, [4]Maasland Ziekenhuis Sittard, [5]St Franciscus Gasthuis Rotterdam, [6]Ziekenhuis de Baronie Breda, [7]Carolus Ziekenhuis Den Bosch, [8]Westfries Gasthuis Hoorn.

Aliment Pharm Therap 1998;12:965-71.

Summary

Background: Ursodeoxycholic acid (UDCA) prolongs transplantation-free survival in primary biliary cirrhosis (PBC). However, the optimal therapeutic dose has not been established.

Aim: To compare the effects of UDCA administered in daily doses of 10 versus 20 mg/kg on symptoms, liver biochemistry and biliary UDCA enrichment.

Methods: A 6-months multi-centre randomised open controlled trial was conducted to assess the effects of an increase in the dose of UDCA to 20 mg/kg/day versus continuation of 10 mg/kg/day for patients who had not achieved biochemical normalisation during treatment for at least 6 months with the 10 mg/kg dose. Clinical and laboratory evaluations were performed at entry and at 3-months intervals. The percentage UDCA in duodenal bile was assessed at entry and at 6 months.

Results: Sixty-one patients were enrolled. No side-effects of UDCA were observed. Within the 20 mg/kg/day group significant decreases were found for alkaline phosphatase (-8%; p=0.003), aspartate aminotransaminase (-11%; p=0.01), alanine aminotransaminase (-17%; p<0.001), γ-glutamyl transferase (-34%; p<0.001), immunoglobulin M (-11%; p=0.002) and cholesterol (-8.1%; p<0.001). In the 10 mg/kg group none of these parameters differed significantly from baseline. No significant differences between the dose groups for symptom scores or serum bilirubin were found. Biliary enrichment with UDCA increased from 37% to 46% in the 20 mg/kg group (p=0.02) while remaining stable in the 10 mg/kg group.

Conclusions: Liver biochemistry improved in PBC patients receiving UDCA 20 mg/kg/day compared to a dose of 10 mg/kg/day. Both doses were equally well tolerated. These results indicate that UDCA 10 mg/kg/day is a suboptimal dose for treating PBC.

Introduction

Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease of unknown aetiology, characterised by immune-mediated destruction of intrahepatic bile ducts (1-3). The beneficial effects of ursodeoxycholic acid (UDCA) on biochemical parameters and transplantation-free survival have been well documented (4-8). However, surprisingly few data on the optimum therapeutic UDCA dose are available (9). In several large controlled trials UDCA was administered in a dose of 13-15 mg/kg/day (4-6), which was based on preliminary findings that this dose was well tolerated and resulted in marked biochemical improvements (10). Others, however, have used lower doses, varying from 7.7 (11, 12) to 10 mg/kg/day (7, 13, 14).
The Dutch Multi-centre PBC Study Group chose a dose of 10 mg/kg/day. When evaluation revealed that biochemical remission was achieved in 11% of patients and combined clinical, biochemical and histological remission occurred in less than 5%, the question arose as to whether the UDCA dose was too low (15).
In view of the paucity of data on the optimum UDCA dose for treatment of PBC, the aim of this study was to assess the dose-response relationship and patient tolerance of relatively low (10 mg/kg) versus relatively high (20 mg/kg) doses of UDCA.

Methods

Patients with an established diagnosis of PBC (16) from 20 centres in the Netherlands were included in this randomised open controlled trial. All patients had compensated disease, defined as the absence of criteria for Child-Pugh class B disease (17), and had been treated with 10 mg UDCA /kg/day for at least 6 months, without achieving normalisation of serum bilirubin, alkaline phosphatase (APh), aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), γ-glutamyl transferase (γ-GT) or immunoglobulin M (IgM). Patients were randomly allocated either to a group which continued to take UDCA 10 mg/kg/day or to a group which received 20 mg/kg/day for a period of 6 months. UDCA (Ursochol®; Zambon BV; Amersfoort, The Netherlands) was given in two divided daily doses. Follow-up data were collected every 3 months. Symptoms, i.e. fatigue and pruritus, were quantified using visual analogue scales (VAS). Patients were asked to indicate the severity of pruritus and fatigue on a horizontal line for the first 5 days following each visit. Blood analyses included: bilirubin, AST, ALT, APh, γ-GT, immunoglobulin G (IgG), IgM, cholesterol and haematological indices. Biochemical remission was defined as normal values for bilirubin, AST and IgM and APh ≤ 1.5 upper limit of normal (ULN). All patients gave informed consent; approval was obtained from the medical ethics committees of the participating centres.

Bile acid analysis

Duodenal bile was collected at entry and at 6 months using the Enterotest® method (18, 19). Patients came to the hospital in the fasting state and swallowed the Enterotest® string with water, the oral end of the string being taped to the corner of the mouth. The string was removed after 4 hours and stored at -20°C until further analysis.
Bile acids were eluted from the distal, absorbent part of the string, usually the last

10–15 cm, by three consecutive washes with 5 ml 0.5 M phosphate buffer, pH 7.0, in an ultrasound bath (18). Bile salts were extracted from the buffer by means of solid-phase extraction using Sep-Pak C18 cartridges (Waters Inc., Milford, MA, USA). Bile acids were eluted from the cartridge with 3 ml of methanol. Total bile salt concentration was analysed enzymatically in the phosphate buffer and in the methanolic extract from the string (20). It appeared that 85% of the total bile salt pool was recovered in the phosphate buffer and additional washes of the string with methanol increased the total recovery to a limited extent only. Therefore, the buffer extract was used for further analysis.
Bile salt species were analysed by capillary gas-liquid chromatography on a CP Sil 19 CB fused silica column (Chrompack BV, Middelburg, The Netherlands). Bile acids were enzymatically hydrolysed (choloylglycine hydrolase; Sigma Chemical Co., St. Louis, MO, USA) and converted to methylester-trimethylsilyl derivatives using acetylchloride/methanol (Merck, Darmstadt, Germany) and hexamethyldisilazane/trimethylchlorosilane (Pierce, Rockford, Il, USA) (21, 22). Chromatograms were analysed with Shimadzu Scientific Instrument Inc. Class-VP software.

Statistical analysis
Data were analysed on an intention-to-treat basis. To detect differences of at least 75% of the standard deviation in the main laboratory parameters between the treatment groups, with a significance of 0.05 and a power of 80%, it was calculated that a sample size of 60 patients was needed. After stratification for the presence of cirrhosis, randomisation was performed centrally at the University Hospital Rotterdam. Laboratory parameters were expressed as multiples of the ULN. Data at 3 months were compared with baseline using the signed-rank test. The change from baseline of logarithmically transformed laboratory parameters was analysed using repeated measurements analysis of variance (rmANOVA). Symptom scores and biliary enrichment with UDCA were compared by means of Student's t-test. Factors determining biochemical values at 6 months were assessed by multiple regression analysis. A *p*-value equal to or less than 0.05 was considered significant.

Results
Of the 61 patients studied, 28 were allocated to the group continuing with UDCA 10 mg/kg/day (actual mean dose 9.8 mg/kg, standard error of the mean (SEM) 0.5) and 33 to the 20 mg/kg/day dose group (actual mean dose 20.3 mg/kg, SEM 0.7). Patients were well matched for baseline characteristics (table 1). During follow-up, 1 patient in the 10 mg group developed progressive liver failure within 1 month and was withdrawn from the study. One patient (20 mg/kg group) was lost to follow-up immediately after entry.
One patient in the 20 mg/kg group complained of diarrhoea during the first 2 weeks of the study. Clinical evidence suggested that this was due to intercurrent viral gastro-enteritis. No other adverse events were noted. No adverse effects on haematological indices or renal function (serum creatinine) were found.
Analysis of scores for fatigue and pruritus, which were comparable at entry, revealed no changes within or between groups.

Table 1: Features at entry.

Variable	UDCA 10 mg/kg/day n=28	UDCA 20 mg/kg/day n=33
Mean age (range)	55 (37-76)	59 (34-73)
Sex (male/female)	M 3 / F 25	M 3 / F 30
Pruritus (n)	8	9
Fatigue (n)	17	20
Cirrhosis (n)	6	6
Bilirubin	0.9 (0.11)	0.9 (0.18)
Albumin	0.9 (0.02)	0.9 (0.01)
APh	1.7 (0.2)	1.9 (0.2)
γ-GT	3.5 (0.77)	3.8 (0.62)
AST	1 (0.07)	1.1 (0.16)
ALT	1.1 (0.11)	1.4 (0.27)
IgG	1 (0.06)	0.87 (0.04)
IgM	1.95 (0.29)	1.7 (0.23)
Cholesterol	0.9 (0.04)	1.0 (0.05)

Biochemical values are expressed in means of ULN with standard error in parentheses.

At 3 months, in the 20 mg/kg group, APh (-8%; p=0.003), γ-GT (-34%; p<0.001), AST (-11%; p=0.01), ALT (-17%; p<0.001), IgM (-11%; p=0.002) and cholesterol (-8.1%; p<0.001) (figure 1) were all significantly decreased compared to baseline values. In the 10 mg/kg group, liver function tests did not change significantly. Compared with the 10 mg/kg group, the decrease at 3 and 6 months was significantly larger for APh (p =0.006), γ-GT (p<0.001), AST (p<0.001), ALT (p<0.001), IgM (p=0.01) and cholesterol (p<0.001) in the 20 mg/kg group (rmANOVA). Bilirubin and IgG remained the same in both groups. At 6 months biochemical remission was observed in 1 (4%) and 4 (12%) patients in the 10 and 20 mg/kg dose groups, respectively (not significant (ns)).

Duodenal bile was collected from 57 patients at entry; 3 patients (one from the 10 mg/kg group) refused to undergo the procedure and the test failed in 1 case (10 mg/kg dose). After 6 months duodenal bile was collected from 52 patients; 7 patients refused to undergo the procedure (five from the 20 mg/kg group) and it was not performed for other reasons in 2 cases. Bile acid analysis was successful for 43/57 (75%) samples obtained at entry and 44/52 (85%) of the 6-month samples. The main reason for failure was sampling of insufficient bile acid via the

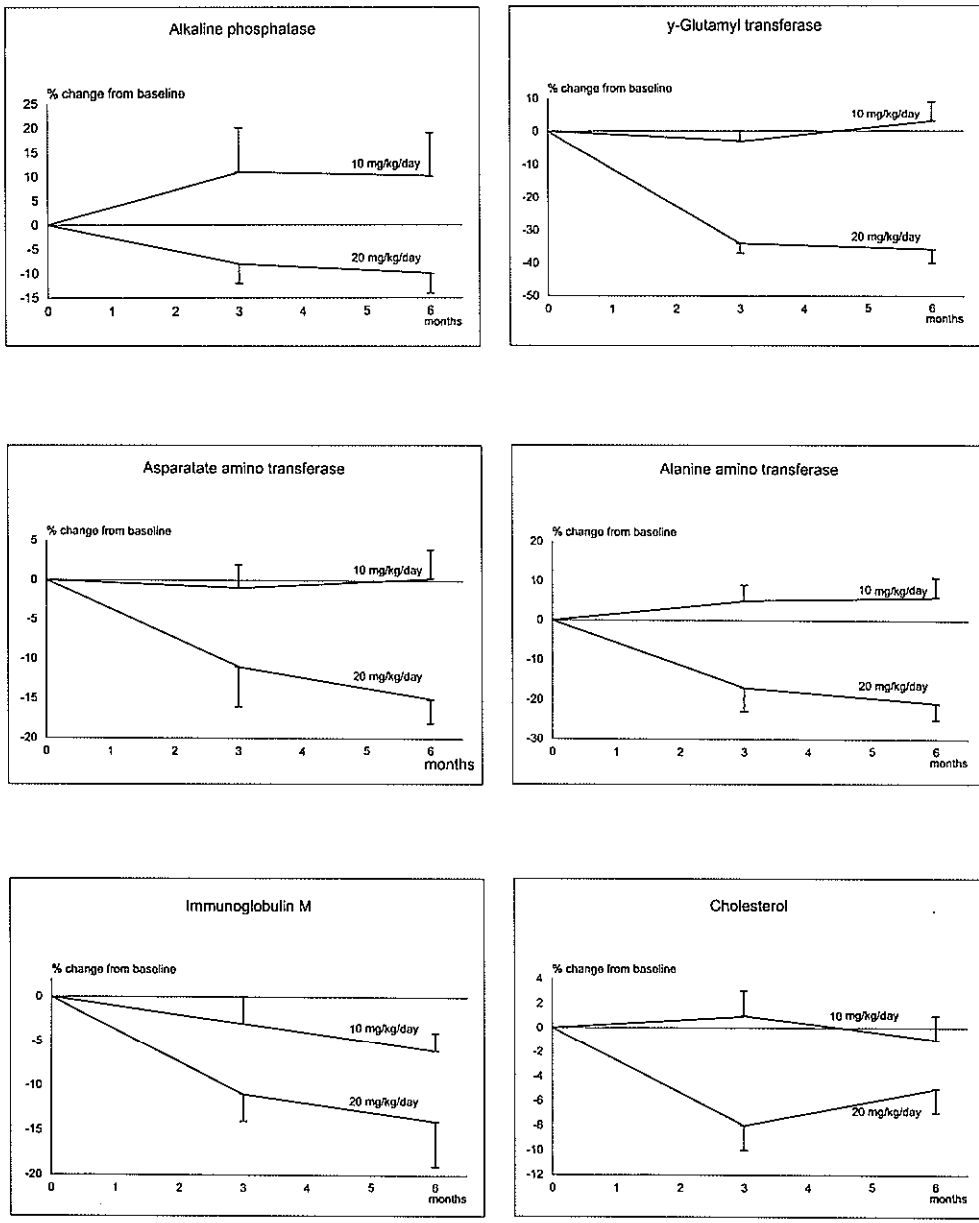
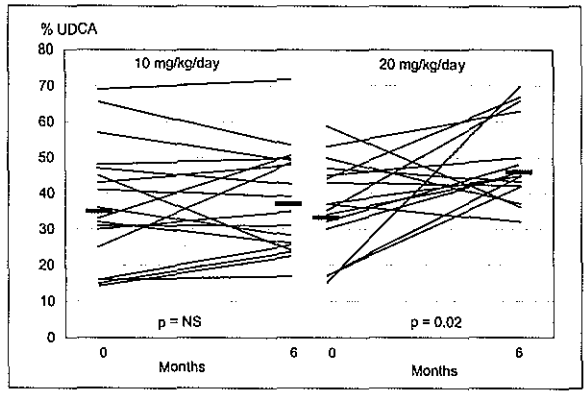


Figure 1: Percentage change from baseline values for alkaline phosphatase, γ-glutamyl transferase, aspartate amino transaminase, alanine amino transaminase, immunoglobulin M and cholesterol for the 10 mg/kg and 20 mg/kg UDCA dose groups. Error bars indicate standard error of the mean. Values for the 20 mg/kg group are significantly lower compared to both the 10 mg/kg group and baseline values.

Figure 2: Percentage UDCA enrichment of duodenal bile at entry and at 6 months for each patient with paired observations. Bars indicate mean percentages of the total group (paired and unpaired data).



string. At entry biliary enrichment with UDCA was 35% (SEM 4.3%) in the 10 mg/kg group and 33% (SEM 2.9%) in the 20 mg/kg group (p=ns.). After 6 months of treatment biliary enrichment remained stable (37%; SEM 3.4) in the 10 mg/kg group and had significantly increased to 46% (SEM 2.6) in the 20 mg/kg group (p=0.02) (figure 2). No significant correlations were found between the changes in any of the laboratory parameters and the change in UDCA enrichment. From further analysis by a multiple regression model, which included dose group, biochemical value at entry and biliary enrichment at 6 months, it appeared that biochemical values at 6 months were primarily associated with the dose group and not with biliary enrichment. The same applied to the increase in biliary enrichment. When UDCA enrichment at entry for patients with and without cirrhosis was compared, no significant differences were found either for the total group of patients or for the 2 dose groups separately. At 6 months, UDCA enrichment in the 20 mg/kg group was 36% for those with established cirrhosis and 49% for those without cirrhosis, respectively (p<0.05).

Discussion

Our data indicate that for patients with PBC, treatment with UDCA in a dose of 20 mg/kg/day results in greater UDCA enrichment of bile and greater improvements in liver biochemistry than treatment with 10 mg/kg/day. Both doses were equally well tolerated, confirming previous experience obtained with UDCA 20 mg/kg/day in other cholestatic disorders (23-25). The further improvements obtained with the higher UDCA dose were, however, not associated with a favourable effect on symptoms.

For patients treated with UDCA, biliary enrichment is determined by the intestinal absorption of UDCA and the subsequent uptake and excretion by the liver. Previous studies of patients with (9, 24-27) and without liver disease (28, 29) have shown that higher doses of UDCA lead to greater biliary enrichment, although the relative intestinal absorption of UDCA may decrease simultaneously (27, 30). Biliary enrichment with UDCA was found to be related to biochemical improvement in

some (9, 31, 32) but not all studies (7, 26). The factors, which ultimately determine the degree of biliary UDCA enrichment that can be obtained in individual patients, have not been clearly defined. It remains to be established whether doses higher than those used in this study will further enhance biliary enrichment with UDCA.
Although the number of cases was limited, our results suggest that the maximum biliary enrichment that can be obtained is lower for patients with cirrhosis than for non-cirrhotics. In advanced stages of PBC, decreased bile production may impair intestinal UDCA absorption. Biliary UDCA enrichment is also determined by the capacity of the liver to excrete UDCA into bile and decreasing enrichment was found to be associated with increasing cholestasis and the presence of cirrhosis (33). Studies of cystic fibrosis have shown that in this disease biliary enrichment is relatively low, due to malabsorption of the bile acids; a 20 mg/kg/day dose has been found to increase biliary UDCA enrichment and the biochemical response compared to a 10 mg/kg/day dose (24, 25).
Our results appear to confirm previous reports (6, 34) that UDCA treatment has no clear effect on symptoms. However, our patients had already been treated for at least 6 months and symptomatic benefit may have occurred earlier. In this context it should also be noted that the further biochemical improvements observed with the higher UDCA dose were quantitatively rather small.
In view of the results of this study one might question whether PBC patients should be treated with a UDCA dose of at least 20/mg/kg. Theoretically, it seems logical to use a dose, which has been found to exert the most pronounced effects. To assess whether this dose is therapeutically more potent in preventing end-points such as death or liver transplantation would require a randomised controlled trial with a long period of follow-up. Moreover, given the already established efficacy of UDCA treatment and the small differences in the effects of 'low' and 'high' doses, many hundreds of patients would be needed. Therefore, studies to actually prove the superiority of one dose regimen over the other hardly seem feasible.
Recently, a study similar to ours (35) was published in abstract form, where UDCA in doses of 5-7, 13-15 and 22-25 mg/kg/day were compared. No differences were found between the 13-15 and 22-25 mg/kg groups while both doses resulted in a significantly greater biochemical improvement than the lower dose. Based on these and our own findings, the conclusion that UDCA at a dose of 13-15 mg/kg/day is the most (cost)-effective dose for treating patients with PBC seems justified.
Our method for collection of duodenal bile by means of the Enterotest® has previously been validated (18, 36). Duodenal bile has been reported to be qualitatively similar to gall-bladder bile (37, 38). The Enterotest method does not require duodenal intubation and therefore may be more easily tolerated and accepted by patients. In several cases we were unable to perform bile acid analysis due to insufficient recovery of bile, a problem also reported by others (39). Therefore, to increase the diagnostic yield when using this method, one might consider stimulating gall-bladder contraction (39). The 37% biliary UDCA enrichment found at baseline is comparable with the findings of others, who reported a 31-40% enrichment with UDCA in bile collected by duodenal intubation from patients treated with UDCA doses ranging from 8 to 15 mg/kg/day (7, 34, 40, 41).

The exact mechanism of action of UDCA in PBC is still subject to debate. UDCA decreases the hydrophobicity index of the bile acid pool and may prevent liver cell damage caused by endogenous hydrophobic bile acids (42-44). Furthermore, stabilisation of cell membranes (45, 46) and a choleretic effect may contribute to the therapeutic effect of UDCA (47). Although the therapeutic effect of UDCA in cholestatic liver disease may correlate with the degree of UDCA enrichment of the bile acid pool, this could not be confirmed in our study, suggesting the importance of other factors in the mechanism of action of UDCA.

In conclusion, for patients with PBC, treatment with UDCA at a dose of 20 mg/kg/day, which is well tolerated, results in greater biliary enrichment with UDCA and has a more pronounced effect on biochemical liver function tests than a 10 mg/kg/day dose. Consequently, the latter dose is not optimal. Given the results of the recently reported trial by Lindor et al., UDCA at a daily dose of 13-15 mg/kg appears to be the preferred dose.

Acknowledgements

This study was supported in part by Zambon Nederland BV, Amersfoort, The Netherlands.

We gratefully acknowledge JJ Braun, CTBM van Deursen, ThJM van Ditzhuijsen, KJ Heering, EW van der Hoek, AWM van Milligen de Wit, CJJ Mulder, IP van Munster, SHA Peters, J Scherpenisse, A Stronkhorst, TG Tan, JC Thijs and SDJ van der Werf for their help with patient recruitment.

References

1. Kaplan MM. Primary biliary cirrhosis. N Engl J Med 1996;335:1570-80.
2. Gershwin ME. The molecular basis of primary biliary cirrhosis: interfacing clinical medicine and wet bench research. Isr J Med Sci 1995;31:22-30.
3. Sherlock S. Primary biliary cirrhosis: clarifying the issues. Am J Med 1994;96:27S-33S.
4. Lindor KD, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology 1996;110:1515-8.
5. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med 1994;330:1342-7.
6. Heathcote EJ, Cauch-Dudek K, Walker V, et al. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
7. Combes B, Carithers R Jr., Maddrey WC, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1995;22:759-66.
8. Poupon RE, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1997;113:884-90.
9. Podda M, Ghezzi C, Battezzati PM, et al. Effect of different doses of ursodeoxycholic acid in chronic liver disease. Dig Dis Sci 1989;34:59S-65S.
10. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet 1987;1:834-6.
11. Eriksson LS, Olsson R, Glauman H, et al. Ursodeoxycholic acid treatment in patients with primary biliary cirrhosis. A Swedish multicentre, double-blind, randomized controlled study. Scand J Gastroenterol 1997;32:179-86.
12. Battezzati PM, Podda M, Bianchi FB, et al. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis. Preliminary analysis of a double-blind multicenter trial. Italian Multicenter Group for the Study of UDCA in PBC. J Hepatol 1993;17:332-8.
13. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OF. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroenterol Hepatol 1994;9:162-8.
14. Leuschner U, Fischer H, Kurtz W, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
15. Wolfhagen FH, van Buuren HR, Schalm SW, et al. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis? The Dutch Multicentre PBC Study Group. J Hepatol 1995;22:381.
16. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology 1983;30:178-82.
17. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646-9.
18. Vonk RJ, Kneepkens CM, Havinga R, Kuipers F, Bijleveld CM. Enterohepatic circulation in man. A simple method for the determination of duodenal bile acids. J Lipid Res 1986;27:901-4.
19. Rosenthal P. Collection of duodenal bile in infants and children by the string test. J Pediatr Gastroenterol Nutr 1985;4:284-5.
20. Turley SD, Dietschy JM. Re-evaluation of the 3 alpha-hydroxysteroid dehydrogenase

assay for total bile acids in bile. J Lipid Res 1978;19:924-8.
21. Setchell KD, Matsui A. Serum bile acid analysis. Clin Chim Acta 1983;127:1-17.
22. Kuipers F, Spanjer HH, Havinga R, Scherphof GL, Vonk RJ. Lipoproteins and liposomes as in vivo cholesterol vehicles in the rat: preferential use of cholesterol carried by small unilamellar liposomes for the formation of muricholic acids. Biochim Biophys Acta 1986;876:559-66.
23. Mitchell S, Bansi D, Hunt N, Christie J, Fleming K, Chapman R. High dose ursodeoxycholic acid (UDCA) in primary sclerosing cholangitis (PSC): Results after two years of a randomised double-blind, placebo-controlled trial. Gastroenterology 1997;112 (suppl):A1335.
24. van de Meeberg PC, Houwen RH, Sinaasappel M, Heijerman HG, Bijleveld CM, Vanberge-Henegouwen GP. Low-dose versus high-dose ursodeoxycholic acid in cystic fibrosis- related cholestatic liver disease. Results of a randomized study with 1-year follow-up. Scand J Gastroenterol 1997;32:369-73.
25. Colombo C, Crosignani A, Assaisso M, et al. Ursodeoxycholic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. Hepatology 1992;16:924-30.
26. Crosignani A, Battezzati PM, Setchell KD, et al. Effects of ursodeoxycholic acid on serum liver enzymes and bile acid metabolism in chronic active hepatitis: a dose-response study. Hepatology 1991;13:339-44.
27. Walker S, Rudolph G, Raedsch R, Stiehl A. Intestinal absorption of ursodeoxycholic acid in patients with extrahepatic biliary obstruction and bile drainage. Gastroenterology 1992;102:810-5.
28. Tint GS, Salen G, Shefer S. Effect of ursodeoxycholic acid and chenodeoxycholic acid on cholesterol and bile acid metabolism. Gastroenterology 1986;91:1007-18.
29. Stiehl A, Raedsch R, Rudolph G, Walker S. Effect of ursodeoxycholic acid on biliary bile acid and bile lipid composition in gallstone patients. Hepatology 1984;4:107-11.
30. Marcus SN, Schteingart CD, Marquez ML, et al. Active absorption of conjugated bile acids in vivo. Kinetic parameters and molecular specificity of the ileal transport system in the rat. Gastroenterology 1991;100:212-21.
31. Jorgensen RA, Dickson ER, Hofmann AF, Rossi SS, Lindor KD. Characterisation of patients with a complete biochemical response to ursodeoxycholic acid. Gut 1995;36:935-8.
32. van de Meeberg PC, Wolfhagen FHJ, van Berge Henegouwen GP, et al. Single or multiple dose ursodeoxycholic acid for cholestatic liver disease: relation with biliary enrichment and biochemical response. J Hepatol 1996;25:887-894.
33. Stiehl A, Rudolph G, Sauer P, Theilmann L. Biliary secretion of bile acids and lipids in primary sclerosing cholangitis. Influence of cholestasis and effect of ursodeoxycholic acid treatment. J Hepatol 1995;23:283-9.
34. Lindor KD, Dickson ER, Baldus WP, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
35. Lindor KD, Jorgensen R, Therneau TM, Smith C, Mahoney DW, Dickson ER. Comparison of three different doses of ursodeoxycholic acid in the treatment of primary biliary cirrhosis: a randomized trial. Hepatology 1997;26:1240(A).
36. Muraca M, Vilei MT, Miconi L, Petrin P, Antoniutti M, Pedrazzoli S. A simple method for the determination of lipid composition of human bile. J Lipid Res 1991;32:371-4.
37. Choudhuri G, Agarwal DK, Saraswat VA, Negi TS, Saxena R, Kapoor VK. Is duodenal bile representative of gallbladder bile? A comparative study. Scand J Gastroenterol 1993;28:920-3.
38. Janowitz P, Swobodnik W, Wechsler JG, Zoller A, Kuhn K, Ditschuneit H. Comparison

of gall bladder bile and endoscopically obtained duodenal bile. Gut 1990;31:1407-10.
39. Muraca M, Pazzi P, Vilei MT, et al. Routine determination of biliary lipid composition with the entero-test. Ann Chir 1994;48:197-200.
40. Crosignani A, Podda M, Battezzati PM, et al. Changes in bile acid composition in patients with primary biliary cirrhosis induced by ursodeoxycholic acid administration. Hepatology 1991;14:1000-7.
41. Batta AK, Salen G, Mirchandani R, et al. Effect of long-term treatment with ursodiol on clinical and biochemical features and biliary bile acid metabolism in patients with primary biliary cirrhosis. Am J Gastroenterol 1993;88:691-700.
42. Heuman DM, Mills AS, McCall J, Hylemon PB, Pandak WM, Vlahcevic ZR. Conjugates of ursodeoxycholate protect against cholestasis and hepatocellular necrosis caused by more hydrophobic bile salts. In vivo studies in the rat. Gastroenterology 1991;100:203-11.
43. Pazzi P, Puviani AC, Libera MD, et al. Bile salt-induced cytotoxicity and ursodeoxycholate cytoprotection: in-vitro study in perfused rat hepatocytes. Eur J Gastroenterol Hepatol 1997;9:703-9.
44. Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. Bile acid-induced liver toxicity: relation to the hydrophobic- hydrophilic balance of bile acids. Med Hypotheses 1986;19:57-69.
45. Guldutuna S, Zimmer G, Imhof M, Bhatti S, You T, Leuschner U. Molecular aspects of membrane stabilization by ursodeoxycholate. Gastroenterology 1993;104:1736-44.
46. Heuman DM, Bajaj R. Ursodeoxycholate conjugates protect against disruption of cholesterol-rich membranes by bile salts. Gastroenterology 1994;106:1333-41.
47. Galan AI, Jimenez R, Munoz ME, Gonzalez J. Effects of ursodeoxycholate on maximal biliary secretion of bilirubin in the rat. Biochem Pharmacol 1990;39:117-80.

CHAPTER 4

TRIPLE THERAPY WITH URSODEOXYCHOLIC ACID, PREDNISONE AND AZATHIOPRINE IN PRIMARY BILIARY CIRRHOSIS: A 1-YEAR RANDOMISED PLACEBO-CONTROLLED STUDY

Frank HJ Wolfhagen[1], Hubert JF van Hoogstraten[1], Henk R van Buuren[1], Gerard P van Berge-Henegouwen[2], Fiebo JW ten Kate[3], Wim CJ Hop[4], Egbert W van der Hoek[5], Marjo J Kerbert[6], Hans H van Lijf[6], Janny W den Ouden-Muller[7], Adelbert M Smit[5], Richard A de Vries[8], Rene AA van Zanten[9], Solko W Schalm[1].

[1]Dept. of Hepatogastroenterology, University Hospital Rotterdam; [2]Dept. of Gastroenterology, University Hospital Utrecht; [3]Dept. of Pathology, Academic Medical Center, Amsterdam; [4]Dept. of Biostatistics, Erasmus University Rotterdam; [5]Dept. of Internal Medicine, Carolus Hospital, Den Bosch; [6]Dept. of Internal Medicine, Medical Center Twente, Enschede; [7]Dept. of Internal Medicine, St. Franciscus Hospital, Rotterdam; [8]Dept. of Internal Medicine, Rijnstate Hospital, Arnhem; [9]Dept. of Internal Medicine, Twenteborg Hospital, Almelo.

J Hepatol 1998;29:736-42.

Summary

Background: Treatment with ursodeoxycholic acid (UDCA) has been shown to decrease the rate of disease progression in patients with primary biliary cirrhosis (PBC), although the effect is modest. Since PBC has many features of an autoimmune disorder, immunosuppressives added to UDCA may be of value in the treatment of PBC.

Methods: A 1 year randomised, double-blind, placebo-controlled trial was carried out in 50 patients with PBC, who had already been treated with UDCA for at least 1 year, but had not achieved complete disease remission. Patients were randomised to additional prednisone (30 mg/day initially, tapered to 10 mg daily after 8 weeks) and azathioprine (50 mg daily) or placebo. A subgroup of patients received cyclical etidronate and calcium. The principal aim of the study was to assess the short-term benefits and risks of the combined bile acid and low-dose immunosuppressive regimen. Primary endpoints were effects on symptoms, liver biochemistry, liver histology, bone mass and the occurrence of adverse events.

Results: Pruritus (p=0.02), alkaline phosphatase, aspartate aminotransaminase, immunoglobulin M and procollagen-III-propeptide improved significantly (all p<0.002) in the combined treatment group as compared to the placebo group. Histological scores for disease activity and disease stage decreased significantly within the combination treatment group (p<0.001).

Conclusions: In patients with PBC receiving UDCA, there is an additional beneficial effect of 1-year treatment with prednisone and azathioprine on symptoms and biochemical, fibrogenetic and histological parameters. These results strongly encourage the evaluation of this triple treatment regimen in long-term controlled trials of adequate size to document its effect on clinical events.

Introduction

Currently, ursodeoxycholic acid (UDCA) is the standard treatment for primary biliary cirrhosis (PBC) (1, 2). However, the potential of UDCA to improve the natural course of the disease is modest and complete remissions are achieved in less than 5% of patients (3). The combination of UDCA with other drugs has therefore been suggested as a logical next step (4, 5). Theoretically, the addition of immunosuppressive drugs to UDCA is attractive because UDCA, interfering mainly with endogenous bile-acid-mediated liver damage, is unlikely to stop the primary immune damage occurring in PBC (4, 5).

Both prednisone (6-8) and azathioprine (9, 10) have been shown to be of some benefit in PBC. Prednisone has been considered contraindicated due to its negative effect on bone status, which was mainly observed in patients with advanced PBC and jaundice (11). In compensated patients, however, no major adverse effects of low-dose prednisone on bone mass was found (12). Moreover, drugs like bisphosphonates may prevent steroid-associated bone loss (13-15).

Azathioprine has not been accepted as therapy for PBC since the observed small benefit in life expectancy did not neutralise the fear for an increased risk of neoplasms (10). Long-term follow-up of patients with autoimmune diseases treated with prednisone/azathioprine indicates that this fear is unwarranted (16, 17).

As in autoimmune hepatitis, combining low doses of prednisone and azathioprine may lead to an increased immunosuppressive potential without enhancing the risk of side effects. Before starting a long-term study, the potential benefits and risks of combined UDCA, prednisone and azathioprine treatment versus UDCA monotherapy, were investigated in a 1-year, multi-centre, double-blind, placebo-controlled trial in PBC patients who had not completely responded to UDCA alone.

Patients and methods

All patients had an established diagnosis of PBC (18). Exclusion criteria were age > 75 years, extrahepatic bile duct disease, (risk of) pregnancy, the use of other potential disease modifying drugs (e.g. corticosteroids, d-penicillamine, azathioprine, colchicine) within 6 months before entry, known intolerance for prednisone or azathioprine, osteoporotic spinal fractures, systemic infections, a psychiatric history or cytopenia (defined as white bloodcell count (WBC) < 2.5 x 10^9/l, platelets < 70 x 10^9/l or haemoglobin (Hb) < 6 mmol/l). Patients with Child-Pugh classification B or C (19) were excluded since they were considered unlikely to benefit from medical treatment.

All patients had been treated with UDCA (ca. 10 mg/kg/day) for at least one year and none of them had achieved a complete remission of the disease, according to previously formulated criteria (5). After stratification (presence versus absence of cirrhosis and centre) patients were allocated at random to additional prednisone/azathioprine or placebo for 1 year. Both clinicians and patients were unaware of the treatment allocation.

Prednisone and identical-looking placebo tablets were taken in a dose of 30 mg daily for the first month, 20 mg in the second month and 10 mg for the remaining 10 months. One tablet containing 50 mg azathioprine or placebo (Glaxo Wellcome

BV, Zeist, The Netherlands) was taken daily. After one year, prednisone was gradually withdrawn during four weeks and azathioprine was stopped.
All patients received calcium carbonate 500 mg daily. In 2 centres in the Rotterdam area, patients were randomly assigned to treatment with 3-monthly cycles of etidronate 400 mg daily for 2 weeks (Procter & Gamble Pharmaceuticals BV, Rotterdam, The Netherlands) or placebo (20). All other patients received open label etidronate. Patients with subnormal 25-OH vitamin D levels received 600,000 IU cholecalciferol intramuscularly once. Patients visited the outpatient department monthly during the first 3 months and at 3-months intervals thereafter. At each visit a physical examination, including weight and blood pressure measurements and laboratory investigations (Hb, WBC, platelets, serum bilirubin, alkaline phosphatase (APh), aspartate aminotransaminase (AST), albumin and immunoglobulin M (IgM)) were performed. For the 5 days following each visit, patients were asked to grade itching and fatigue on a scale from 0 (absent) to 4 (severe) for the morning, afternoon, evening and night, resulting in a total score ranging from 0-16 per day. Analysis was based on the total score of 5 consecutive days (minimal score 0; maximal score 80).
In the two Rotterdam centres (24 patients), fasting serum aliquots were taken before start of the immuno-suppressive treatment and at 3, 6 and 12 months for radioimmunoassay of procollagen-III aminoterminal propeptide concentrations (P-III-P), using a commercially available kit (Orion Diagnostica, Espoo, Finland).
Prior to the study and after 1 year, liver biopsies and Dual Energy X-ray Absorptiometry (DXA) measurements of Bone Mineral Density (BMD, in grams hydroxyapatite/cm^2) of the lumbar spine (L2-L4) and femur neck were performed. The biopsies were reviewed by one pathologist (FJWtK), who was unaware of the treatment allocation and clinical state of the patients. The biopsies were staged according to Ludwig et al. (21). For a more refined histological grading and staging of the disease (22, 23), piecemeal necrosis, inflammatory bile duct lesions (affection by lymphocytes and degenerative changes of the bile duct epithelium), portal infiltrate, lobular infiltrate, fibrosis and copper accumulation were scored on a qualitative scale (0-3). An overall histological score combined all individual scores.
Power calculations had led to a study size of 62 patients. However, after no more patients could be recruited during a 6-months period, entry was closed after 50 patients had entered. With this study size, differences in changes from baseline of biochemical values can be detected (two-sided alpha: 0.05; power: 80%) for differences of means equalling 0.8 standard deviations. Data are presented as means with standard errors, unless indicated otherwise. Laboratory data are expressed as multiples of the upper limit of normal (ULN) or percentage change from baseline. Changes in laboratory parameters were analysed using Repeated Measurements ANOVA, after logarithmic transformation. Symptoms and histological scores were compared by non-parametric tests (signed-ranks test and rank-sum test). χ^2-tests were used for qualitative data. A p-value ≤ 0.05 was considered significant. All analyses were performed on an intention-to-treat basis. The study was approved by all local Medical Ethical Committees. All patients gave written informed consent.

Results

Entry characteristics in the UDCA/prednisone/azathioprine (U-PA group, n=26) and the UDCA/placebo group (U-PL, n=24) were comparable (table 1).

Symptoms

At entry, mean scores for fatigue and pruritus were higher in the U-PL group although the differences were not significant (table 2). No changes in the severity of fatigue were observed, neither between nor within the 2 groups. In the U-PA group, pruritus decreased although not significantly. A significant difference (p=0.02) between the 2 groups was found when changes in pruritus between entry and 12 months of treatment were compared. All 4 patients with arthralgia in the U-PA group noted improvement during treatment and/or deterioration after stopping treatment, while no change was reported by 8 patients with arthralgia receiving U-PL.

Biochemistry

In the U-PA group serum APh, AST and IgM improved significantly as compared with the U-PL group (all p<0.002). The differences between changes were constant

Table 1: Patient characteristics at entry.

		U-PA (n=26)	U-PL (n=24)
Age	(years)	52.7 (8)	52 (10.8)
Sex	(M/F)	3 / 23	2 / 22
APh	(x ULN)	2.6 (1.6)	2.8 (1.6)
AST	(x ULN)	1.2 (0.6)	1.2 (0.7)
ALT	(x ULN)	1.5 (0.8)	1.7 (1.4)
γ-GT	(x ULN)	4.7 (4)	4.7 (4.7)
Bilirubin	(x ULN)	1 (0.6)	1.1 (0.7)
Albumin	(x ULN)	0.9 (0.1)	0.8 (0.1)
IgM	(x ULN)	1.7 (0.9)	1.9 (1.9)
P-III-P*	g/L (n<4.2)	6.1 (3.5)	4.9 (3.0)
Histological stage			
I / II / III / IV		3 / 7 / 11 / 5	1 / 7 / 9 / 6
Bone mineral density			
L2-L4	g HA/cm^2	1.077 (0.221)	1.028 (0.175)
Femur neck	g HA/cm^2	0.883 (0.128)	0.819 (0.147)

Data are expressed as means with standard deviation in parentheses.
*Measurements performed in 24 patients.

Table 2: Scores for fatigue and pruritus.				
	U-PA		U-PL	
	entry	12 months	entry	12 months
Fatigue	20 (18)	20 (18)	27 (19)	27 (16)
Pruritus	8 (12)	5 (7)	12 (18)	12 (15) *
Means with standard deviations in parentheses. * p=0.02, comparison between groups for change in pruritus score.				

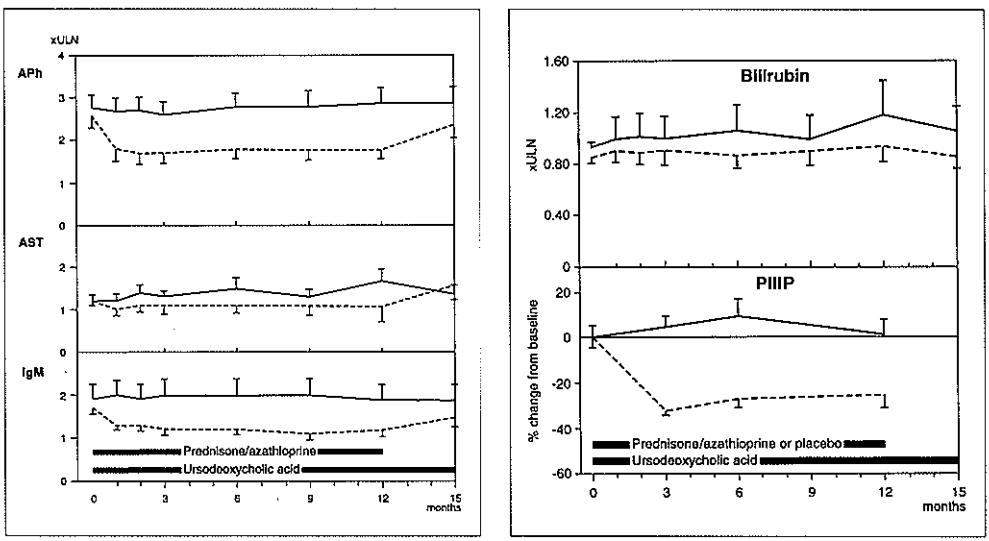


Figure 1. Mean serum alkaline phosphatase (APh), aspartate transaminase (AST) and gM during 1 year of UDCA+prednisone/azathioprine (- - -) versus UDCA+placebo treatment (—) and after 3 months of prednisone/azathioprine withdrawal. Data are expressed as multiples of the upper limit of normal (ULN). Error bars indicate standard errors. Significantly greater decreases occurred in the U-PA group as compared to placebo (all p<0.002; rmANOVA after logarithmic transformation). In the U-PA group values significantly increased and returned to their pre-treatment levels (all p<0.002) at 15 months.

Figure 2. Means of bilirubin (geometric) and P-III-P (measured in 24 patients) during 1 year of UDCA + prednisone/azathioprine (- - -) versus UDCA + placebo treatment (—) and after 3 months of prednisone/azathioprine withdrawal. Data are expressed as multiples of the upper limit of normal (ULN) for bilirubin and as percentage change from baseline for P-III-P. Error bars indicate standard errors. No significant change in bilirubin occurred. A significantly greater decrease of P-III-P occurred in the U-PA group as compared to placebo (p<0.001).

Table 3: Histological grading and staging.

	U-PA			U-PL		
	Entry	End	*p*	Entry	End	*p*
Disease activity						
Piecemeal necrosis (0-3)	1.14 (0.6)	1 (0.5)	0.35	1.3 (0.7)	1.2 (0.7)	0.71
Biliary lesions (0-3)	2.1 (0.8)	1.6 (0.9)	0.11	1.9 (1.1)	1.4 (1)	0.14
Portal infiltrate (0-3)	1.8 (0.6)	1.4 (0.6)	0.01	1.8 (0.6)	1.7 (0.7)	0.54
Lobular infiltrate (0-3)	1.4 (0.5)	0.7 (0.8)	0.006	1.4 (0.7)	1 (0.8)	0.11
Composite score (0-12)	6.4 (1.6)	4.6 (2.1)	0.002	5.8 (1.7)	4.8 (2.4)	0.11
Disease stage						
Copper accumulation (0-3)	1.95 (1.3)	1.64 (1.2)	0.13	2 (1.1)	2 (1.2)	0.78
Fibrosis (0-3)	2.23 (1.2)	2.0 (1.1)	0.22	2.55 (0.9)	2.55 (1)	1
Composite score (0-6)	4.18 (2.2)	3.6 (2.1)	0.05	4.63 (1.9)	4.63 (1.9)	0.86

Data expressed as means with standard deviation between parentheses. Comparison between both groups with respect to change from baseline in the histological scores revealed no significant differences.

during the whole study period (figure 1). Bilirubin (figure 2) and albumin did not change significantly and no differences between the 2 groups were observed. The degree of the improvements did not differ between early (I-II) and late stage (III-IV) disease. These effects were achieved on top of decreases in APh, AST and IgM induced by the preceding UDCA treatment as compared with values at the start of UDCA (-23%, -44% and -23%, respectively; all $p<0.001$).

Three months after cessation of prednisone and azathioprine, APh, AST and IgM significantly increased and returned to pre-treatment values, while bilirubin remained unchanged.

Biochemical remission, defined as sustained normalisation of AST, IgM and bilirubin, and APh ≤ 1.5x ULN), present on at least 2 consecutive follow-up visits, was observed in 3 U-PA patients and 1 U-PL patient. In the U-PA group, more patients with normalisations of AST (3 vs. 1; $p=0.3$), APh (APh ≤ 1.5 x ULN: 7 vs. 1; $p=0.03$) and IgM (11 vs. 0; $p<0.01$) were observed. In none of the patients (8 in each group) elevated bilirubin levels normalised.

After 3 months of treatment, a sustained and significant decrease in P-III-P levels (figure 2) was found in the U-PA group as compared with the U-PL group ($p<0.001$). Abnormal P-III-P levels normalised in 4 of 8 U-PA patients, but in none of 5 U-PL patients.

Table 4: Non-hepatic events

	U-PA	U-PL
Weight gain		
2.5 kg	10	4
> 5 kg	8	1
Cushing face	8	0
Hypertension*	4	1
Ecchymosis	3	3
Hirsutism	2	0
NIDDM	1	2
Cytopenia**	2	2
Infections	2	7
urinary tract	1	2
upper respiratory tract	0	2
sinusitis	0	2
parotitis	0	1
spontaneous bacterial peritonitis	1	0
Gastrointestinal complaints	7	3
Peritonitis (laparotomy)	0	1
Partial portomesenterial thrombosis	1	0
Traumatic vertebral fracture	0	1
Oedema	2	0
Angina pectoris	0	1
Events leading to dose reduction of		
- prednisone	2	0
- azathioprine	1	1
Treatment withdrawal	3	1

* Diastolic pressure increased to ≥ 95 mmHg or by 20 ≥ mmHg on 2 consecutive visits.
** WBC < 2.5×10^9/l and/or platelets < 70×10^9/l.

Histology

Pre- and posttreatment liver biopsies were available in 42 patients (22 U-PA, 20 U-PL). In 1 patient the entry biopsy was insufficient, 5 patients refused a follow-up biopsy and 2 were referred for transplantation. Histological stage according to Ludwig et al. did not change significantly in either group. The overall histological score decreased significantly in the U-PA group by 2.3 ± 2.4 points ($p<0.001$) and remained stable in the U-PL group (-0.9 ± 3; $p=0.21$). Scores for disease activity, disease stage and for lobular and portal infiltrates improved significantly in the U-PA group (table 3). These changes, however, did not differ significantly between the 2 study groups.

Adverse effects

Forty-four patients were included in the evaluation of bone mineral density; in 6 patients paired DXA measurements were not available. Twenty-four patients participated in a randomised controlled trial evaluating the effect of cyclic etidronate and calcium. The main conclusion of this trial, which has been published (20), was that in prednisone-treated patients a small but significant difference in lumbar bone mass was observed after 1 year in favour of patients receiving etidronate/calcium.

Non-hepatic events, observed during the study, are summarised in table 4. Evident weight gain and hypertension were more frequent in the U-PA group. There was no clear difference between the groups in the number of patients showing hepatic deterioration using the criteria of Mitchison (8)(table 5).

Treatment was interrupted in 3 patients treated with U-PA because of persistent general malaise (n=2) and spontaneous bacterial peritonitis (n=1) and in 1 U-PL-treated patient who was referred for transplantation.

Table 5: Incidence of hepatic deterioration.

	U-PA	U-PL
Doubling of bilirubin	1	2
Albumin decrease of > 6 g/l	0	0
De novo signs of portal hypertension (ascites, variceal bleeding)	2	0
New appearance of cirrhosis	1	3
Accepted for liver transplantation	1	2
Hepatic death	0	0
Number of patients fulfilling one or more of these criteria:	3	5

Discussion

This study shows an additional effect of treatment with prednisone and azathioprine on itching, on serum markers of liver cell damage, cholestasis, immune activity and fibrogenesis and on liver histology in PBC patients who had already been treated with UDCA for at least 1 year. Our data indicate that side effects of this triple therapy are limited.

The effects of the combined immuno-suppressive treatment were more pronounced than those reported for azathioprine alone (9, 24). The results of this study are in agreement with previous studies, evaluating prednisone monotherapy and combined prednisone/UDCA therapy in previously untreated patients (7, 8). In contrast to these studies, however, the observed benefits in this study were achieved on top of improvements already obtained with UDCA alone. When the study was initiated the standard UDCA dose in our country was 10/mg/kg/day. Current available data suggest that this may not be the optimal dose. Since patients of both treatment

groups were taking the same UDCA dose, it seems unlikely that this has affected the main results.
The finding that bilirubin, a major prognostic parameter in PBC, remained stable in both groups, is readily explained by considering the entry characteristics of the patients, the slow progression of the disease and the duration of this study. P-III-P, a serum marker of fibrogenesis (25), which has also been reported by several groups to be of prognostic significance in PBC (26-29), normalised in 50% of patients receiving triple therapy. In patients receiving UDCA, P-III-P may be a more appropriate prognostic marker than bilirubin since UDCA may directly increase bilirubin excretion (28), but does not seem to affect P-III-P levels (30). Obviously, larger trials of longer duration are required to establish whether the triple regimen can delay clinical, biochemical and histological disease progression.
The adverse effect of corticosteroids on bone mass has limited their use in PBC, although low-dose prednisone has been shown to be relatively safe in patients with non-advanced disease (8, 12). The maximal bone loss during corticosteroid treatment occurs during the first year of therapy (31). Previously we have reported (20) that treatment with bisphosphonates can prevent this initial bone loss, which is in agreement with studies in other diseases (13-15). Whether this approach will prevent bone loss during long-term corticosteroid treatment remains to be established.
In the triple treatment group weight gain occurred frequently, which may have been related to the relatively high initial prednisone dose. A minority of these patients experienced significant cosmetic changes and increased blood pressures. The immuno-suppressive treatment was not clearly associated with infections or other intercurrent medical problems. As all adverse effects leading to treatment withdrawal occurred during the first weeks, diminishing the high induction doses of prednisone in future studies should be considered. Other measures such as appropriate dietary advice and timely dose adjustments could further decrease the incidence of adverse effects. In this context it should be recognised that from the present study no conclusion can be drawn with respect to the relative contribution of the "induction" prednisone doses to the overall treatment effect. At least in theory, an induction-maintenance regimen could be more effective than regimen without initial higher doses.
Previous studies, combining UDCA with colchicine, have failed to show additional benefit of colchicine (32-34). Additive beneficial effects of methotrexate to UDCA have been reported (35, 36) but serious side effects (37), especially interstitial pneumonitis, have also been noted (38). The combination of UDCA and methotrexate is now the subject of a large controlled trial.
We conclude that there is a synergistic beneficial effect of low-dose prednisone/azathioprine treatment with UDCA in PBC. The short-term benefit/risk ratio appears positive and justifies studies to establish the efficacy of long-term triple therapy on the incidence of major clinical events of the disease.

Acknowledgements
We thank J van Hattum, FM Lalisang, JC Thijs, PHGM Stadhouders, R Zwertbroek, LRKW Lie, JJ. Braun, PL Tjepkema, W Lesterhuis and SDJ van der Werf for their contribution to patient recruitment and follow up.

References

1. Heathcote EJ, Lindor KD, Poupon R, Cauch-Dudek K, Dickson ER, Trout R, et al. Combined analysis of French, American and Canadian randomized trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1995;108:A1082.
2. Lindor KD, Therneau TM, Jorgensen RA, Malinchoc M, Dickson ER. Effects of ursodeoxycholic acid on survival in patients with primary biliary cirrhosis. Gastroenterology 1996;110:1515-8.
3. Wolfhagen FH, van Buuren HR, Schalm SW, Kate FJW ten, Hattum J van, Eskens FA, et al. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis? The Dutch Multi-centre PBC Study Group. J Hepatol 1995;22:381.
4. Kaplan MM. Primary biliary cirrhosis - a first step in prolonging survival. N Engl J Med 1994;330:1386-7.
5. Beukers R, Schalm SW. Immunosuppressive therapy for primary biliary cirrhosis. J Hepatol 1992;14:1-6.
6. Wolfhagen FH, van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.
7. Leuschner M, Guldutuna S, You T, Hubner K, Bhatti S, Leuschner U. Ursodeoxycholic acid and prednisone versus ursodeoxycholic acid and placebo in the treatment of early stages of primary biliary cirrhosis. J Hepatol 1996;25:49-57.
8. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 1992;15:336-44.
9. Heathcote J, Ross A, Sherlock S. A prospective controlled trial of azathioprine in primary biliary cirrhosis. Gastroenterology 1976;70:656-60.
10. Christensen E, Neuberger J, Crowe J, Altman DG, Popper H, Portmann B, et al. Beneficial effect of azathioprine and prediction of prognosis in primary biliary cirrhosis. Final results of an international trial. Gastroenterology 1985;89:1084-91.
11. Long RG, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biochemical, and histological studies of osteomalacia, osteoporosis, and parathyroid function in chronic liver disease. Gut 1978;19:85-90.
12. Van Berkum FN, Beukers R, Birkenhager JC, Kooij PP, Schalm SW, Pols HA. Bone mass in women with primary biliary cirrhosis: the relation with histological stage and use of glucocorticoids. Gastroenterology 1990;99:1134-9.
13. Reid IR, King AR, Alexander CJ, Ibbertson HK. Prevention of steroid-induced osteoporosis with (3-amino-1-hydroxypropylidene)-1,1-bisphosphonate (APD). Lancet 1988;1:143-6.
14. Mulder H, Struys A. Intermittent cyclical etidronate in the prevention of corticosteroid-induced bone loss. Br J Rheumatol 1994;33:348-50.
15. Adachi JD, Bensen WG, Brown J, Hanley D, Hodsman A, Josse R, Kendler DL, et al. Intermittent etidronate therapy to prevent corticosteroid-induced osteoporosis. N Engl J Med 1997;337:382-7.
16. Singh G, Fries JF, Spitz P, Williams CA. Toxic effects of azathioprine in rheumatoid arthritis. A national post-marketing perspective . Arthritis Rheum 1989;32:837-43.
17. Wang KK, Czaja AJ, Beaver SJ, Go VL. Extrahepatic malignancy following long-term immunosuppressive therapy of severe hepatitis B surface antigen-negative chronic active hepatitis. Hepatology 1989;10:39-43.
18. Taal BG, Schalm SW, ten Kate FW, Hermans J, Geertzen RG, Feltkamp BE. Clinical diagnosis of primary biliary cirrhosis: a classification based on major and minor criteria. Hepatogastroenterology 1983;30:178-82.
19. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Br J Surg 1973;60:646.
20. Wolfhagen FHJ, van Buuren HR, den Ouden JW, Hop WCJ, Leeuwen JPTM van, Schalm

SW, et al. Cyclical etidronate in the prevention of bone loss in corticosteroid-treated primary biliary cirrhosis. J Hepatol 1997;26:325-330.
21. Ludwig J, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). Virchows Arch A Pathol Anat Histol 1978;379:103-12.
22. Ishak K, Baptista A, Bianchi L, Callea F, Groote J de, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-9.
23. Attili AF, Rusticali A, Varriale M, Carli L, Repice AM, Callea F. The effect of ursodeoxycholic acid on serum enzymes and liver histology in patients with chronic active hepatitis. A 12-month double-blind, placebo-controlled trial. J Hepatol 1994;20:315-20.
24. Crowe J, Christensen E, Smith M, Cochrane M, Ranek L, Watkinson G, et al. Azathioprine in primary biliary cirrhosis: a preliminary report of an international trial. Gastroenterology 1980;78:1005-10.
25. Frei A, Zimmermann A, Welgand K. The N-terminal propeptide of collagen type III in serum reflects activity and degree of fibrosis in patients with chronic liver disease. Hepatology 1984;4:830-4.
26. Eriksson S, Zettervall O. The N-terminal propeptide of collagen type III in serum as a prognostic indicator in primary biliary cirrhosis. J Hepatol 1986;2:370-8.
27. Babbs C, Smith A, Hunt LP, Rowan BP, Haboubi NY, Warnes TW. Type III procollagen peptide: a marker of disease activity and prognosis in primary biliary cirrhosis. Lancet 1988;1:1021-4.
28. Poupon RE, Balkau B, Guechot J, Heintzmann F. Predictive factors in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: role of serum markers of connective tissue. Hepatology 1994;19:635-40.
29. Mutimer DJ, Bassendine MF, Kelly P, James OF. Is measurement of type III procollagen amino propeptide useful in primary biliary cirrhosis? J Hepatol 1989;9:184-9.
30. Floreani A, Zappala F, Mazzetto M, Naccarato R, Plebani M, Chiaramonte M. Different response to ursodeoxycholic acid (UDCA) in primary biliary cirrhosis according to severity of disease. Dig Dis Sci 1994;39:9-14.
31. Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OF. A pilot, double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-9.
32. Raedsch R, Stiehl A, Walker S, Scherrmann JM, Kommerell B. Kombinierte Ursodeoxycholsaure plus Colchizin Behandlung bei primar biliarer Zirrhose: Ergebnisse einer Placebo-kontrollierten Doppelblindstudie. Z Gastroenterol 1992;1:55-7.
33. Goddard C, Hunt L, Smith A, Fallowfield G, Rowan B, Warnes T. A trial of ursodeoxycholic acid (UDCA) and colchicine in primary biliary cirrhosis (PBC). Hepatology 1994;20:151A.
34. Poupon RE, Huet PM, Poupon R, Bonnand A, Tran Van Nhieu J, Zafrani ES. A randomized trial comparing colchicine and ursodeoxycholic acid to ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1996;24:1098-1103.
35. Buscher HP, Zietzschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
36. Van Steenbergen W, Sciot R, Van Eyken P, Desmet V, Fevery J. Combined treatment with methotrexate and ursodeoxycholic acid in non-cirrhotic primary biliary cirrhosis. Acta Clin Belg 1996;51:8-18.
37. Lindor KD, Dickson ER, Jorgensen RA, Anderson ML, Wiesner RH, Gores GJ, et al. The combination of ursodeoxycholic acid and methotrexate for patients with primary biliary cirrhosis: the results of a pilot study. Hepatology 1995;22:1158-62.
38. Sharma A, Provenzale D, McKusick A, Kaplan MM. Interstitial pneumonitis after low-dose methotrexate therapy in primary biliary cirrhosis. Gastroenterology 1994;107:266-70.

CHAPTER 5

URSODEOXYCHOLIC ACID THERAPY FOR PRIMARY SCLEROSING CHOLANGITIS: RESULTS OF A 2-YEAR RANDOMIZED CONTROLLED TRIAL TO EVALUATE SINGLE VERSUS MULTIPLE DAILY DOSES

Hubert JF van Hoogstraten[1], Frank HJ Wolfhagen[1], Paul C van de Meeberg[2], Hillechien Kuiper[3], Gerard AJJ Nix[1], Marco CJM Becx[4], Aad C Hoek[5], Dennis PF van Houte[6], Marco CM Rijk[7], Jan MJI Salemans[8], Joost Scherpenisse[9], Max Schrijver[10], Adelbert M Smit[11], Pieter Spoelstra[12], Paul HGM Stadhouders[13], T Gie Tan[14], Wim CJ Hop[1], Fiebo JW ten Kate[3], Gerard P van Berge-Henegouwen[2], Solko W Schalm[1], Henk R van Buuren[1].

Erasmus University Hospital Rotterdam[1]; University Hospital Utrecht[2]; Academic Medical Centre, Amsterdam[3]; Ziekenhuis Overvecht[4], Utrecht; van Weel-Bethesda Ziekenhuis[5], Dirksland; Streekziekenhuis Oranjeoord[6], Harlingen; Ziekenhuis de Baronie[7], Breda; University Hospital Nijmegen[8]; Reinier de Graaf Gasthuis[9], Delft; Ziekenhuis Bronovo[10], The Hague; St Carolus Ziekenhuis, Den Bosch[11] Medisch Centrum Leeuwarden[12]; Canisius-Wilhelmina Ziekenhuis[13], Nijmegen; Streekziekenhuis Midden-Twente[14], Hengelo.

J Hepatol 1998;29:417-23

Summary

Background/aims: Ursodeoxycholic acid (UDCA) has been reported to be of potential benefit for primary sclerosing cholangitis (PSC) but little is known about the long-term biochemical, histological and radiological efficacy or the optimum frequency of UDCA administration.

Methods: A 2-year multi-centre randomised controlled trial was initiated to assess the effects of UDCA (10 mg/kg/day), given in either single or multiple daily doses, on symptoms, serum liver test, cholangiographic and histological findings and the occurrence of treatment failure. Liver biopsies were taken and endoscopic retrograde cholangiography (ERC) was performed at entry and after 2 years, follow-up examinations were at 3-months intervals. Treatment failure was defined as death, liver transplantation, 4-fold increase in serum bilirubin, variceal bleeding, *de novo* ascites or cholangitis. Actuarial survival was compared with predicted survival using the revised Mayo natural history model for PSC.

Results: Forty-eight patients were enrolled. In one case, UDCA had to be discontinued because of gastro-intestinal complaints. No other side-effects were observed. After 2 years of follow-up, treatment was not associated with a beneficial effect on either symptoms or liver histology. Serum liver tests (alkaline phosphatase, γ-glutamyl transferase, aspartate aminotransferase) improved significantly in both groups while serum bilirubin (which was near normal at entry) and immunoglobulin G remained stable. No major changes in radiographic bile duct appearance seemed to be present. After 2 years, actuarial survival was 91% (95 confidence interval 83%-99%), which is comparable to the predicted 97% survival rate. Treatment failure occurred in 15% of cases. No significant differences in any of the study endpoints (symptoms, serum liver tests, cholangiographic findings, histology, disease progression) were found between the 2 groups.

Conclusions: UDCA is well tolerated in PSC. Significant effects on biochemical parameters were found and symptoms, bilirubin and histology did not deteriorate. No advantage of a multiple daily dose over a single dose was observed.

Introduction

As a rule, primary sclerosing cholangitis (PSC) is a slowly progressing cholestatic liver disease, potentially leading to cirrhosis and liver failure (1, 2). For patients with advanced disease, liver transplantation is a therapeutic option. Currently, no effective medical treatment is available. The relatively few controlled trials did not reveal a clear benefit for d-penicillamine (3), methotrexate (4) or colchicine (5). After encouraging results were obtained with ursodeoxycholic acid (UDCA) treatment of primary biliary cirrhosis (PBC) (6, 7) several uncontrolled (8, 9) and controlled (10-12) studies were carried out; the findings suggested beneficial effects of this agent on serum liver biochemistry and histology for patients with PSC. Others have suggested that the effect of UDCA on survival in PSC is only marginal (13).
Several studies have indicated that biochemical improvement in patients with cholestatic liver diseases treated with UDCA can be attributed to UDCA enrichment of bile (14-17). Previously our group demonstrated that biliary enrichment in patients with PBC and PSC is independent of administration as a single-dose or divided daily UDCA doses (14). Others, however, have suggested that multiple doses are needed to achieve maximum intestinal uptake and biliary UDCA concentration (18). Since no information is available on the clinical efficacy of single versus multiple daily UDCA administration, we initiated a 2-year randomised controlled trial to compare the effects of a single-dose versus 3 divided daily doses of UDCA (10 mg/kg/day) on symptoms, serum liver test, histology, cholangiographic findings and survival in PSC patients.

Patients and methods

Patients were selected on the basis of a set of major and minor diagnostic criteria. Characteristic findings on endoscopic retrograde cholangiography (ERC) and the presence of typical histological lesions (pericholangiolar "onion-skin" fibrosis) were considered major criteria. Minor criteria were serum alkaline phosphatase (APh) 2 x upper limit of normal (ULN) and the presence of inflammatory bowel disease (IBD). The diagnosis of PSC was considered definitive if at least one major criterion and one other (major or minor) criterion were present. Exclusion criteria were the presence of anti-mitochondrial antibodies, renal failure, malignancy, age < 18 years, pregnancy, advanced or decompensated disease (defined as Child-Pugh class B or C) and evidence of another concomitant liver disease or secondary sclerosing cholangitis. Only patients who had never used UDCA or had discontinued UDCA at least 3 months before entry were included. Patients taking other potentially disease-modifying drugs, e.g. corticosteroids or azathioprine, were allowed to continue this therapy.

Study design

This multi-centre trial, performed in 14 centres in The Netherlands, started in January 1993. The study was approved by the ethics committees of all participating centres. Patient recruitment was stopped after 2 years because further inclusion of patients within the next 6 months seemed unlikely. All patients provided written informed consent. Randomisation was performed at 1 centre by opening consecu-

tively numbered opaque envelopes. UDCA capsules (Ursofalk® 250 mg; Tramedico B.V., Weesp, The Netherlands) were administered either in a single dose at bedtime or in 3 divided doses with meals, at a dose of approximately 10 mg/kg/day.
Patients visited the hospital every 3 months for a general clinical examination and assessment of symptoms, IBD activity and adverse effects. Blood analyses included serum bilirubin, APh, γ-glutamyl transferase (γ-GT), aspartate amino transaminase (AST) and immunoglobulin G (IgG). Pruritus and fatigue were quantified using a self-administered questionnaire. At, and for 5 days after each visit, patients were asked to indicate on a 5-point scale the intensity of pruritus and fatigue in the morning, afternoon and evening and at night separately. The activity of IBD was classified according to the criteria of Truelove and Witts (19, 20).
Liver biopsy and ERC were performed at entry and after 2 years. All liver biopsies were reviewed blind by 2 pathologists (FJWtK and HK). Liver biopsies were staged according to Ludwig et al. (21). Furthermore, a numerical scoring system was applied for fibrosis (0-5) as the main characteristic of disease stage. Grade of the disease was quantified on the basis of bile duct obstruction characterised by copper accumulation (0-3), keratin-7 expression (0-3) (22), portal oedema (0-3) and hepatocellular cholestasis (0-3); bile duct lesions characterised by pericholangiolar fibrosis (0-1), biliary inflammatory affection (0-3), biliary piecing (0-3) and ductular proliferation (0-3); and inflammatory lesions, characterised by portal infiltrate (0-4), piecemeal necrosis (0-4), lobular infiltrate (0-3) and focal necrosis (0-4).
ERC's were reviewed by the trial radiologist (GAJJN) and one of the investigators (HRvB), both of whom were unaware of the dose regimen. Radiological features at 2 years and at entry were compared and scored as deteriorated (increased length or number of stenoses), improved (decreased length or number of stenoses) or stable (no changes).

Statistical analysis
To detect differences of at least 75% of the standard deviation of the main laboratory parameters between the single- and the multiple-dose groups, with a significance of 0.05 and a power of 80%, a sample size of 60 patients was needed. With the numbers achieved, differences of at least 80% of the standard deviation can be detected. All analyses were performed on the intention-to-treat basis. The laboratory parameters were expressed as multiples of the ULN. The course of the laboratory parameters was analysed using repeated measurement analysis of variance (rmANOVA) after logarithmic transformation of the data. Treatment failure was defined as death, liver transplantation, 4-fold increase in serum bilirubin, variceal bleeding, *de novo* ascites and cholangitis. Survival and time to treatment failure were analysed by the Kaplan-Meier method; the results for the 2 groups were compared with the log-rank test. Predicted survival was calculated using the recently revised Mayo natural history model (23) and compared with actuarial survival rates. The Wilcoxon rank-sum test was used to compare data between groups; within-group comparisons were performed with the signed-ranks test. A *p*-value equal to or less than 0.05 was considered statistically significant.

Results

Forty-eight patients were enrolled. In 5 patients (3 single-dose group) the diagnosis was not based on cholangiographic abnormalities but on typical histological lesions, biochemical abnormalities and the presence of IBD (small-duct PSC). At entry, the 2 groups were comparable in terms of clinical, biochemical and histological indices (table 1). The mean age was 38.3 years; 71% of the patients were men and 60% had a history of IBD. IBD was in remission in 17 cases, mild in 6 cases and severe in another 5 cases. Because of missing data for one patient the intensity of IBD could not be classified. The mean daily UDCA dose for both groups was 10.4 mg/kg.

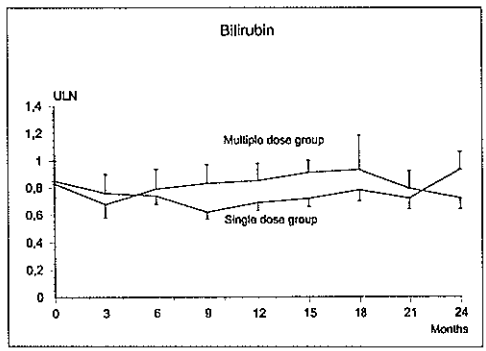
One patient was lost to follow-up (multiple-dose group). Three patients stopped therapy: one (multiple-dose group) because of evidence of autoimmune hepatitis for which prednisone was instituted after 3 months (patient censored at that time)

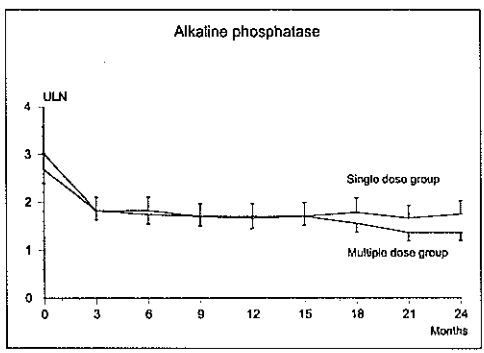
Table 1: Patient characteristics at entry.

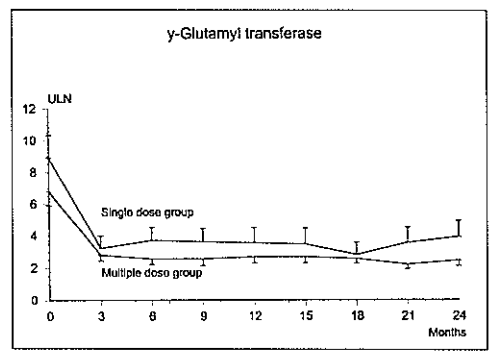
	single dose (n=23)	multiple dose (n=25)
Mean age (years)	36.9 (11.6)	39.5 (13.1)
Sex (M/F)	M 17/ F 6	M 17/ F 8
Inflammatory bowel disease (n)	15	14
Histological stage of disease (n)		
1	4	5
2	3	6
3	13	13
4	3	1
Pruritus (n)	10	10
Fatigue (n)	15	17
Concurrent treatment (n)		
Predniso(lo)ne	2	1
5-aminosalicylic acid	13	10
Loperamide		1
Cholestyramine		1
Bilirubin (ULN)	1.1 (1)	1.0 (0.6)
Alkaline phosphatase (ULN)	3.9 (2.9)	3.1 (1.6)
γ-GT (ULN)	11 (7.3)	8.1 (4.3)
AST (ULN)	1.8 (1.1)	2.2 (1.7)
Albumin (ULN)	0.8 (0.1)	0.8 (0.1)
IgG (ULN)	1.1 (0.3)	1.1 (0.4)

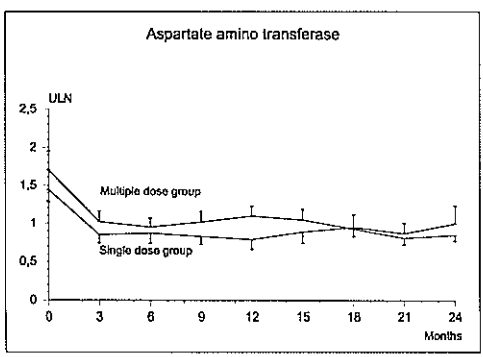
Data are expressed as means with standard deviation in parentheses.

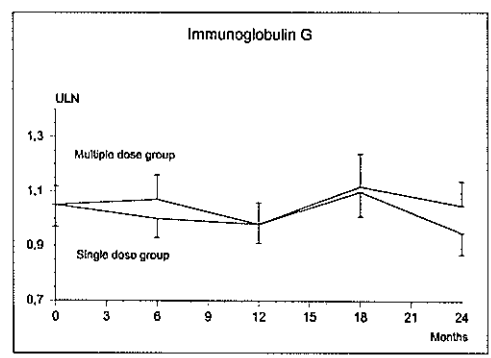
Figure 1: Geometric means of serum bilirubin, alkaline phosphatase (APh), γ-glutamyl transferase (γ-GT), aspartate aminotransaminase (AST) and immuno-globulin G (IgG). Data are expressed as multiples of the upper limit of normal (ULN). After start of treatment a significant decrease in APh, γ-GT and AST was observed. No significant differences were found between the 2 treatment groups.











and 2 because of diarrhoea and nausea (1 from the multiple-dose group). In one of the latter (multiple-dose group), UDCA therapy was gradually reinstated. No other adverse events were reported. In 3 patients in the single-dose group and 2 in the multiple-dose group, corticosteroid therapy was instituted because of increased activity of IBD. In 1 patient corticosteroids could be discontinued and in another case the dose was tapered. In 1 case multiple sclerosis was diagnosed; the patient was treated with corticosteroids. During follow-up, cholestyramine therapy was instituted in 4 patients (1 single-dose group).
Throughout the study the scores for pruritus and fatigue were comparable for both groups. Changes in severity of symptoms within the groups were not observed.

Analysis of biochemical, histological and cholangiographic findings
At entry APh and γ-GT were significantly elevated compared to normal and the mean levels were higher, although the difference was not significant, in the single

Table 2: Histological indices for all patients combined.

	Total group		
	entry	end	*p*
Bile duct obstruction			
Copper accumulation (0-3)	1.3 (0.9)	1.3 (1.1)	0.6
Keratin-7 expression (0-3)	1.3 (0.8)	1.1 (0.9)	0.4
Portal oedema (0-3)	0.7 (0.7)	0.6 (0.7)	0.7
Cholestasis (0-3)	0.6 (0.8)	0.5 (0.8)	0.8
Composite score (0-12)	4 (2.2)	3.6 (2.8)	0.7
Bile duct lesions			
Pericholangiolar fibrosis (0-1)	0.8 (0.4)	0.7 (0.5)	0.4
Affection by inflammation (0-3)	1.1 (0.5)	1 (0.4)	0.6
Biliary piecing (0-3)	1.2 (0.8)	1 (0.8)	0.2
Ductular proliferation (0-3)	1.5 (0.8)	1.6 (0.8)	0.4
Composite score (0-10)	4.6 (2.1)	4.2 (1.9)	0.6
Inflammation			
Portal infiltrate (0-4)	1.1 (0.5)	1.3 (0.5)	0.2
Piecemeal necrosis (0-4)	0.6 (0.8)	0.3 (0.7)	0.07
Focal necrosis (0-4)	1.6 (1.2)	0.9 (0.6)	0.1
Lobular infiltrate (0-3)	0.9 (0.8)	0.5 (0.6)	0.1
Composite score (0-15)	4.2 (2.5)	2.9 (1.8)	0.2

Data are expressed as means with standard deviation in parentheses.

dose group. Serum bilirubin levels were normal or only slightly elevated; 2 patients in the single- and 2 patients in the multiple-dose group had bilirubin levels that exceeded more than twice the ULN. After 3 months of treatment with UDCA, APh had decreased by 31% and 25%, γ-GT by 58% and 57% and AST by 33% and 24% in the single- and multiple-dose groups respectively (all $p0.002$ compared to values at entry; figure 1). Subsequent values remained stable. Serum bilirubin and IgG did not change significantly (figure1). There were no significant differences in response to therapy between the 2 groups.

Paired entry and follow-up liver biopsies were available for 36 patients (19 in the single-dose group). Six patients refused a follow-up biopsy. One patient died before the end of the trial, 1 patient was lost to follow-up, UDCA therapy was discontinued in 1 case and 3 patients were referred for transplantation. Histological stage according to Ludwig (21) did not change significantly in either group. Almost all histological indices improved, although the differences were not significant (table 2). A trend towards improvement, however, was noted for piecemeal necrosis, focal necrosis and lobular infiltrates. No significant differences were found between the 2 study groups.

Paired ERC's were available for 31 patients; 7 patients refused a follow-up ERC (3 single-dose group), in 6 patients (3 single-dose group) the procedure failed or was inadequate for evaluation, 1 patient died and 3 received a liver transplant. Findings at ERC suggested deterioration in 10 patients (4 single dose group), stable conditions in 17 patients (11 single dose group) and improvement in 4 patients (1 single dose group).

One patient with small duct PSC died of post-colectomy complications. Serum bilirubin remained stable in all other patients with small duct PSC. The histological stage increased from stage 2 to 4 in 1 patient with small duct PSC.

Analysis of survival and treatment failure

During the 2 year follow-up, 3 patients (1 single-dose group) received a liver transplant. One patient (single-dose group) died from post-colectomy complications. In 4 patients (1 in the single-dose group) serum bilirubin increased to at least four times the ULN. Two of these patients responded favourably to endoscopic biliary stenting and one to dilatation of dominant bile duct strictures. In 1 case intervention failed and the patient was referred for liver transplantation. One patient suffered repeated bouts of cholangitis, for which he received repeated

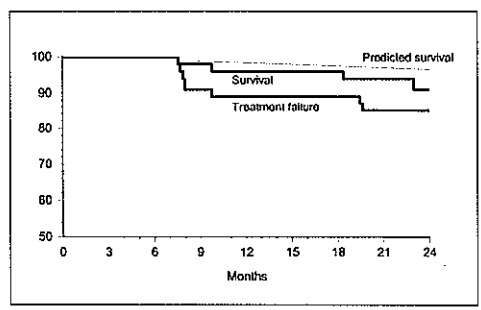


Figure 2: Predicted and actuarial transplantation-free survival and time to treatment failure (actuarial) for all patients. No significant differences were found between predicted and actuarial survival.

courses of antibiotics and endoscopic therapy. Variceal bleeding, ascites and other events indicating progressive disease were not observed. Actuarial transplantation-free survival at 1 and 2 years was 96% (95% confidence interval (95 CI) 100%-90%) and 91% (95 CI 83%-99%), respectively, for both groups. Predicted survival was 98% at 1 year and 97% at 2 years, neither of which differed significantly from observed survival. Survival without treatment failure was 87% for the single-dose group and 83% for the multiple-dose group (not significant) (figure 2).

Discussion

The results of the present study show that the effects of treatment with UDCA on serum liver test and symptoms in PSC patients are comparable when UDCA is administered as a single or as multiple daily doses. Previously our group had found that in UDCA-treated patients with cholestatic liver disease, biliary UDCA enrichment is independent of the dose regimen (14). Therefore, the present results seem to confirm indirectly the hypothesis that, during prolonged UDCA treatment, multiple daily doses do not result in higher levels of UDCA in the bile acid pool than can be achieved with a single daily dose.

This study confirms previous reports that UDCA is well tolerated by PSC patients (8-11, 13).

Like others (8-13), we found prompt improvement in serum liver tests after initiation of UDCA therapy. Serum bilirubin remained stable; the (near) normal levels at entry may explain why we, unlike others (8, 10, 13), were unable to document a decrease in serum bilirubin following UDCA treatment.

Our data suggest that UDCA had no clear effect on pruritus and fatigue. This observation should be interpreted with caution because this was not a placebo-controlled trial.

Although some parameters improved, a consistent beneficial effect on liver histology, as reported by others (10, 11), was not apparent. In the single-dose group liver inflammatory activity decreased significantly, but this was observed neither in the multiple-dose group nor in the 2 groups combined.

A number of qualitative classifications has been proposed for the cholangiographic appearances in PSC (24, 25). A quantitative scoring system has also been developed (26), based on the grade, length and extent of strictures and the degree of dilatation of intrahepatic and extrahepatic bile ducts. In the present study, a serious attempt was made to apply this classification system. However, major difficulties, including the frequent inability to establish presumed 'normal' bile duct diameters and the precise length of strictures or to distinguish dilatation from the pre-existing normal calibre, were encountered. In addition, technical variations such as magnification and bile duct filling contributed significantly to our eventual failure to assess the bile duct abnormalities by means of this quantitative approach, a problem also experienced by others (12 and R. Chapman, personal communication). Therefore, a crude non-quantitative assessment was made of the cholangiographic features and this suggested that there was not a major beneficial effect of UDCA on the anatomical bile duct lesions. Moreover, in view of the potential morbidity and the

invasive nature of ERC, this procedure does not seem to be suitable for documenting the effects of medical therapies in PSC.
Recently, the Mayo group reported the results of a randomised placebo-controlled trial to evaluate the effects of UDCA in 105 patients; the median and maximum follow-up were 2.2 and 6 years, respectively (13). This study showed absence of a favourable effect of prolonged UDCA treatment on symptoms and histology; more importantly the authors were unable to detect an effect on the clinically relevant endpoints. The 2-year 85% transplantation-free survival rate reported in that study is a little lower than the 91% survival rate observed in the present study. However, in contrast to the patients in the Mayo study, the mean serum bilirubin level in our patients was near normal, suggesting that our patients had less advanced disease. Since our study was not placebo-controlled, the effects of UDCA on transplantation-free survival were estimated by comparing observed and predicted survival. No significant difference was found. However, given the slow progressive nature of the disease, the duration of all studies published so far has been fairly short and a true benefit of UDCA, possibly restricted to subgroups of patients, remains a realistic possibility.
Could higher doses than used in the Mayo- and in the present study be more effective? Although, higher doses of UDCA lead to higher biliary enrichment (27), the proportion of UDCA absorbed may decrease with higher doses (18). A recent two-year controlled study (12) found that high-dose UDCA (20 mg/kg/day) treatment was well tolerated and had a pronounced, beneficial effect on liver histology. Further studies are required to confirm this important finding and to assess the long-term clinical efficacy of higher doses.
In conclusion, we found that serum liver tests improved significantly and the histology remained stable in PSC patients on UDCA therapy, irrespective of the treatment regimen. A practical implication is that single-dose administration may be preferable, since patient compliance is likely to be better when the dose regimen is simpler.

Acknowledgements
This research was supported by Tramedico B.V., Weesp, The Netherlands and The Netherlands Digestive Diseases Foundation.

References

1. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med 1995;332:924-33.
2. Broome U, Eriksson LS. Assessment for liver transplantation in patients with primary sclerosing cholangitis. J Hepatol 1994;20:654-9.
3. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. Gastroenterology 1988;95:1036-42.
4. Knox TA, Kaplan MM. A double-blind controlled trial of oral-pulse methotrexate therapy in the treatment of primary sclerosing cholangitis. Gastroenterology 1994;106:494-9.
5. Olsson R, Broome U, Danielsson A, Hagerstrand I, Jarnerot G, Loof L, Prytz H, et al. Colchicine treatment of primary sclerosing cholangitis. Gastroenterology 1995;108:1199-203.
6. Leuschner U, Fischer H, Kurtz W, Guldutuna S, Hubner K, Hellstern A, Gatzen M, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
7. Poupon RE, Balkau B, Eschwege E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med 1991;324:1548-54.
8. O'Brien CB, Senior JR, Arora-Mirchandani R, Batta AK, Salen G. Ursodeoxycholic acid for the treatment of primary sclerosing cholangitis: a 30-month pilot study. Hepatology 1991;14:838-47.
9. Chazouilleres O, Poupon R, Capron JP, Metman EH, Dhumeaux D, Amouretti M, Couzigou P, et al. Ursodeoxycholic acid for primary sclerosing cholangitis. J Hepatol 1990;11:120-3.
10. Beuers U, Spengler U, Kruis W, Aydemir U, Wiebecke B, Heldwein W, Weinzierl M, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatology 1992;16:707-14.
11. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Theilmann 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.
12. Mitchell S, Bansi D, Hunt N, Christie J, Fleming K, Chapman R. High dose ursodeoxycholic acid (UDCA) in primary sclerosing cholangitis(PSC): Results after two years of a randomised double-blind, placebo-controlled trial. Gastroenterology 1997;112 (suppl):A1335.
13. Lindor KD. Ursodiol for primary sclerosing cholangitis. N Engl J Med 1997;336:691-5.
14. van de Meeberg PC, Wolfhagen FHJ, van Berge Henegouwen GP, Salemans JMJI, Tangerman A, van Buuren HR, van Hattum J, et al. Single or multiple dose ursodeoxycholic acid for cholestatic liver disease: relation with biliary enrichment and biochemical response. J Hepatol 1996;25:887-894.
15. Lacerda MA, Lindor KD, Jorgensen RA, Rossi S, Hofmann A, Dickson ER. Ursodiol in primary biliary cirrhosis (PBC): enrichment correlates with biochemical improvement. Hepatology 1993;104:A933.
16. Jorgensen RA, Dickson ER, Hofmann AF, Rossi SS, Lindor KD. Characterisation of patients with a complete biochemical response to ursodeoxycholic acid. Gut 1995;36:935-8.
17. Colombo C, Crosignani A, Assaisso M, Battezzati PM, Podda M, Giunta A, Zimmer-Nechemias L, et al. Ursodeoxycholic acid therapy in cystic fibrosis-associated liver disease: a dose-response study. Hepatology 1992;16:924-30.

18. Walker S, Rudolph G, Raedsch R, Stiehl A. Intestinal absorption of ursodeoxycholic acid in patients with extrahepatic biliary obstruction and bile drainage. Gastroenterology 1992;102:810-5.
19. Truelove SC, Witts LJ. Cortisone in ulcerative colitis. BMJ 1955;2:1041-1048.
20. Hodgson HJ, Mazlam MZ. Review article: assessment of drug therapy in inflammatory bowel disease. Aliment Pharmacol Ther 1991;5:555-84.
21. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1:632-40.
22. Van Eyken P, Desmet VJ. Cytokeratins and the liver. Liver 1993;13:113-22.
23. Kim WR, Therneau TM, Wiesner RH, Malinchoc M, Kaplan MM, Williams R, Lindor KD, et al. A revised natural history model for primary sclerosing cholangitis obviates the need for liver histology. Hepatology 1997;26:362A.
24. Chen LY, Goldberg HI. Sclerosing cholangitis: broad spectrum of radiographic features. Gastrointest Radiol 1984;9:39-47.
25. Majoie CB, Reeders JW, Sanders JB, Huibregtse K, Jansen PL. Primary sclerosing cholangitis: a modified classification of cholangiographic findings. AJR Am J Roentgenol 1991;157:495-7.
26. 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;157:959-64.
27. Podda M, Ghezzi C, Battezzati PM et al. Effect of different doses of ursodeoxycholic acid in chronic liver disease. Dig Dis Sci 1989;34:59S-65S.

CHAPTER 6

THE AUTOIMMUNE HEPATITIS - PRIMARY SCLEROSING CHOLANGITIS OVERLAP SYNDROME: A SERIES OF 8 PATIENTS

Henk R. van Buuren, Hubert JF van Hoogstraten, Türkan Terkivatan,
Solko W Schalm
Dept. of Hepatogastroenterology, Erasmus University Hospital Rotterdam,
The Netherlands

Abstract
Background: Traditionally, autoimmune hepatitis (AIH) and primary sclerosing cholangitis (PSC) are regarded as separate disease entities. We report on a group of patients that suggests the existence of an overlap syndrome of the two conditions.
Methods: Retrospective analysis of patients of a tertiary referral centre for liver disease, who fulfil the diagnostic criteria for consecutively AIH and PSC. The diagnosis of PSC was based on cholangiographic findings. The diagnosis of AIH was based on elevated serum transaminase levels, increased immunoglobulin G levels and the presence of serum auto-antibodies, in combination with liver histology showing interface hepatitis and a predominantly lymphoplasmacytic infiltrate. The diagnosis was tested against a diagnostic scoring system defined by an international consensus group in 1993.
Results: Diagnosis of the overlap syndrome was established for eight patients. Four patients presented with features of AIH and in four cases PSC was diagnosed first. Seven patients also suffered from inflammatory bowel disease. All patients responded to immunosuppressive therapy, in three cases long-term remission was achieved. During prolonged follow-up two patients underwent liver transplantation.
Conclusions: Patients with overlapping features of AIH and PSC may be more common than is currently assumed. Recognition of this syndrome is of clinical significance because the therapeutic consequences are so important. Our findings support the concept that AIH and PSC are phenotypes of an immune-mediated liver disease with varying degrees of biliary and parenchymal involvement.

Introduction

Autoimmune hepatitis (AIH), primary sclerosing cholangitis (PSC) and primary biliary cirrhosis (PBC) are usually regarded as separate disease entities. On the basis of clinical, biochemical, sero-immunological, histological and radiological criteria, a clear distinction between these conditions can readily be made in the majority of cases. However, AIH, PSC and PBC also have many features in common. In particular, autoimmune-mediated necro-inflammatory damage of hepatocytes and bile ducts seems to be of primary pathogenic importance (1-5).

In other autoimmune diseases the occurrence of patients with overlap syndromes has been clearly established (6). In recent years, syndromes with overlapping features of AIH and PBC have also been recognised increasingly (7-11). Furthermore, the simultaneous presence of AIH and PSC has been described, especially in children (12) but also in a number of adults (13-16). Recent review articles, however, (4, 5, 17) do not refer to this syndrome, illustrating that it is still believed to be rare. Our senior authors (HRvB; SWS) have noticed over a 20-year period a change in the phenomenology of AIH, in particular an increase in incidence in young males, often with cholestatic features and regular occurrence together with PSC. To verify this clinical impression, a retrospective study was initiated. We report here our single-centre experience with eight patients with an AIH and PSC overlap syndrome.

Patients and methods

A survey was performed to identify cases of the concomitant diagnoses of AIH and PSC in our centre since 1980. The diagnosis PSC was based on cholangiography showing (multi)focal stricturing of intrahepatic and/or extrahepatic bile ducts with intervening normal or dilated segments, producing a beaded bile duct appearance. Originally, cholangiograms were studied by a senior radiologist with a special interest in endoscopic retrograde cholangiography (ERC); for the purpose of this study all cholangiograms were reviewed by the first author. The diagnosis of AIH was based on the finding of at least three-fold elevations in serum transaminase activities, elevated concentrations of IgG or γ-globulins and positive tests for antinuclear antibodies (ANA), smooth muscle antibodies (SMA) or liver-kidney microsomal antibodies in combination with liver histology showing moderate or severe chronic active hepatitis with interface hepatitis and a predominantly lymphoplasmacytic infiltrate. Patients were assumed not to have AIH if there was evidence for viral hepatitis or some other concomitant liver disease, except for PSC, and if antimitochondrial antibodies (AMA) were present. PSC was not diagnosed if bile duct abnormalities could possibly be attributed to such causes as previous bile-duct surgery, ischaemia or portal vein thrombosis. Furthermore, a numerical scoring system for the diagnosis AIH, proposed by an international group of experts during a consensus meeting in 1993 (18), was used to test the diagnosis retrospectively. In accordance with others, who applied this diagnostic system to patients with PSC (19), scores were obtained before therapy and IBD was not considered an autoimmune disorder. Furthermore, despite the fact that "biliary lesions" are to be expected in patients with PSC, the score was decreased by one point in cases with his-

tological features such as cholangiolitis, pericholangiolar ("onionskin") fibrosis and bile duct proliferation.
Data relevant for this study were extracted from patient files and data-banks of the radiological, pathological, biochemical and immunological departments. All patients were followed until December 1997 or until death.
Determination of serum bilirubin, aspartate aminotransaminase (AST), alanine aminotransaminase (ALT), alkaline phosphatase (APh), immunoglobulin G (IgG) and γ-globulins was performed by means of standard automated procedures. Immunofluorescence techniques were used to detect auto-antibodies. Perinuclear antineutrophile cytoplasmic antibodies (pANCA) were detected on granulocytes, ANA on Hep2 cells, AMA on mouse and rat kidney cells and SMA on rat stomach cells. Markers for hepatitis A, B and C were determined by standard commercially available tests.

Case reports

Case one:

In 1977 this man underwent cholecystectomy at the age of 50. At that time he was jaundiced. In 1982, at the age of 55, he was admitted elsewhere with jaundice. Endoscopic retrograde cholangiography (ERC) revealed multiple intrahepatic biliary stenoses and two strictures in the common bile duct; PSC was diagnosed. Common bile duct stenoses were considered to be the most likely cause of the jaundice and side-to-side hepatico-jejunostomy was carried out. Postoperative T-drain cholangiography confirmed the presence of two common bile duct strictures and several intrahepatic strictures (figure 1). Histological examination of a biopsy specimen of the common bile duct was consistent with sclerosing cholangitis. Five months after the surgical procedure he was readmitted for persistent jaundice. Clinical evidence of inflammatory bowel disease (IBD) was not present. Serum bilirubin (117 µmol/l, normal < 16), transaminases (AST 432 U/l, ALT 337 U/l; normal < 30) and IgG (53.1 g/l, normal < 16 g/l) were elevated and SMA were present. HLA typing revealed the presence of the B8-DR3 haplotype. A liver biopsy showed extensive fibrosis, portal and lobular lymphocytic and plasma cellular infiltrates and periportal piecemeal necrosis (figure 2). AIH in association with PSC was diagnosed and the patient received prednisone/azathioprine combination therapy. Full clinical remission, but incomplete bio-

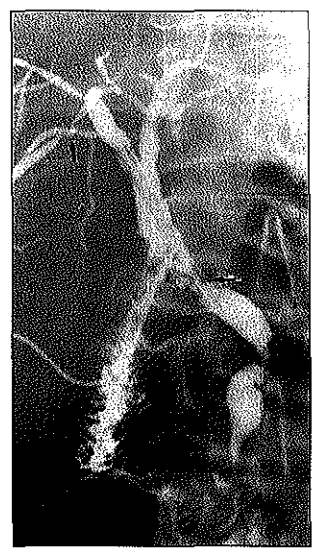


Figure 1: T-tube cholangiography (case 1) showing intrahepatic and extrahepatic bile duct strictures. The wall of the common bile duct is irregular and small diverticula are present near the strictures (lower two arrows).

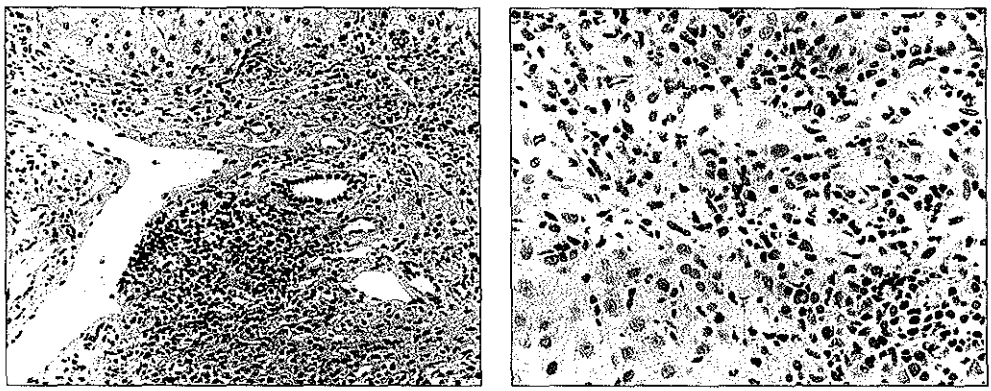


Figure 2: Liver biopsy (case 1).
A shows an enlarged portal triad with a predominantly lymphoplasmacytic infiltrate with an ill-defined limiting plate and (top) inflammatory cells infiltrating into the adjacent parenchyma. Original magnification x 20
B Portal lymphoplasmacytic infiltrate expanding into the liver lobulus with piecemeal necrosis. Original magnification x 40.

chemical response (normal bilirubin and IgG, transaminases ± 1.5 x upper limit of normal) followed. In subsequent years, despite maintenance treatment with prednisone and azathioprine, episodic hepatitis flares occurred and a predominantly cholestatic biochemical profile emerged. In 1990 ursodeoxycholic acid (UDCA) was added to the therapeutic regimen. Gradual disease progression necessitated liver transplantation in 1992. Colonoscopy before this procedure revealed abnormalities indicative of long-standing, inactive IBD. At present the patient is doing well.

Case two:
This male patient was diagnosed with AIH at the age of 7 in 1985. Serum bilirubin was 52 µmol/l, AST 364 U/l, ALT 115 U/l and IgG 29 g/l. ANA (titre > 1:640) and low titres of SMA were detected. A liver biopsy showed chronic active hepatitis with features of AIH (figure 3). Treatment consisted of 5 mg prednisone/day; complete biochemical remission was achieved after one year. In 1987 he was reassessed because of rectal blood loss. Colonoscopy and biopsy specimens showed left-sided ulcerative colitis which was treated with salazopyrine. Subse-

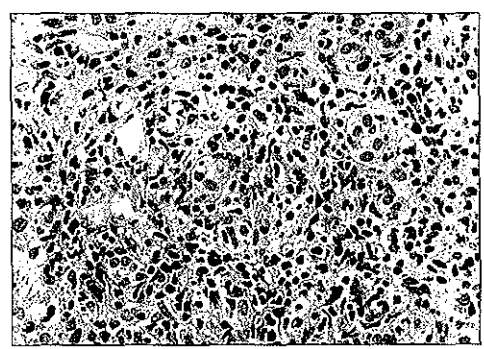


Figure 3: Liver biopsy (case 2)
Portal area with moderately dense lymphoplasmacytic infiltrate and periportal piecemeal necrosis. Original magnification x 40.

quently, while on maintenance treatment with prednisone, liver biochemical tests showed mild fluctuations. In 1993 azathioprine was initiated because the transaminase levels were increased 4-5 fold. In 1996 he was referred to our department. Serum bilirubin (41 µmol/l) and APh (630 U/l, n < 75) were increased. Intrahepatic bile duct abnormalities characteristic of PSC were found at ERC (figure 4). The liver biopsy showed marked fibrosis, bile duct proliferation and minimal inflammatory activity. UDCA therapy was started three months later.

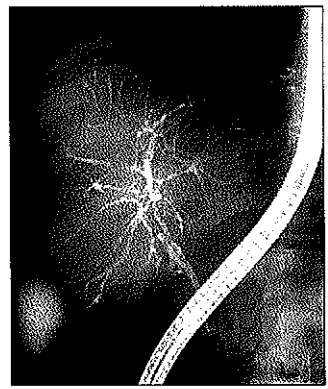


Figure 4: Endoscopic retrograde cholangiogram (case 2) showing multiple areas of intrahepatic strictures and ductal dilatation.

Results

In the period 1980-1997 eight patients who fulfilled the diagnostic criteria for both AIH and PSC were identified. The age at presentation with either one of these conditions varied from 7 to 54 years (mean 24.7). An overlap syndrome was diagnosed after intervals varying from 6 months to 11.5 years (mean 6.3 years). The duration of follow-up ranged from 1 to 16.8 years. Seven patients had concomitant IBD; in the one remaining case endoscopic investigations were not performed.

Four patients (2 male, 2 female), mean age 16 years (range 7-22), were initially diagnosed with AIH (table 1). In all cases the serum transaminase levels were elevated at least ten-fold (mean 18 times the upper limit of normal, IgG or γ-globulin concentrations were raised, SMA and/or ANA were detected and the liver biopsy showed interface hepatitis with a lymphoplasmacytic infiltrate. Marked biochemical improvement was observed after treatment with prednisone and azathioprine (table 2). Mainly because a predominantly cholestatic biochemical profile emerged during follow-up, ERC was performed at intervals after the initial diagnosis, varying from 5.9 to 11.5 years (mean 8.9 years).

Four patients, all men with a mean age of 33.5 years (range 20-54), were initially diagnosed with PSC. Liver histology showed pericholangiolar fibrosis in one case; pANCA were detected in 2 cases and 3 suffered from concomitant IBD. AIH was diagnosed after a mean interval of 3 years (range: 0.5-5.8).

Of the patients in whom PSC was diagnosed first, one patient (number 3) exhibited the classical features of AIH: transaminases elevated ten-fold, IgG 49 g/l, high titres of SMA and histologically severe chronic active hepatitis. PSC had been diagnosed elsewhere six years earlier; in the course of time he exhibited persistently elevated transaminases and underwent an episode of severe hepatitis, which resolved spontaneously. In retrospect, the diagnosis of AIH could have been established six years earlier. Another patient (number 6) developed liver failure with variceal bleeding and ascites when corticosteroids were withdrawn following colec-

Table 1: Patient characteristics at presentation and laboratory findings at the time of diagnosis of AIH.

patient	sex	age	IBD	initial diagnosis	diagnostic interval PSC-AIH (years)	AIH score	histology	IgG (g/l)	γ-globulin (g/l)	SMA	ANA
1	M	7	UC	AIH	11	16	ifh,f	29.4		1:10	>1:640
2	M	14	UD	AIH	11.5	17	ifh,f		49.2	1:40	1:80
3	M	20	UC	PSC	5.6	17	pcf, ifh	49.4		1:320	negative
4	F	21	UC	AIH	5.9	17	ifh,f		40	negative	1:2560*
5	F	22	CD	AIH	7.3	18	ifh		30	1:320*	1:80*
6	M	23	UC	PSC	2.9	15	ifh,c	34.8		negative	1:180
7	M	37	no	PSC	5.8	15	ifh,f	20.9		negative	1:2560
8	M	54	UD	PSC	0.5	17	ifh,f	53.1		1:80	1:40

IBD: inflammatory bowel disease; UC: ulcerative colitis; CD Crohn's disease; UD undetermined colitis.
AIH score: score according to international autoimmune hepatitis group before immunosuppressive treatment (18). A score > 15 indicates "definite" AIH and a score of 10 to 15 indicates "probable" AIH.
ifh: interface hepatitis; pcf: pericholangiolar fibrosis; f:fibrosis; c:cirrhosis
* data 3-4 years after diagnosis AIH

Table 2: Serum liver tests and IgG/γ-globulins during the first year of immunosuppressive treatment.

Patient	bilirubin (µmol/l)			AST (U/l)			APh (U/l)			IgG/γ-glob (g/l)		
	start	1 month	1 year	start	1 month	1 year	start	1 month	1 year	start	1 month	1 year
1	52	10	9	364	38	72	458	146	397	29.4		
2	22	19	18	470	30	172	338	223	458	49.2*		
3	459	148	29	410	81	45	166	151	95	49.4	36	13.9
4	123	n.a.	10	918	149	12	35	33	17	40*		18*
5	46	11	15	348	82	97	135	80	97	34*		
6	307	450	36	750	332	112	87	100	122	34.8	19	21
7	54	27	11	303	23	14	117	55	120	23.2	12.8	9.9
8	117	20	9	432	99	106	119	90	82	53.1	21.6	28.4

reference values: APh < 75 U/l; AST < 30 U/l; bilirubin < 12 µmol/l; IgG < 16 g/l; γ-globulins 9-18 g/l
* γ-globulins

tomy for ulcerative colitis. Renewed immunosuppressive treatment resulted in complete clinical and biochemical remission. Another patient (number 7) developed AIH when the corticosteroids, which he had received for 8 weeks in the context of a controlled clinical trial for PSC, were tapered off. The fourth patient (number 8; case 1) remained jaundiced after hepatico-jejunostomy for common bile duct strictures. During immunosuppressive treatment bilirubin, transaminases, APh and immunoglobulins normalised or decreased significantly (table 2).
Five patients, four with an initial diagnosis of PSC, received UDCA during the course of their disease at various intervals after the date of diagnosis. This treatment appeared to result in slight improvements in the serum liver tests in some cases.
ERC revealed bile duct changes characteristic of PSC in all cases. Mild abnormalities of both intrahepatic and extrahepatic ducts were found in 3 patients while only the intrahepatic ducts were affected in 2 patients. In 2 patients marked intrahepatic bile duct deformities were found, one was associated with intrahepatic stones while the extrahepatic ducts appeared to be normal. Dominant strictures were observed in one patient (no. 8, case 1).
Despite an initially favourable therapeutic response to immunosuppressive therapy, one patient initially diagnosed with AIH (no. 5) and one with an initial diagnosis of PSC (no. 8) had slowly progressive disease and received a liver transplant after 7 and 9 years, respectively. The remaining five patients are alive and are still being followed. In three cases long-term biochemical remission was achieved with continued immunosuppressive treatment.

Discussion

In this report 8 patients with an overlap syndrome of AIH and PSC are described. In view of the number of cases reported in the literature (table 3) this represents a relatively large group. It is noteworthy that four more patients have been identified with AIH who had marked cholestatic liver function test abnormalities, negative test results for AMA, and histologically chronic active hepatitis with ductular changes, pericholangiolar fibrosis and copper deposits. A diagnosis of PSC, however, could not be made because cholangiography was not performed.
Specific referral patterns and increased diagnostic awareness in recent years may have contributed to the number of cases reported here. The prevalence of the overlap syndrome among patients with AIH remains to be established because none of the studies reported, including the present one, included cholangiography in all cases. Among children with AIH a prevalence of 27% was reported, but also in this study cholangiography was not performed uniformly (20). The prevalence of AIH among patients with PSC was assessed by Boberg et al. (19), who applied the 1993 AIH scoring system to a group of 114 PSC patients. Two (2%) patients were classified as "definite" AIH and 38 (33%) as "probable" AIH.
Establishment of the diagnosis of AIH is difficult since there is no single diagnostic test or validated set of criteria. This applies specifically for patients with a concomitant liver disease. The diagnostic aspects of AIH were addressed by an international forum of experts during a consensus meeting in 1993. Their efforts resulted in a diagnostic system, which, however, still may not be ideal and should be further

Table 3: Case series of the PSC-AIH overlap syndrome.

author	year	n	IBD	treatment	therapeutic response
Minuk (13)	1988	2	UC	prednisone	poor
Rabinovitz (14)	1992	1	UC	prednisone/azathioprine	good
Perdigoto (22)	1992	5	UC	prednisone/azathioprine	treatment failure (n=4); transplantation (n=2)
Lawrence (15)	1994	1	UC	cyclosporine	good
Gohlke (16)	1996	3	UC (n=1)	prednisolone/azathioprine/UDCA	remission (n=1)
Boberg (19)	1996	2	UC	corticosteroids (n=1)	death (n=1); transplantation (n=1)
Luketic (23)	1997	5	UC (n=2)	corticosteroids/azathioprine	liver transplantation (n=4)

improved (21). In our centre we rely mostly on the positive criteria of more than 3-10 fold elevations of serum transaminases, two-fold elevation of IgG or γ-globulins, presence of auto-antibodies and compatible liver histology. Negative factors such as alcohol use, positive viral serology, hepatotoxic medication and biliary lesions on biopsy are clearly considered, but we do not attribute a decisive significance to these factors. The report of the 1993 meeting (18) states that patients with biliary changes identified either histologically or by cholangiography should not be assumed to have AIH. This point of view should be modified in the light of cumulating evidence that true overlap syndromes do occur (21). With these reservations in mind, our patients were evaluated according to the 1993 system. The scoring system was applied conservatively and resulted in scores indicating "definite" AIH for 6 patients and "probable" AIH for 2. Both of the latter patients exhibited a complete response to therapy. The observed response to immunosuppressive therapy in the other cases further strengthens our belief that all of our patients had AIH.

When the clinical features of patients in the present series are compared with those of previously reported adult patients (table 3) with AIH-PSC overlap, several similarities are found: percentage males is 75% versus 63% in the literature, mean age at presentation 25 versus 27 years and prevalence of IBD 88% versus 70%, respectively. With respect to other laboratory, histological and radiological characteristics, these patients seem to be very similar. The presently available cumulative experience indicates that the AIH-PSC overlap syndrome is mainly, but not exclusively, a disorder encountered in children and young adults, with a predominance of males. In the majority of cases the overlap syndrome is associated with ulcerative colitis or Crohn's disease.

The therapeutic response to immunosuppressives can be excellent. Our experience as well as that of others (14-16) indicates that proper therapy can lead to complete remission of disease activity and indeed may be life-saving. However, our experience seems to be in accordance with that of earlier reports (12, 13, 22, 23) suggesting that the thera-

peutic benefit for patients with an overlap syndrome may be less than for those with "genuine" AIH. In fact, two of our patients needed a liver transplant 7-9 years after the start of immunosuppressive therapy. This finding is in contrast to current results for genuine AIH (24).

The cause of AIH and PSC is unknown. Most findings seem to indicate that these disorders may develop in patients with a genetic susceptibility. Human leukocyte antigen (HLA) studies have shown that both disorders are closely associated with B8, DR3-positive haplotypes (19, 25) and with the DRB3 allele DRB3*0101, which encodes for DR52a (26, 27). The results of detailed HLA typing have not been reported for patients with the overlap syndrome and this therefore requires further study. The many similarities between the clinical, histological, genetic and immunological features of AIH and PSC (19, 28) and the existence of an overlap syndrome may suggest that these conditions represent phenotypic expressions of a common underlying process with aberrant autoreactivity. The role of IBD within this concept is even more speculative. IBD is associated with both PSC (4, 5, 29) and AIH (22) and the majority of overlap patients had IBD. In theory, IBD could be another phenotypic manifestation of a common underlying disease process. On the other hand IBD could be a condition that facilitates the development of AIH and PSC.

In conclusion, we report on eight patients with an overlap syndrome of AIH and PSC, which brings the total number of reported cases to 27. Although the true prevalence remains to be established, our experience suggests that this entity may be less rare than currently assumed. Clinicians should be well aware of this syndrome since recognition of these cases can have major therapeutic implications.

Acknowledgement

We gratefully acknowledge the help of Pieter E Zondervan and Monica Seijbel of the Department of Pathology of the University Hospital Rotterdam.

References

1. Kaw M, Silverman WB, Rabinovitz M, Schade RR. Biliary tract calculi in primary sclerosing cholangitis. Am J Gastroenterol 1995;90:72-5.
2. Lo SK, Fleming KA, Chapman RW. A 2-year follow-up study of anti-neutrophil antibody in primary sclerosing cholangitis: relationship to clinical activity, liver biochemistry and ursodeoxycholic acid treatment. J Hepatol 1994;21:974-8.
3. Davis PA, Leung P, Manns M, Kaplan M, Munoz SJ, Gorin FA, Dickson ER, et al. M4 and M9 antibodies in the overlap syndrome of primary biliary cirrhosis and chronic active hepatitis: epitopes or epiphenomena? Hepatology 1992;16:1128-36.
4. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med 1995;332:924-33.
5. Harnois DM, Lindor KD. Primary sclerosing cholangitis: evolving concepts in diagnosis and treatment. Dig Dis 1997;15:23-41.
6. Bernhard GC. Mixed connective tissue disease and other overlap syndromes. In: Katz WA, ed. Diagnosis and management of rheumatic diseases. 2nd ed. Philadelphia: J.B. Lippincot Company, 1988:530-4.
7. Michieletti P, Wanless IR, Katz A, Scheuer PJ, Yeaman SJ, Bassendine MF, Palmer JM, et al. Antimitochondrial antibody negative primary biliary cirrhosis: a distinct syndrome of autoimmune cholangitis. Gut 1994;35:260-5.
8. Ben-Ari Z, Dhillon AP, Sherlock S. Autoimmune cholangiopathy: part of the spectrum of autoimmune chronic active hepatitis. Hepatology 1993;18:10-5.
9. Colombato LA, Alvarez F, Cote J, Huet PM. Autoimmune cholangiopathy: the result of consecutive primary biliary cirrhosis and autoimmune hepatitis? Gastroenterology 1994;107:1839-43.
10. Flora K, Benner K, Lee R, Fennimore S. Primary autoimmune cholangitis as an early variant of primary biliary cirrhosis. Hepatology 1994;20:152A.
11. Chazouilleres O, Montembault S, Poupon R. Primary biliary cirrhosis (PBC)-autoimmune hepatitis (AH) overlap syndrome: Clinical features and response to therapy. Hepatology 1996;24:167A.
12. el-Shabrawi M, Wilkinson ML, Portmann B, Mieli-Vergani G, Chong SK, Williams R, Mowat AP. Primary sclerosing cholangitis in childhood. Gastroenterology 1987;92:1226-35.
13. Minuk GY, Sutherland LR, Pappas SC, Kelly JK, Martin SE. Autoimmune chronic active hepatitis (lupoid hepatitis) and primary sclerosing cholangitis in two young adult females. Can J Gastroenterol 1988;2:22-27.
14. Rabinovitz M, Demetris AJ, Bou-Abboud CF, Van Thiel DH. Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis. Dig Dis Sci 1992;37:1606-11.
15. Lawrence SP, Sherman KE, Lawson JM, Goodman ZD. A 39 year old man with chronic hepatitis. Semin Liver Dis 1994;14:97-105.
16. Gohlke F, Lohse AW, Dienes HP, Lohr H, Marker-Hermann E, Gerken G, Meyer zum Buschenfelde K. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. J Hepatol 1996;24:699-705.
17. Mitchell SA, Chapman RW. Review article: the management of primary sclerosing cholangitis. Aliment Pharm Therap 1997;11:33-43.
18. Johnson PJ, McFarlane IG. Meeting report: International Autoimmune Hepatitis Group. Hepatology 1993;18:998-1005.
19. Boberg KM, Fausa O, Haaland T, Holter E, Mellbye OJ, Spurkland A, Schrumpf E. Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of

114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. Hepatology 1996;23:1369-76.
20. Gregorio GV, Portmann B, Reid F, Donaldson PT, Doherty DG, McCartney M, Mowat AP, et al. Autoimmune hepatitis in childhood: a 20-year experience. Hepatology 1997;25:541-7.
21. Mackay IR. Toward diagnostic criteria for autoimmune hepatitis. Hepatology 1993;18:1006 8.
22. Perdigoto R, Carpenter HA, Czaja AJ. Frequency and significance of chronic ulcerative colitis in severe corticosteroid-treated autoimmune hepatitis. J Hepatol 1992;14:325-31.
23. Luketic VA, Gomez DA, Sanyal AJ, Shiffman ML. An atypical presentation for primary sclerosing cholangitis. Dig Dis Sci 1997;42:2009-16.
24. Schvarcz R, Glaumann H, Weiland O. Survival and histological resolution of fibrosis in patients with autoimmune chronic hepatitis. J Hepatol 1993;18:15-23.
25. Donaldson PT, Doherty DG, Hayllar KM, McFarlane IG, Johnson PJ, Williams R. Susceptibility to autoimmune chronic active hepatitis: human leukocyte antigens DR4 and A1-B8-DR3 are independent risk factors. Hepatology 1991;13:701-6.
26. Doherty DG, Donaldson PT, Underhill JA, Farrant JM, Duthie A, Mieli-Vergani G, McFarlane IG, et al. Allelic sequence variation in the HLA class II genes and proteins in patients with autoimmune hepatitis. Hepatology 1994;19:609-615.
27. Boberg KM, Schrumpf E, Fausa O, Leivestad T, Vartdal F, Spurkland A. Increased frequency of HLA-DRB3*0101(DR52a) associated DQA1,DQB1 haplotypes in primary sclerosing cholangitis (PSC). J Hepatol 1994;21(suppl):S71.
28. Wiesner RH, LaRusso NF. Clinicopathologic features of the syndrome of primary sclerosing cholangitis. Gastroenterology 1980;79:200-6.
29. Mitchell SA, Chapman RW. Review article: the management of primary sclerosing cholangitis. Aliment Pharm Therap 1997;11:33-43.

CHAPTER 7

BUDESONIDE OR PREDNISONE IN COMBINATION WITH URSODEOXYCHOLIC ACID IN PRIMARY SCLEROSING CHOLANGITIS: A RANDOMIZED DOUBLE-BLIND PILOT STUDY

Hubert JF van Hoogstraten[1], Frank P Vleggaar[1], Greet J Boland[2], Werner van Steenbergen[3], Pieter Griffioen[4], Wim CJ Hop[5], Jan van Hattum[2], Gerard P van Berge-Henegouwen[2], Solko W Schalm[1] and Henk R van Buuren[1], representing the Belgian-Dutch PSC study group.

Dept. of Gastroenterology and Hepatology, University Hospitals [1]Rotterdam, [2]Utrecht, [3]Leuven, The Netherlands & Belgium; [4]Dept. of Clinical Chemistry, [5]Dept. of Epidemiology and Biostatistics, University Hospital Rotterdam, The Netherlands.

Submitted Am J Gastroenterol

Summary
Introduction: PSC has characteristics of an (auto)immune-mediated disease; however, few studies have evaluated corticosteroid therapy for this disorder.
Methods: We performed an 8-week double-blind randomised pilot study to assess the effects of additional treatment with 9 mg budesonide (n=6) versus 3 mg budesonide (n=6) versus 10 mg prednisone (n=6) in patients who had been treated with UDCA (mean dose 12 mg/kg/day) for at least five months without achieving biochemical remission. Pruritus and fatigue were evaluated using visual analogue scales. Serum liver biochemistry was measured every four weeks. At entry and at the end of the trial, adrenocorticotrophic hormone (ACTH) and dehydroepiandrosterone (DHEA) were measured to assess effects on the pituitary-adrenal axis. Duodenal bile was collected for assessment of biliary corticosteroid activity.
Results: Pruritus decreased significantly more in the prednisone group compared to both the 3 mg and the 9 mg budesonide groups ($p<0.05$). Alkaline phosphatase (mean: -23.4%; $p=0.03$) and immunoglobulin G (mean: -16.2%; $p=0.04$) decreased in the prednisone group, while bilirubin, γ-glutamyl transferase, aspartate aminotransaminase and alanine aminotransaminase did not change significantly. No significant clinical and liver biochemical changes were observed in the 3 mg and 9 mg budesonide groups. Significantly larger drops in serum ACTH were found in the 10 mg prednisone group (-40.7%; $p=0.04$) and 9 mg budesonide group (-36.6%; $p=0.02$) compared to the 3 mg budesonide group (+19.0%). No significant differences in percentage change in baseline values for DHEA between the three treatment arms were found. Mononuclear cell proliferation assays did not demonstrate corticosteroid activity in bile. Autoimmune hepatitis was observed in one case (9 mg budesonide) when corticosteroids were tapered off.
Conclusion: The results of this pilot study suggest only minor beneficial short-term effects of prednisone but not budesonide on symptoms and serum liver tests in UDCA-treated PSC patients.

Introduction

The aetiology of primary sclerosing cholangitis (PSC) is unclear. An (auto)immune background is suggested (1,2) by the strong association between PSC and inflammatory bowel disease (IBD) (3,4), the presence of perinuclear antineutrophil cytoplasmic antibobodies (pANCA) (5-8) and an increased prevalence of HLA B8 DR3 DR52a genotypes (9-11). Furthermore, an overlap syndrome between autoimmune hepatitis (AIH) and PSC has been described (12-14).

Few studies have evaluated the effects of corticosteroid treatment in PSC. Prednisone therapy has only been assessed in open-label studies (15,16). The use of corticosteroids in cholestatic liver disease has been discouraged because of potential side-effects, in particular osteoporosis (17). Systemic side-effects of corticosteroids may be avoided by replacing prednisone by budesonide, which is a potent corticosteroid with a high first-pass effect (18), resulting in low systemic availability and potentially fewer side-effects (19).

Improvements in serum liver function tests and histology have been observed following ursodeoxycholic acid (UDCA) monotherapy in PSC (20-22). Although effects on disease progression were absent after 2 years of treatment (23), the drug is still being used extensively in PSC. In view of these facts, a double-blind randomised pilot study was initiated to evaluate the efficacy and safety of budesonide and prednisone in addition to UDCA maintenance therapy for PSC patients.

Patients and methods

A total of 104 PSC patients was screened for eligibility; a flow diagram according to the CONSORT statement (24) is given in figure 1. Reasons for not participating are listed in table 1. Eighteen patients were recruited. The diagnosis of PSC was based on characteristic findings on endoscopic retrograde cholangiography (ERC) (25) in 17 cases. Typical histological lesions (pericholangiolar "onion-skin" fibrosis) (26) in combination with both a serum alkaline phosphatase (APh) level elevated to more than twice the upper limit of normal (ULN) and the presence of IBD led to the diagnosis of PSC in one patient. Prior to the study, all patients had been treated with UDCA (mean dose 12 mg/kg body-weight/day) for at least five months, without achieving biochemical remission, defined as normalisation of APh, aspartate aminotransaminase

Table 1: Reasons for not randomising a patient.

patient refused	13
use of immunosuppressive medication	13
biochemical remission	33
no UDCA treatment	8
previous cholecystectomy	4
biliary stent	2
Child-Pugh score > 6	3
presence of malignancy/other severe disease	6
PSC associated AIH	1
age > 65 years	3

AIH: autoimmune hepatitis.

(AST) and alanine aminotransaminase (ALT). Exclusion criteria were: age > 65 years, use of immunosuppressive drugs such as corticosteroids, azathioprine, cyclosporine or methotrexate, pregnancy, evidence of PSC-associated AIH, previous cholecystectomy, presence of a biliary stent and cirrhosis with a Child-Pugh score > 6 (27).

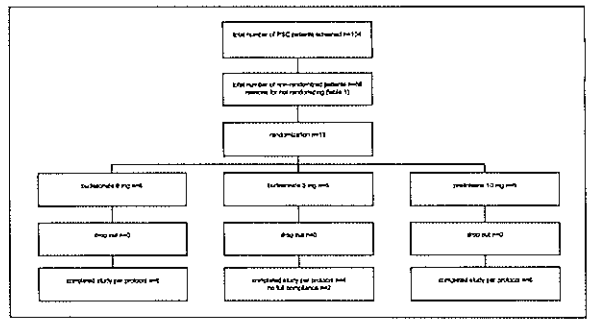


Figure 1: Study profile.

Patients were randomised by assigning consecutive treatment numbers, which corresponded to the trial medication. Patients were randomised to take 10 mg/day prednisone (two 5 mg tablets of prednisone and three placebo capsules Budenofalk®), 3 mg/day budesonide (one 3 mg Budenofalk® capsule, two placebo capsules and two placebo tablets of 5 mg prednisone) or 9 mg/day budesonide (three 3 mg Budenofalk® capsules and two placebo tablets of 5 mg prednisone) for eight weeks. Verum and placebo medication was of identical appearance. Treatment with UDCA was continued. Three blocks of six patients were generated. In each block, the three treatment options were equally and randomly distributed. One block of six treatment numbers was allocated to each participating centre: University Hospitals Rotterdam, Utrecht and Leuven. The randomisation list was generated by Allphamed, Goettingen, Germany; the medication was packed accordingly. Follow-up data were collected every 4 weeks. Blood analysis including bilirubin, APh, γ-glutamyl transferase (γ-GT), AST, ALT, immunoglobulin G (IgG), immunoglobulin M (IgM), albumin and prothrombin time was performed by standard automated procedures. Separation of APh isoenzymes was performed by agarose gel electrophoresis (Titan Gel®, Helena Laboratories, Gateshead, United Kingdom). Quantitation of APh isoenzyme fractions was performed by computerised scanning techniques (Sharp, JX330P). At baseline and after eight weeks, blood was drawn to assess adrenocorticotrophic hormone (ACTH) and dehydroepiandrosterone (DHEA) after 30 minutes of bedrest, at 09.00 a.m., to determine effects on the pituitary-adrenal axis. The severity of pruritus and fatigue was quantified with visual analogue scales (VAS), starting one week before administration of the study medication. Patients were asked to indicate daily the severity of pruritus and fatigue on a 10 cm scale during the complete study period. After the 8-week study period all patients received treatment with 5 mg prednisone per day; subsequently, prednisone was tapered off in three weeks.

Poor compliance was defined as taking less than 90% of the study medication. The study was approved by the Medical Ethics Committees of each of the three participating hospitals and all patients gave written informed consent. All assessments, except the quantitation of APh isoenzymes, were performed before the randomisation code was broken.

Collection of bile and determination of biliary corticosteroid activity
Bile was collected using duodenal intubation under fluoroscopic control at entry and on the last day of treatment. Gall-bladder contraction was induced by intravenous injection of 2 g of ceruletide (Takus®, Pharmacia & Upjohn GmbH, Erlangen, Germany). A maximum dose of 6 g ceruletide was given in steps of 2 g. Duodenal bile was collected in ice chilled tubes and stored at -20°C until further analysis.
Mononuclear cells (MNCs) were isolated by Ficoll density centrifugation of heparinized blood from one healthy individual. 10^5 MNCs were incubated in RPMI culture medium supplemented with 10% AB-serum in the presence of pre-treatment and post-treatment bile in a final dilution of 1:1000 (optimal dilution determined in previous experiments). A mixture of diphtheria/tetanus toxoid was used for antigenic stimulation of MNC proliferation. To obtain reference values, prednisolone was added to the cultures in final dilutions of 10^{-4}, 10^{-6} and 10^{-8} mol/l in the presence of bile collected at entry. Cultures without diphtheria/tetanus toxoid were used for determination of background MNC proliferation. MNCs were incubated at 37°C in 5% CO_2-atmosphere for five days; ^{3}H-thymidine was added 24 hours before harvesting. ^{3}H-thymidine incorporation was measured with a liquid scintillation counter and MNC proliferation was expressed as counts per minute (cpm). Phytohaemagglutinin was used to check the proliferative potential of the MNCs. Every culture was performed in triplicate.

Statistical analysis
Since no data on the effects of budesonide and prednisone on liver biochemistry in PSC patients receiving UDCA were available and because this was a pilot study, a statistical power analysis was not performed. A group of six patients per treatment arm was considered sufficient for this pilot study to get an impression of the efficacy of the drugs. Data were analysed according to the intention-to-treat principle. Mean daily VAS scores per week for pruritus and fatigue were calculated by dividing the total score for one week by seven. Serum liver tests are expressed as multiples of the upper limit of normal (ULN). The Kruskal-Wallis test was used to assess overall differences between the three treatment arms. Paired t-tests were applied after verifying that differences between pre-treatment and post-treatment values exhibited an approximately normal distribution; otherwise, Wilcoxon's signed-rank test was used. A p-value ≤ 0.05 was considered statistically significant.

Results
At entry, the three groups were largely comparable in terms of clinical and biochemical characteristics (table 2). The mean age was 43 years (range 26-65); 14 patients were male and 11 (61%) had a history of IBD. No significant differences in biochemical and clinical values at entry between the three groups existed. Compliance was good; two patients used 88% of the study medication, all others used 100% as indicated by pill count.

Pruritus and fatigue
The number of patients reporting pruritus at entry and after eight weeks was four

Table 2: Features at entry.

	Prednisone 10 mg n = 6	Budesonide 9 mg n = 6	Budesonide 3 mg n = 6
Age (years)	44.5 (10.9)	46.4 (9.7)	38.9 (12.3)
Sex (male/female)	M 6 / F 0	M 4 / F 2	M 4 / F 2
UDCA dose (mg/kg)	11.8 (2.2)	10.4 (2.2)	14.6 (6.8)
IBD (n)	3 UC / 1 CD	2 UC / 1 CD	4 UC / 0 CD
Treatment with			
-5-ASA (n)	4	3	3
- Rifampicin (n)	0	1	0
Bilirubin (ULN)	1.1 (0.3)	0.9 (0.4)	0.8 (0.3)
APh (ULN)	2.4 (0.9)	1.8 (1.4)	2.8 (1.4)
γ-GT (ULN)	6.5 (5.0)	6.4 (7.9)	7.8 (6.6)
AST (ULN)	1.2 (0.4)	1.2 (1.0)	1.3 (0.4)
ALT (ULN)	2.0 (0.9)	1.7 (2.2)	1.9 (0.6)
Albumin (ULN)	1.0 (0.3)	1.0 (0.3)	1.0 (0.2)
IgG (ULN)	1.2 (0.7)	0.9 (0.3)	1.0 (0.3)

Means with standard deviation in parentheses.
IBD: inflammatory bowel disease; UC: ulcerative colitis; CD: Crohn's disease; 5-ASA: 5-amino salicylic acid.

Table 3: Effects of prednisone and budesonide on the pituitary-adrenal axis.

	Entry	End	Δ	p^*
ACTH (pg/l)				
Prednisone 10 mg	48.0 (14.6)	27.5 (21.9)	-40.7 (23.0)	0.130
Budesonide 9 mg	35.8 (7.3)	21.7 (9.1)	-36.6 (13.4)	0.064
Budesonide 3 mg	38.1 (13.4)	44.0 (14.5)	+19.0 (13.6)	0.219
DHEA (μmol/l)				
Prednisone 10 mg	5.4 (2.8)	2.6 (1.0)	-39.2 (15.9)	0.081
Budesonide 9 mg	5.8 (3.3)	3.7 (3.1)	-40.9 (10.4)	0.052
Budesonide 3 mg	3.7 (1.5)	3.5 (1.4)	-7.1 (8.4)	0.359

Means with standard deviation in parentheses.
Δ: percentage change from baseline with standard error of the mean in parentheses.
* Within group comparison

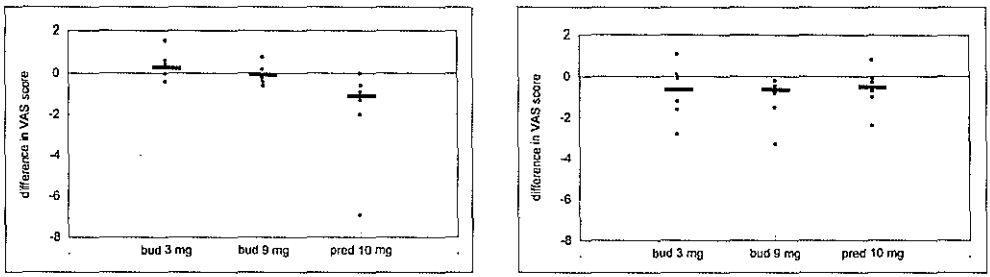


Figure 2: The difference between pre-treatment and 8-week VAS pruritus score in 3 mg budesonide (bud 3 mg), 9 mg budesonide (bud 9 mg) and 10 mg prednisone (pred 10 mg) groups. Horizontal bars indicate medians.

Figure 3: The difference between pre-treatment and 8-week VAS fatigue score in 3 mg budesonide (bud 3 mg), 9 mg budesonide (bud 9 mg) and 10 mg prednisone (pred 10 mg) groups. Horizontal bars indicate medians.

and three in the 10 mg prednisone group, two and none in the 3 mg budesonide group and one and two in the 9 mg budesonide group, respectively. The median difference between baseline and 8 weeks score was +0.15 points for the 3 mg budesonide group, -0.1 for the 9 mg budesonide group and -1.1 for the 10 mg prednisone group. The median decrease from baseline in the 10 mg prednisone group was significantly greater compared to the other groups (both $p<0.05$) (figure 2).

Eleven patients complained of fatigue at entry: three in the 10 mg prednisone group, three in the 3 mg budesonide group and five in the 9 mg budesonide group. After eight weeks, eight patients complained of fatigue: two in the 10 mg prednisone group, three in the 3 mg budesonide group and three in the 9 mg budesonide group. The median change from baseline of the fatigue score was similar in all groups (all 0.6) (figure 3).

Serum liver tests

In the 10 mg prednisone group, the mean changes from baseline in APh (-23.4%, $p=0.03$; 95%-confidence interval (95 CI): -42.7% to -4.2%) (figure 4) and IgG (-16.2%, $p=0.04$; 95% CI: -24.8% to -7.5%) were significantly less than zero after eight weeks of treatment. The liver and bone APh isoenzyme fractions decreased by a mean of 27.6% (95% CI: -54.5% to -0.8%; $p=0.04$)

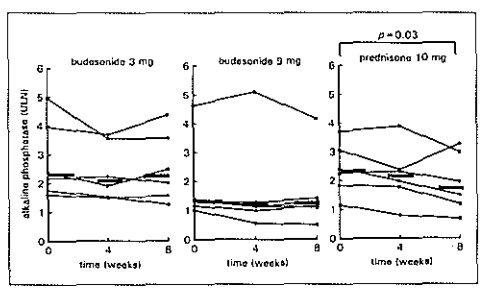


Figure 4: Course of serum alkaline phosphatase in the 10 mg prednisone, 9 mg budesonide and 3 mg budesonide groups. Data are expressed as multiples of the upper limit of normal (ULN). Horizontal bars indicate medians.

and 10.0% (95% CI: -38.1% to +18.0%; p=0.9), respectively. No significant changes in serum liver tests were observed in the 3 mg budesonide and 9 mg budesonide groups. The mean changes in APh and IgG in the 10 mg prednisone group, however, did not differ significantly from the changes in the 3 mg and 9 mg budesonide groups. Serum bilirubin (figure 5), AST, ALT, γ-GT and IgM did not change significantly in the 10 mg prednisone group. Albumin and prothrombin time remained stable in all three groups.

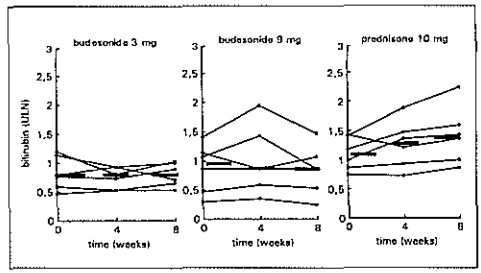


Figure 5: Course of serum bilirubin in the 10 mg prednisone, 9 mg budesonide and 3 mg budesonide groups. Data are expressed as multiples of the upper limit of normal (ULN). Horizontal bars indicate medians.

Pituitary-adrenal axis

Significantly greater decreases in serum ACTH were found in the 10 mg prednisone group (-40.7%; p=0.04) and 9 mg budesonide group (-36.6%; p=0.02) compared to the 3 mg budesonide group (+19.0%)(table 3). No significant differences in percentage change in baseline values for DHEA between the three treatment arms were found.

Adverse events

A 42-years old male patient was diagnosed with PSC in 1991. At regular follow-up examinations transaminases were mildly elevated and IgG was normal. He was allocated to the 9 mg budesonide group; he had also taken rifampicin for pruritus in the preceding 6 months. When prednisone was tapered off, marked hepatitis developed: serum bilirubin 105 μmol/l (normal < 17 μmol/l); ALT 595 IU/l (normal < 30); IgG 23.2 g/l (normal < 16). Antinuclear antibodies (titre 1:2560), anti-DNA (> 50 IE/ml), anti SS-A and anti SS-B antibodies tested positive. Serological markers for hepatitis A, B and C were negative. A liver biopsy revealed dense portal mononuclear infiltrates with interface hepatitis, numerous Councilman bodies and collapse of liver parenchyma. Combination treatment with prednisone (30 mg/day) and azathioprine (50 mg/day) was instituted, resulting in rapid normalisation of serum bilirubin, ALT and IgG. An overlap syndrome of PSC and AIH was diagnosed.

One patient, who received 10 mg prednisone per day, reported slightly impaired vision and itching of the eyes. In the 9 mg budesonide group, one patient reported increased facial acne and another patient complained of nausea. These side-effects were found to be transient. No other adverse events were noted.

Biliary corticosteroid activity

Duodenal bile collection was successful in 15 cases; it failed in two patients of the 9 mg budesonide group and one patient of the 3 mg budesonide group. Mean cpm of diphtheria/tetanus toxoid-stimulated MNCs incubated with bile collected at entry

with and without *in vitro* addition of 10^{-8} mol/l prednisolone was 11504.0 (sd 6235.1) and 15614.6 (sd 6188.1), respectively (p=0.01). Thus, it was possible to measure an inhibitory effect comparable to that of prednisolone at a concentration of 10^{-8} mol/l, corresponding to a concentration of 10^{-5} mol/l in undiluted bile. Mean cpm of diphtheria/tetanus toxoid-stimulated MNCs incubated with bile collected at entry and at the end of the study was 15614.6 (sd 6188.1) and 15073.9 (sd 8586.3), respectively (p=0.36). In none of the groups could an inhibitory effect of post-treatment bile compared with pre-treatment bile on MNC proliferation be demonstrated.

Discussion

This study indicates that in PSC patients receiving treatment with ursodeoxycholic acid additional therapy with 10 mg prednisone per day or 3 or 9 mg budesonide per day does not result in major short-term improvements in clinical and laboratory parameters of the disease, although treatment with 10 mg prednisone per day was associated with a significant decrease in pruritus as assessed by visual analogue scores.

Currently, no effective medical treatment has been found for PSC. This study was based on the hypothesis that both immunologically mediated inflammatory activity (1) as well as bile acid-related liver damage contribute to the gradually progressive liver disease that characterises PSC. Consequently, a therapeutic approach based on combined treatment with ursodeoxycholic acid and low-dose immunosuppressives was considered of interest. The results of this 8-week study do not allow conclusions as to the possible long-term effects of such a treatment regimen but they do seem to indicate an absence of major short-term benefits. In this context, it should be noted that the patients included in this study had relatively mild liver disease, as indicated by the biochemical test results at entry, which may a priori have limited the chance to observe significant changes in liver biochemical parameters upon treatment with immunosuppressives.

Interpretation of the effect of corticosteroids on pruritus is markedly hampered by the considerable variation in both the number of symptomatic patients and the severity of pruritus among the treatment groups. Moreover, this study was not placebo-controlled. Therefore, the observed beneficial effect of prednisone should be interpreted with caution and would require confirmation in further studies. Interestingly, a positive effect of corticosteroids on pruritus has also been reported for another cholestatic liver disease, primary biliary cirrhosis (28,29). This effect could be related to the reported ability of corticosteroids to induce cytochrome P450-3A activity (30), which is analogous to the action of the well-known antipruritic drug rifampicin.

Budesonide is a potent corticosteroid with high receptor affinity. It is rapidly metabolised in the liver into almost inactive metabolites (31). In theory, a high concentration of active drug could be delivered to the biliary tract, whereas systemic concentrations remain low. On the other hand, budesonide might be inactivated before exerting its immunosuppressive effect. The first hypothesis is not supported by the results of our experiments with duodenal bile, showing absence of increased

immunosuppressive action in bile of patients treated with budesonide. This study demonstrates that suppression of the pituitary-adrenal axis by 10 mg prednisone per day and 9 mg budesonide per day was comparable while 3 mg budesonide per day had no such effect. Although the systemic availability of budesonide is reported to be low (31,32), we, in accordance with others (19,33), found that in patients treated with 9 mg budesonide as well as 10 mg prednisone the pituitary-adrenal axis was significantly suppressed.

In general, patients tolerated the low-dose corticosteroid regimen well. One exceptional serious adverse event was observed in a patient treated with 9 mg budesonide per day who developed severe, corticosteroid-responsive hepatitis when corticosteroids were tapered off. We are not aware of any similar cases. Although the aetiopathogenesis of this event remains speculative, available data suggest that in this patient manipulation of the immune system triggered the development of an autoimmune-mediated liver disease.

Relatively few studies have evaluated the effects of immunosuppressive drugs in PSC and the findings are inconsistent. No evidence was found for beneficial effects of D-penicillamine (34), cyclosporine (35), methotrexate (36,37) or combination therapy with colchicine and prednisone (16). Conversely, serum liver tests appeared to respond favourably to treatment with tacrolimus (38) whereas some benefit of treatment with prednisone monotherapy has been reported for a series of ten patients (15).

In conclusion, in this pilot study only minor short-term beneficial effects of treatment with prednisone but not budesonide were noted in PSC. The apparent benefit-risk ratio of immunomodulating agents, as assessed in this and previous studies, does not therefore support long-term evaluation of these agents in PSC.

Acknowledgement

We gratefully acknowledge the ongoing support of MCJM Becx, Ziekenhuis Overvecht, Utrecht; J Scherpenisse, Reinier de Graaf Gasthuis, Delft; JJ Uil, Ziekenhuis de Gelderse Vallei, Wageningen; MCM Rijk, Ziekenhuis de Baronie, Breda; PC van de Meeberg, Slingeland Ziekenhuis, Doetinchem; DJ Bac, Ikazia Ziekenhuis, Rotterdam; and G Ghillebert, H. Hartziekenhuis, Roeselare; HJL Dieteren, Tramedico B.V., Weesp; HD Tauschel, Dr. Falk Pharma GmbH, Freiburg, Germany.

References

1. Lee YM, Kaplan MM. Primary sclerosing cholangitis. N Engl J Med 1995;332:924-33.
2. Harnois DM, Lindor KD. Primary sclerosing cholangitis: evolving concepts in diagnosis and treatment. Digest Dis Sci 1997;15:23-41.
3. Wiesner RH, Grambsch PM, Dickson ER, Ludwig J, MacCarty RL, Hunter EB, Fleming TR, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989;10:430-6.
4. Broome U, Olsson R, Loof L, Bodemar G, Hultcrantz R, Danielsson A, Prytz H, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38:610-5.
5. Chapman RW. Role of immune factors in the pathogenesis of primary sclerosing cholangitis. Semin Liver Dis 1991;11:1-4.
6. Mulder AH, Horst G, Haagsma EB, Limburg PC, Kleibeuker JH, Kallenberg CG. Prevalence and characterization of neutrophil cytoplasmic antibodies in autoimmune liver diseases. Hepatology 1993;17:411-7.
7. Seibold F, Slametschka D, Gregor M, Weber P. Neutrophil autoantibodies: a genetic marker in primary sclerosing cholangitis and ulcerative colitis. Gastroenterology 1994;107:532-6.
8. Bansi DS, Bauducci M, Bergqvist A, Boberg K, Broome U, Chapman R, Fleming K, et al. Detection of antineutrophil cytoplasmic antibodies in primary sclerosing cholangitis: a comparison of the alkaline phosphatase and immunofluorescent techniques. Eur J Gastroen Hepat 1997;9:575-80.
9. Chapman RW, Varghese Z, Gaul R, Kokkinon N, Sherlock S. Association of primary sclerosing cholangitis with HLA-B8. Gut 1983;24:38-41.
10. Prochazka EJ, Terasaki PI, Park MS, Goldstein LI, Busuttil RW. Association of primary sclerosing cholangitis with HLA-DRw52a. N Engl J Med 1990;322:1842-4.
11. Mehal WZ, Lo YM, Wordsworth BP, Neuberger JM, Hubscher SC, Fleming KA, Chapman RW. HLA DR4 is a marker for rapid disease progression in primary sclerosing cholangitis. Gastroenterology 1994;106:160-7.
12. Gohlke F, Lohse AW, Dienes HP, Lohr H, Marker-Hermann E, Gerken G, Meyer zum Buschenfelde K. Evidence for an overlap syndrome of autoimmune hepatitis and primary sclerosing cholangitis. J Hepatol 1996;24:699-705.
13. Rabinovitz M, Demetris AJ, Bou-Abboud CF, Van Thiel DH. Simultaneous occurrence of primary sclerosing cholangitis and autoimmune chronic active hepatitis in a patient with ulcerative colitis. Digest Dis Sci 1992;37:1606-11.
14. Boberg KM, Fausa O, Haaland T, Holter E, Mellbye OJ, Spurkland A, Schrumpf E. Features of autoimmune hepatitis in primary sclerosing cholangitis: an evaluation of 114 primary sclerosing cholangitis patients according to a scoring system for the diagnosis of autoimmune hepatitis. Hepatology 1996;23:1369-76.
15. Burgert SL, Brown BP, Kirkpatrick RB, LaBrecque DR. Positive corticosteroid response in early sclerosing cholangitis. Gastroenterology 1984;86:1037.
16. Lindor KD, Wiesner RH, Colwell LJ, Steiner B, Beaver S, LaRusso NF. The combination of prednisone and colchicine in patients with primary sclerosing cholangitis. Am J Gastroenterol 1991;86:57-61.
17. Long RG, Meinhard E, Skinner RK, Varghese Z, Wills MR, Sherlock S. Clinical, biochemical, and histological studies of osteomalacia, osteoporosis, and parathyroid function in chronic liver disease. Gut 1978;19:85-90.
18. Ryrfeldt A, Andersson P, Edsbacker S, Tonnesson M, Davies D, Pauwels R. Pharmacokinetics and metabolism of budesonide, a selective glucocorticoid. Eur Respir J Suppl 1982;122:86-95.
19. Rutgeerts P, Lofberg R, Malchow H, Lamers C, Olaison G, Jewell D, Danielsson A, et al. A comparison of budesonide with prednisolone for active Crohn's disease. N Engl J Med

1994;331:842-5.
20. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Theilmann 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.
21. Beuers U, Spengler U, Kruis W, Aydemir U, Wiebecke B, Heldwein W, Weinzierl M, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatology 1992;16:707-14.
22. Hoogstraten HJF van, Wolfhagen FHJ, Meeberg PC van de, Kuiper H, Nix GAJJ, Becx MCJM, Hoek AC, et al. Ursodeoxycholic acid therapy for primary sclerosing cholangitis: results of a 2-year randomized controlled trial to evaluate single versus multiple daily doses. J Hepatol 1998;29:417-23.
23. Lindor KD. Ursodiol for primary sclerosing cholangitis. N Engl J Med 1997;336:691-5.
24. Begg C, Cho M, Eastwood S, Horton R, Moher D, Olkin I, Pitkin R, et al. Improving the quality of reporting of randomized controlled trials. The CONSORT statement. JAMA 1996;276:637-9.
25. MacCarty RL, LaRusso NF, Wiesner RH, Ludwig J. Primary Sclerosing Cholangitis: findings on cholangiography and pancreatography. Radiology 1983;149:39-44.
26. Ludwig J, Barham SS, LaRusso NF, Elveback LR, Wiesner RH, McCall JT. Morphologic features of chronic hepatitis associated with primary sclerosing cholangitis and chronic ulcerative colitis. Hepatology 1981;1:632-40.
27. Pugh RNH, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. Brit J Surg 1973;60:646-9.
28. Mitchison HC, Bassendine MF, Malcolm AJ, Watson AJ, Record CO, James OF. A pilot, double-blind, controlled 1-year trial of prednisolone treatment in primary biliary cirrhosis: hepatic improvement but greater bone loss. Hepatology 1989;10:420-9.
29. Wolfhagen FHJ, Hoogstraten HJF van, Buuren HR van, Berge Henegouwen GP van, Kate FJW ten, Hop WCJ, Hoek EW van der, et al. Triple therapy with ursodeoxycholic acid, prednisone and azathioprine in primary biliary cirrhosis: a 1-year randomized, placebo-controlled study. J Hepatol 1998;29:736-42.
30. Pichard L, Fabre I, Daujat M, Domergue J, Joyeux H, Maurel P. Effect of corticosteroids on the expression of cytochromes P450 and on cyclosporin A oxidase activity in primary cultures of human hepatocytes. Mol Pharmacol 1992;41:1047-55.
31. Brattsand R. Overview of newer glucocorticosteroid preparations for inflammatory bowel disease. Can J Gastroenterol 1990;4:407-14.
32. Ryrfeldt A, Andersson P, Edsbaecker S, Toennesson M, Davies D, Pauwels R. Pharmacokinetic and metabolism of budesonide, a selective glucocorticoid. Eur Resp J 1993;63(Suppl 122):86-95.
33. Greenberg GR, Feagan BG, Martin F, Sutherland LR, Thomson AB, Williams CN, Nilsson LG, et al. Oral budesonide for active Crohn's disease. Canadian Inflammatory Bowel Disease Study Group. N Engl J Med 1994;331:836-41.
34. LaRusso NF, Wiesner RH, Ludwig J, MacCarty RL, Beaver SJ, Zinsmeister AR. Prospective trial of penicillamine in primary sclerosing cholangitis. Gastroenterology 1988;95:1036-42.
35. Wiesner RH, Steiner B, LaRusso NF, Lindor KD, Baldus WP. A controlled trial evaluating cyclosporine in the treatment of primary sclerosing cholangitis. Hepatology 1991;14:63A.
36. Knox TA, Kaplan MM. A double-blind controlled trial of oral-pulse methotrexate therapy in the treatment of primary sclerosing cholangitis. Gastroenterology 1994;106:494-9.
37. Lindor KD, Jorgensen RA, Anderson ML, Gores GJ, Hofmann AF, LaRusso NF. Ursodeoxycholic acid and methotrexate for primary sclerosing cholangitis: a pilot study. Am J Gastroenterol 1996;91:511-5.
38. Thiel DH van, Carroll P, Abuelmagd K, Rodriguezrilo H, Irish W, Mcmichael J, Starzl TE. Tacrolimus (FK 506), a treatment for primary sclerosing cholangitis: Results of an open-label preliminary trial. Am J Gastroenterol 1995;90:455-9.

CHAPTER 8

MULTI-DRUG TREATMENT FOR PRIMARY BILIARY CIRRHOSIS AND PRIMARY SCLEROSING CHOLANGITIS: IS MORE BETTER?

Hubert JF van Hoogstraten

Parts of this chapter have been published in:
H.J.F. van Hoogstraten, G. P. vanBerge-Henegouwen, H.R. van Buuren. Multi-drug treatment for Primary Biliary Cirrhosis, is more better ? In: Paumgartner G, Stiehl A, Gerok W, editors. Proceedings of the XIV International Bile Acid Meeting (Falk symposium No. 93). London: Kluwer Academic Publishers; 1997:291-8

Introduction

After the initial report by Poupon et al (1), Leuschner and co-workers published the results of the first randomised controlled trial with ursodeoxycholic acid (UDCA) in primary biliary cirrhosis (PBC) in 1989 (2). Since then, numerous studies (2-12) (table 1) have confirmed their findings, showing beneficial effects of UDCA on liver-biochemistry, especially on serological markers of cholestasis. However, no significant improvements in histology are observed. In 1997, a meta-analysis combining data of the French, Canadian and American studies showed that UDCA significantly increases transplantation-free survival (13). It was concluded that UDCA should be considered as a safe, effective and life-extending treatment for patients with PBC.

Although these results were encouraging, the efficacy of UDCA seems only moderate because induction of complete disease remission is achieved in less than 5% of patients (14). Furthermore, the beneficial effects on transplantation-free survival are mainly observed in stage 4 of the disease (13) and UDCA has not been found to improve invalidating symptoms, such as pruritus and fatigue.

In primary sclerosing cholangitis (PSC), identical results were found regarding serum liver function tests and histology (15-21). In 1997, results from a large double-blind randomised controlled study comprising 105 patients with a mean follow-up of 2 years have become available; no significant differences in disease progression were observed in the UDCA treated group when compared with placebo (22). However, as in PBC, effects of UDCA on survival may only become apparent after a mean follow-up of at least 4 years, and larger patient-numbers may be required. Currently, a Scandinavian group is performing a large randomised controlled trial with a follow-up of at least 4 years, comparing UDCA with a placebo. The results of this trial are not yet available.

Table 1: Major clinical trials in PBC with UDCA mono-therapy

	year	design	follow-up	n	Results biochemistry	histology	survival
Leuschner (2)	1989	RCT	9 months	20	improved	trend	n.a.
Battezzati (8)	1993	RCT	6 months	88	improved	n.a.	n.a.
Poupon (6)	1994	RCT	4 years	145	improved	n.a.	improved
Lindor (5)	1994	RCT	2 years	180	improved	n.s.	n.s.
Heathcote (4)	1994	RCT	2 years	222	improved	trend	n.s.
Turner (10)	1994	RCT	2 years	64	improved	n.s.	n.s.
Combes (11)	1995	RCT	2 years	151	improved	trend	n.s.
Eriksson (64)	1997	RCT	2 years	116	improved	n.s.	n.s.
Poupon (13)	1997	meta	4 years	548	n.a.	n.a.	improved

Improved: significantly improved; trend: improvements were reported, which were not significant; n.a.: not assessed; n.s.: not significant.
UDCA did not improve symptoms in any of the trials.

Although the exact pathogenesis of PBC and PSC is unknown, there is growing evidence of an autoimmune mediated aetiology (23-27). The most important mechanism of action of UDCA is probably its reduction of bile acid mediated damage (28-30), by replacing endogenous toxic hydrophobic bile salts with the more hydrophilic and less toxic UDCA. In view of the supposed immunogenetic background of PBC and PSC, the evaluation of combinations of UDCA with other drugs, especially immunosuppressives, seems to be a logical next step. It is the purpose of the final chapter of this thesis to evaluate several combination therapies in PBC and PSC and to discuss strategies for further studies. Furthermore, various prognostic markers and models of prognosis will be reviewed.

Combination therapy in PBC

Combination treatment of UDCA with colchicine

The anti-fibrotic and anti-inflammatory effects of colchicine in combination with UDCA were assessed in several studies (31-37). Significant decreases in pruritus, fatigue and alkaline phosphatase (APh) and bilirubin values were found in a randomised controlled trial comparing the efficacy of the combination of UDCA with colchicine versus a placebo (31). However, the design of this study does not allow to compare the efficacy of the combination of UDCA and colchicine with UDCA monotherapy, which is nowadays considered as the standard treatment of PBC.

A randomised controlled trial from Japan showed significant beneficial effects of colchicine added to UDCA versus UDCA monotherapy on serum liver function tests (37). However, in 3 other randomised controlled trials assessing the efficacy of additional colchicine (1 mg/day) in UDCA treated PBC patients, no beneficial effect of colchicine on serum-transaminases, APh, bilirubin or clinical signs and symptoms was established (33, 35, 38). Furthermore, effects of additional colchicine on histological markers were absent (33).

Vuoristo et al (36) performed a trial comparing the efficacy of UDCA monotherapy with colcichine monotherapy and with a placebo. Colchicine only modestly improved liver function tests, whereas UDCA significantly improved liver-biochemistry when compared with colchicine and with placebo. In conclusion, all available data strongly suggest that added colchicine does not improve the results that can be achieved by UDCA treatment alone.

Combination therapy of UDCA with methotrexate

In 1991, Kaplan et al presented data suggesting beneficial effects of methotrexate in the treatment of PBC (39). Since then, several studies assessing the effects of additional methotrexate in UDCA-treated patients have become available (40-45).

In 2 randomised controlled trials no additional improvement in clinical signs, symptoms, biochemistry and histology in patients treated with UDCA/methotrexate combination therapy when compared with UDCA monotherapy were found (42, 44). In an open label trial, Belgian investigators showed beneficial effects of methotrexate monotherapy on liver biochemistry, without concomitant improvements in histology

(46). In the following 2-year randomised controlled trial, significant improvements in liver biochemistry were found in patients treated with the combination of UDCA and methotrexate when compared with UDCA monotherapy. However, no concurrent improvements in clinical and histological parameters were observed (40). In the Belgian study, methotrexate toxicity consisted of interstitial pneumonitis (one patient), a transient rise in serum transaminases at three months of follow-up (five patients) and of a significant decrease of blood platelets and white blood cells in the methotrexate group after 2 years. All investigators concluded, that in view of the possible side effects and the limited therapeutic value, the use of methotrexate cannot be recommended in PBC at present.

Combination therapy of UDCA with corticosteroids

The use of corticosteroids has long been considered contraindicated in PBC (47) because of fear of increased bone-loss (48-50). Consequently, only few studies examined their therapeutic potential (51-54). In 1992 Mitchison et al. were the first to report the results of a randomised controlled trial, evaluating prednisolone in PBC (51). A significant decrease in APh and immunoglobulins in the prednisolone group was found when compared with controls. Histological examination of paired liver biopsies taken at the start of the trial and after 3 years suggested a beneficial effect of prednisolone treatment. No deterioration in bone mass was detected.
Leuschner et al. performed a 9-months randomised controlled trial in 30 previously untreated PBC patients assessing the effects of prednisolone added to UDCA versus UDCA monotherapy 52). Bone mass density remained stable in both groups. Serum liver function tests improved significantly in both groups, but no significant differences in biochemical response between both groups were observed. The most important finding was that liver histology improved significantly in the group treated with UDCA and prednisolone. By the same investigators, the effects of budesonide added to UDCA were evaluated with a similar study design and the results were identical; histology improved significantly in patients treated with UDCA and budesonide, whereas histological markers did not change in patients treated with UDCA monotherapy 55).
In this thesis the results are presented of a 1-year multi-centre placebo controlled, double-blind trial to assess the effects of a combination of prednisone and azathioprine in addition to UDCA, showing beneficial effects on symptoms, liver biochemistry and histology. Bone mass remained stable in patients treated with etidronate/calcium. Currently, strong efforts are made to start a large European multicentre trial assessing the long-term effects of this combination therapy on transplantation-free survival and to evaluate its possible side-effects, especially the effects on bone-mass.

Combination therapy in PSC

Compared to PBC, relatively few trials were performed in PSC and studies evaluating combination therapy are even fewer.

Immunosuppressive drugs

Lindor et al. have assessed the efficacy of combination therapy with prednisone and colchicine in PSC (56). Twelve patients received 10 mg prednisone and 1.2 mg colchicine per day for a period of 24 months. Data were compared with a group of concurrent historic controls, which were matched with respect to age, sex, histological stage and serum bilirubin. After 2 years of treatment no significant differences in biochemical tests were found between the treated group and their controls. Evaluation of serial biopsies showed no significant changes between both groups. In the untreated group, ascites developed in 4 patients, gastro-intestinal bleeding in 3 patients and 2 patients died. In contrast, in the treated group, the combination of variceal bleeding and ascites occurred in only 1 patient and no deaths occurred. The authors concluded that these results warrant a larger randomised controlled trial with this medication.

In this thesis we describe the results of a pilot-study assessing the effects of prednisone or budesonide added to UDCA in PSC. Small but significant improvements in scores for pruritus and in APh were found in patients treated with prednisone. No effects of budesonide were observed. In view of a severe adverse event in one patient, who developed autoimmune hepatitis after the trials drugs were discontinued (budesonide), and the limited benefit of the drugs, we consider further studies with this treatment regimen unattractive in PSC.

Results of a study evaluating combination therapy with UDCA and methotrexate in PSC have become available in 1996 (57). Nineteen patients received 13-15 mg/kg UDCA per day in combination with 0.25 mg/kg methotrexate per week. A control group consisted of a concurrently studied but non-randomised group of patients receiving UDCA monotherapy and was matched for age, sex, serum liver function tests and histological stage. In the patients receiving combination therapy, no changes in scores for fatigue and pruritus occurred. No significant difference in biochemical response between patients receiving UDCA monotherapy or the combination of UDCA and methotrexate was observed. After the withdrawal of methotrexate no changes in serum liver function tests occurred. Histological changes in the group with combination therapy were as follows: 2 patients progressed, 6 remained the same and 4 improved. There was a considerable number of drop-outs: 3 were referred for transplantation, 1 died from a carcinoma of the small bowel, 1 withdrew voluntarily, 3 patients were withdrawn from the study because of hair-loss and 2 because of sever pulmonary problems requiring hospitalisation. The authors conclude that the combination of UDCA and methotrexate offers little benefit over UDCA monotherapy, whereas substantial side-effects occur. Considering the results of all trials with immuno-suppressive drugs in PSC, the addition of an immunosuppressant to UDCA therapy becomes less and less attractive.

Endoscopic intervention therapy

The combination of UDCA with endoscopic intervention therapy could be promising. Stiehl and co-workers performed an 8-year prospective study in 65 patients, evaluating the efficacy of UDCA in combination with endoscopic dilatation of major bile duct stenoses (58). In all patients, enodoscopic retrograde cholangiography

(ERC) was performed at entry. In patients without narrowing of the common bile duct, repeat ERC was performed with 2-year intervals and whenever serum liver function tests deteriorated with at least 20%. In patients with major bile duct stenoses, endoscopic sphincterotomy of the papilla and rigid dilatation and subsequent balloon dilatation was performed until opening of the stenosis, assessed by repeat ERC. If necessary, a biliary stent was placed. In 23 patients with major duct-stenoses, 91 endoscopic dilatations were needed to obtain opening of the stenoses. In 5 patients intermittent stenting was performed. Pancreatitis occurred after 7 procedures, bacterial cholangitis after 3 procedures and perforation of the common bile duct occurred once. No patients died because of the procedure. It appeared that the actuarial survival in both the total group (p=0.001) and in the subgroup of patients who had undergone endoscopic intervention (p=0.006) was much better than predicted survival. Finally, the Amsterdam group has reported that short-term stent placement of only 9 days leads to the same results as leaving the stent in situ for 2 to 3 months, and greatly reduces the associated complications of stent clogging and cholangitis (59).

Biochemical remission

Since PBC and PSC are both diseases with a slow progression, trials - which investigate the potential of a drug to improve survival - need to include large patient numbers and a long follow-up period. In PBC, a number of easy to determine surrogate markers to predict the effects of medical intervention have therefor been used such as serum transaminases, bilirubin, histological markers and biochemical remission. Based on a multiple regression analysis of a large cohort of PBC patients, serum bilirubin, serum albumin, prothrombin time, age and the presence or absence of oedema were combined in the Mayo model for the prediction of prognosis of PBC. This model has the advantage of not requiring a liver biopsy 60).

In the Dutch cohort of PBC patients treated with UDCA, bilirubin, albumin, aspartate amino transaminase, normalisation of bilirubin, biochemical remission and bilirubin during follow-up were found to be of predictive significance for both treatment failure and death in an univariate model. In a multivariate model, including all before-mentioned variables, it appeared that only bilirubin at entry or during follow-up and age were significant prognostic factors of death and transplantation. Bilirubin at entry or during follow-up together with the presence of cirrhosis significantly predicted treatment failure (multivariate model).

Although serum bilirubin, age, histological stage, presence or absence of inflammatory bowel disease, hepatomegaly and splenomegaly (61-63) have been found to be associated with survival, the Mayo model for the prediction of prognosis in PSC only includes age, serum bilirubin, albumin, aspartate amino transaminase and the presence or absence of variceal bleeding. The predictive value of biochemical remission in PSC has not been evaluated.

Conclusions

UDCA has now been established as an effective medical treatment for PBC, although its effects are modest. The first large randomised trial, assessing the effects of UDCA in PSC did not show a favourable effect of UDCA on disease progression. Other studies with UDCA in PSC are currently being performed but results are not yet available. In PBC, available data indicate that combination therapy of UDCA and methotrexate is not more effective than treatment with UDCA alone, whereas the side effects of methotrexate can be severe. The addition of colchicine did not improve the effects of UDCA monotherapy. The results obtained with corticosteroids either given as monotherapy or in combination with UDCA are promising. In this thesis, we show that triple therapy with UDCA and low dose prednisone and azathioprine was clearly superior to UDCA monotherapy. The addition of a bisphosphonate to this triple regimen may be bone-protective.

Further studies are needed to establish the long term benefit-risk ratio of combined bile acid and immunosuppressive treatment, as well as the effects of bisphosphonates on bone-mass. To obtain a sample size that is large enough a joint initiative of several centres in Europe is currently initiating such a trial in PBC.

In PSC, results of various trials were disappointing and there are less therapeutic options. Endoscopic stenting and dilation of bile-duct strictures, may be important to alleviate symptoms and to potentiate the effects of medical treatment.

References

1. Poupon R, Chretien Y, Poupon RE, Ballet F, Calmus Y, Darnis F. Is ursodeoxycholic acid an effective treatment for primary biliary cirrhosis? Lancet 1987;1:834-6.
2. Leuschner U, Fischer H, Kurtz W, et al. Ursodeoxycholic acid in primary biliary cirrhosis: results of a controlled double-blind trial. Gastroenterology 1989;97:1268-74.
3. Jones DE, James OF, Bassendine MF. Ursodeoxycholic acid therapy in primary biliary cirrhosis. Hepatology 1995;21:1469-73.
4. Heathcote EJ, Cauch-Dudek K, Walker V, et al. The Canadian Multicenter Double-blind Randomized Controlled Trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1994;19:1149-56.
5. Lindor KD, Dickson ER, Baldus WP, et al. Ursodeoxycholic acid in the treatment of primary biliary cirrhosis. Gastroenterology 1994;106:1284-90.
6. Poupon RE, Poupon R, Balkau B. Ursodiol for the long-term treatment of primary biliary cirrhosis. The UDCA-PBC Study Group. N Engl J Med 1994;330:1342-7.
7. Osuga T, Tanaka N, Matsuzaki Y, Aikawa T. Effect of ursodeoxycholic acid in chronic hepatitis and primary biliary cirrhosis. Dig Dis Sci 1989;34:49S-51S.
8. Battezzati PM, Podda M, Bianchi FB, et al. Ursodeoxycholic acid for symptomatic primary biliary cirrhosis. Preliminary analysis of a double-blind multicenter trial. Italian Multicenter Group for the Study of UDCA in PBC. J Hepatol 1993;17:332-8.
9. Lotterer E, Stiehl A, Raedsch R, Foelsch UR, Bircher J. Ursodeoxycholic acid in primary biliary cirrhosis: no evidence for toxicity in the stages I to III. J Hepatol 1990;10:284-90.
10. Turner IB, Myszor M, Mitchison HC, Bennett MK, Burt AD, James OF. A two year controlled trial examining the effectiveness of ursodeoxycholic acid in primary biliary cirrhosis. J Gastroenterol Hepatol 1994;9:162-8.
11. Combes B, Carithers R, Jr., Maddrey WC, et al. A randomized, double-blind, placebo-controlled trial of ursodeoxycholic acid in primary biliary cirrhosis. Hepatology 1995;22:759-66.
12. Poupon RE, Balkau B, Eschwege E, Poupon R. A multicenter, controlled trial of ursodiol for the treatment of primary biliary cirrhosis. UDCA-PBC Study Group. N Engl J Med 1991;324:1548-54.
13. Poupon RE, Lindor KD, Cauch-Dudek K, Dickson ER, Poupon R, Heathcote EJ. Combined analysis of randomized controlled trials of ursodeoxycholic acid in primary biliary cirrhosis. Gastroenterology 1997;113:884-90.
14. Wolfhagen FH, van Buuren HR, Schalm SW, et al. Can ursodeoxycholic acid induce disease remission in primary biliary cirrhosis? The Dutch Multicentre PBC Study Group. J Hepatol 1995;22:381.
15. Hayashi H, Higuchi T, Ichimiya H, Hishida N, Sakamoto N. Asymptomatic primary sclerosing cholangitis treated with ursodeoxycholic acid. Gastroenterology 1990;99:533-5.
16. O'Brien CB, Senior JR, Arora-Mirchandani R, Batta AK, Salen G. Ursodeoxycholic acid for the treatment of primary sclerosing cholangitis: a 30-month pilot study. Hepatology 1991;14:838-47.
17. Chazouilleres O, Poupon R, Capron JP, et al. Ursodeoxycholic acid for primary sclerosing cholangitis. J Hepatol 1990;11:120-3.
18. Beuers U, Spengler U, Kruis W, et al. Ursodeoxycholic acid for treatment of primary sclerosing cholangitis: a placebo-controlled trial. Hepatology 1992;16:707-14.
19. Stiehl A, Walker S, Stiehl L, Rudolph G, Hofmann WJ, Theilmann 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.
20. De Maria N, Colantoni A, Rosenbloom E, Van Thiel DH. Ursodeoxycholic acid does not improve the clinical course of primary sclerosing cholangitis over a 2-year period. Hepatogastroenterology 1996;43:1472-9.
21. Mitchell S, Bansi D, Hunt N, Christie J, Fleming K, Chapman R. High dose ursodeoxycholic acid (UDCA) in primary sclerosing cholangitis(PSC): Results after two years of a randomised double-blind, placebo-controlled trial. Gastroenterology 1997;112 (suppl):A1335.
22. Lindor KD. Ursodiol for primary sclerosing cholangitis. N Engl J Med 1997;336:691-5.
23. Bjorkland A, Totterman TH. Is primary biliary cirrhosis an autoimmune disease? Scand J Gastroenterol Suppl 1994;204:32-9.
24. Gershwin ME. The molecular basis of primary biliary cirrhosis: interfacing clinical medicine and wet bench research. Isr J Med Sci 1995;31:22-30.
25. Laurin JM, Lindor KD. Primary biliary cirrhosis. Dig Dis 1994;12:331-50.
26. Harnois DM, Lindor KD. Primary sclerosing cholangitis: evolving concepts in diagnosis and treatment. Dig Dis 1997;15:23-41.
27. Chapman RW. Role of immune factors in the pathogenesis of primary sclerosing cholangitis. Semin Liver Dis 1991;11:1-4.
28. Heuman DM, Bajaj R. Ursodeoxycholate conjugates protect against disruption of cholesterol-rich membranes by bile salts. Gastroenterology 1994;106:1333-41.
29. Heuman DM. Hepatoprotective properties of ursodeoxycholic acid. Gastroenterology 1993;104:1865-70.
30. Queneau PE, Montet JC. Hepatoprotection by hydrophilic bile salts. J Hepatol 1994;21:260-8.
31. Bobadilla J, Vargas F, Dehesa M, et al. Colchicine and ursodiol in the treatment of primary biliary cirrhoss. Hepatology 1994;20:332A.
32. Shibata J, Fujiyama S, Honda Y, Sato T. Combination therapy with ursodeoxycholic acid and colchicine for primary biliary cirrhosis. J Gastroenterol Hepatol 1992;7:277-82.
33. Poupon R, Niard A, Huet P, et al. A randomized trial comparing the combination of ursodeoxycholic acid and colchicine to ursodeoxycholic acid alone in primary biliary cirrhosis. Hepatology 1994;20:151A.
34. Almassio P, Provenzano G, Battezzatti P, et al. The Italian multi-centre randomized controlled trial of ursodeoxycholic acid versus ursodeoxycholic acid plus colchicine in symptomatic primary biliary cirrhosis. Hepatology 1994;20:267A.
35. Goddard C, Hunt L, Smith A, Fallowfield G, Rowan B, Warnes T. A trial of ursodeoxycholic acid (UDCA) and colchicine in primary biliary cirrhosis (PBC). Hepatology 1994;20:151A.
36. Vuoristo M, Farkkila M, Karvonen AL, et al. A placebo-controlled trial of primary biliary cirrhosis treatment with colchicine and ursodeoxycholic acid. Gastroenterology 1995;108:1470-8.
37. Ikeda T, Tozuka S, Noguchi O, et al. Effects of additional administration of colchicine in ursodeoxycholic acid-treated patients with primary biliary cirrhosis: a prospective randomized study. J Hepatol 1996;24:88-94.
38. Raedsch R, Stiehl A, Walker S, Scherrmann JM, Kommerell B. Kombinierte Ursodeoxycholsaure plus Colchizin—Behandlung bei primar biliarer Zirrhose: Ergebnisse einer Placebo-kontrollierten Doppelblindstudie. Z Gastroenterol 1992;1:55-7.
39. Kaplan MM, Knox TA. Treatment of primary biliary cirrhosis with low-dose weekly methotrexate. Gastroenterology 1991;101:1332-8.
40. Van Steenbergen W, Sciot R, Van Eyken P, Desmet V, Fevery J. Combined treatment

with methotrexate and ursodeoxycholic acid in non-cirrhotic primary biliary cirrhosis. Acta Clin Belg 1996;51:8-18.
41. Buscher HP, Zietzschmann Y, Gerok W. Positive responses to methotrexate and ursodeoxycholic acid in patients with primary biliary cirrhosis responding insufficiently to ursodeoxycholic acid alone. J Hepatol 1993;18:9-14.
42. Lindor KD, Dickson ER, Jorgensen RA, et al. The combination of ursodeoxycholic acid and methotrexate for patients with primary biliary cirrhosis: the results of a pilot study. Hepatology 1995;22:1158-62.
43. Kaplan MM. The therapeutic effects of ursodiol and methotrexate are additive and well tolerated in primary biliary cirrhosis (PBC). Hepatology 1992;16:92A(abstract).
44. Gonzalez-Koch A, Brahm J, Antezana C, Smok G, Cumsille MA. The combination of ursodeoxycholic acid and methotrexate for primary biliary cirrhosis is not better than ursodeoxycholic acid alone. J Hepatol 1997;27:143-9.
45. Bergasa NV, Jones A, Kleiner DE, et al. Pilot study of low dose oral methotrexate treatment for primary biliary cirrhosis. Am J Gastroenterol 1996;91:295-9.
46. van Steenbergen W, Sciot R, van Eyken P, Desmet V, Fevery J. Methotrexate alone or in combination with ursodeoxycholic acid as possible treament in primary biliary cirrhosis. In: Cholestatic liver diseases: New strategies in the prevention and treatment of hepatobiliary and cholestatic liver diseases, 75th Falk Symposium, Maastricht, The Netherlands, 1994. Kluwer Academic Publishers.
47. Sherlock S, Dooley J. Primary Biliary Cirrhosis. In: Sherlock S, Dooley J, eds. Diseases of the Liver and Biliary System. Oxford: Blackwell Scientific Publications, 1993:244.
48. Rosen H. Primary biliary cirrhosis and bone disease. Hepatology 1995;21:253-5.
49. Eastell R, Dickson ER, Hodgson SF, et al. Rates of vertebral bone loss before and after liver transplantation in women with primary biliary cirrhosis. Hepatology 1991;14:296-300.
50. Lindor KD, Janes CH, Crippin JS, Jorgensen RA, Dickson ER. Bone disease in primary biliary cirrhosis: does ursodeoxycholic acid make a difference? Hepatology 1995;21:389-92.
51. Mitchison HC, Palmer JM, Bassendine MF, Watson AJ, Record CO, James OF. A controlled trial of prednisolone treatment in primary biliary cirrhosis. Three-year results. J Hepatol 1992;15:336-44.
52. Leuschner M, Guldutuna S, You T, Hubner K, Bhatti S, Leuschner U. Ursodeoxycholic acid and prednisolone versus ursodeoxycholic acid and placebo in the treatment of early stages of primary biliary cirrhosis. J Hepatol 1996;25:49-57.
53. Wolfhagen FH, van Buuren HR, Schalm SW. Combined treatment with ursodeoxycholic acid and prednisone in primary biliary cirrhosis. Neth J Med 1994;44:84-90.
54. Wolfhagen FHJ, van Hoogstraten HJF, van Buuren HR, et al. Triple therapy with ursodeoxycholic acid, prednisone and azathioprine in primary biliary cirrhosis: a 1-year randomized, placebo-controlled study. J Hepatol 1998;29:736-742.
55. Leuschner M, Maier K, Schlichting J, Herrmann G, Dahm H, Leuschner U. Combination of ursodeoxycholic acid (UDCA) with budesonide (BUD) is superior to UDCA-monotherapy in primary biliary cirrhosis (PBC). J Hepatol 1999;30 (suppl 1):57.
56. Lindor KD, Wiesner RH, Colwell LJ, Steiner B, Beaver S, LaRusso NF. The combination of prednisone and colchicine in patients with primary sclerosing cholangitis. Am J Gastroenterol 1991;86:57-61.
57. Lindor KD, Jorgensen RA, Anderson ML, Gores GJ, Hofmann AF, LaRusso NF. Ursodeoxycholic acid and methotrexate for primary sclerosing cholangitis: a pilot study. Am J Gastroenterol 1996;91:511-5.

58. Stiehl A, Rudolph G, Sauer P, et al. Efficacy of ursodeoxycholic acid treatment and endoscopic dilation of major duct stenoses in primary sclerosing cholangitis. An 8-year prospective study. J Hepatol 1997;26:560-6.
59. van Milligen de Wit AW, Rauws EA, van Bracht J, et al. Lack of complications following short-term stent therapy for extrahepatic bile duct strictures in primary sclerosing cholangitis. Gastrointest Endosc 1997;46:344-7.
60. Dickson ER, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. Hepatology 1989;10:1-7.
61. Farrant JM, Hayllar KM, Wilkinson ML, et al. Natural history and prognostic variables in primary sclerosing cholangitis. Gastroenterology 1991;100:1710-7.
62. Wiesner RH, Grambsch PM, Dickson ER, et al. Primary sclerosing cholangitis: natural history, prognostic factors and survival analysis. Hepatology 1989;10:430-6.
63. Broome U, Olsson R, Loof L, et al. Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis. Gut 1996;38:610-5.
64. Eriksson LS, Olsson R, Glauman H, et al. Ursodeoxycholic acid treatment in patients with primary biliary cirrhosis. A Swedish multicentre, double-blind, randomized controlled study. Scand J Gastroenterol 1997;32:179-86.

SUMMARY

In Rotterdam, the pathofysiology of cholestatic liver diseases has been investigated since 1976. This is the second thesis in which the results are presented of research that was performed within the framework of the Dutch Multi-Centre PBC Study Group and the Belgium-Dutch Multi-Centre PSC Study Group.

Chapter 1
In this chapter an overview is given of the scientific knowledge about cholestatic liver diseases at the time that the work on this thesis began. The pathofysiology of primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) is described as well as the biochemical and histological characteristics of both diseases. An overview is given of the effects of various drugs that were evaluated in randomised controlled trials. Especially the treatment of PBC with ursodeoxycholic acid (UDCA) seemed promising at that time; no effective medical treatment for PSC was available. Finally, the aims of this thesis are described.

Chapter 2
In 1997 a study was reported, combining individual data from three large randomised controlled trials, confirming that UDCA significantly improves transplantation-free survival in PBC. However, it was debated whether prognostic factors, which changed as a result of the use of UDCA (especially serum bilirubin), could still be used. After analysis of 203 PBC patients that were followed within the framework of the Dutch Multi-Centre PBC Study Group, it appeared that serum bilirubin remains the strongest independent prognostic factor both before and during treatment with UDCA.
In autoimmune hepatitis (AIH), biochemical remission, defined as the simultaneous normalisation of serum bilirubin and aspartate amino transaminase (AST) and alkaline phosphatase (APh) $\leq$ 1.5 x upper limit of normal (ULN), serves as a prognostic factor. In our cohort of PBC patients, biochemical remission was significantly associated with a better prognosis (univariate); this association was not found in a multi-variate analysis.
Except for serum bilirubin that slightly increased after 4 years of treatment all other serum liver function tests remained stable at their improved levels, indicating a gradual progression of the disease.

Chapter 3
The dose of UDCA, which has been applied in various trials assessing the effects of UDCA in PBC, ranges from 7.7 mg/kg/day to 15 mg/kg/day. The protocol of the Dutch Multi-Centre PBC Study Group dictates a dose of 10 mg/kg/day. It was hypothesised that a higher dose of UDCA would lead to a higher biliary enrichment, whereby the effectiveness of UDCA would increase. Therefore, a 6-months randomised controlled trial assessing the effects of UDCA 10 mg/kg/day versus 20 mg/kg/day was initiated. The higher dose was well tolerated. Patients, who were treated with the higher dose, achieved significant additional improvements in serum liver function tests. However, no changes in serum bilirubin occurred in both groups. There was a significant increase of the percentage of UDCA in the bile acid pool. A UDCA dose of 13-15 mg/kg/day seems to be the preferred dose.

Chapter 4
The main mechanism of action of UDCA is to reduce liver cell damage caused by cholestasis. The autoimmune process that is present in PBC is not influenced by UDCA. Thus, additional beneficial effects may be achieved by adding an immunosuppressive drug to the treatment with UDCA. In chapter 4, we report the findings of a 1-year double blind randomised controlled trial in 50 PBC patients, who were treated with prednisone and azathioprine in addition to UDCA. In patients treated with prednisone and azathioprine, significant improvements were found in the score for pruritus and in biochemical markers for cholestasis, liver cell damage, immune activity and fibrogenesis when compared with placebo (UDCA mono-therapy). Histological scores for disease activity and disease stage improved significantly in the group treated with prednisone and azathioprine, whereas these changes were absent in the placebo-group. Treatment with cyclic etidronate and calcium protected against corticosteroid induced bone-loss (not in this thesis).

Chapter 5
The long-term effects of UDCA on biochemical, histological and cholangiographic characteristics of PSC patients were not well described, when the study, described in chapter 5, was initiated. Previously our group had shown that biliary enrichment with UDCA and the effects on serum liver function tests was independent of the dose scheme. Others however suggested differently.
After treatment with UDCA for 2 years of 48 PSC-patients according to the protocol of the Dutch Multi-Centre PSC Study Group, no changes in symptoms, histology, or cholangiographic findings were observed. Biochemical parameters of cholestasis and liver cell necrosis significantly improved. Serum bilirubin remained stable. UDCA was well tolerated. Actuarial transplantation-free survival was 91% at 2 years and did not differ significantly from predicted survival in untreated patients (MAYO model). No significant differences in any of the study endpoints (symptoms, serum liver function tests, cholangiographic findings, histology, disease progression) were found between patients who took the UDCA as a single bed-time dose or divided during the day (randomised controlled study design).

Chapter 6
An overlap syndrome between PBC and AIH has been described previously. In PSC, a similar syndrome might be found. In chapter 6, we describe the biochemical, histological and cholangiographic features of 8 patients who were diagnosed with both PSC and AIH. Initially, all patients responded well to treatment with corticosteroids. However, two patients needed a liver transplantation. The overlap syndrome of PSC and AIH might be more common than hitherto assumed. The true incidence, however, is unknown.

Chapter 7
As the short-term beneficial effects of corticosteroids added to UDCA became apparent in PBC patients, a pilot-study with a similar treatment regimen in PSC patients was initiated. In chapter 7, the results of an 8-week double-blind randomised pilot study in 18 patients assessing the effects of prednisone (10 mg/day) or budesonide (3 mg/day or 9 mg/day) added to UDCA (12 mg/kg/day) are reported. No effects of treatment with budesonide were found. APh, immunoglobulin G (IgG) and score for pruritus significantly improved in patients, who were treated with prednisone. After the medication was discontinued, a serious adverse event (autoimmune hepatitis) was observed in one patient who was treated with 9 mg budesonide. The empirical use of corticosteroids in PSC can not be recommended.

Chapter 8
It is now clear that treatment of cholestatic liver diseases with UDCA has limited potential and additional medical treatment is needed. In chapter 8, an overview of studies is given that were performed to assess the effects of combination therapy. In PBC, a combination of UDCA with immunosuppressives seems promising. In PSC, less therapeutic options are available although endoscopic procedures in combination with UDCA could prove to be valuable. Finally, the use of prognostic factors, especially biochemical remission, is discussed.

SAMENVATTING

In Rotterdam wordt sedert 1976 onderzoek verricht naar de klinische vormen en behandeling van cholestatische leverziekten. Dit proefschrift is het tweede met de resultaten van onderzoek dat werd uitgevoerd in het kader van de Dutch Multi-Centre PBC Study Group en de Belgium-Dutch Multi-Centre PSC Study Group.

Hoofdstuk 1
In dit hoofdstuk wordt een overzicht gegeven van de kennis over cholestatische leverziekten op het moment dat met het werk aan dit proefschrift werd begonnen. Er wordt ingegaan op de pathofysiologie van primaire biliaire cirrose (PBC) en primaire scleroserende cholangitis (PSC) en op de biochemische en histologische kenmerken van beide ziektebeelden. Er wordt een overzicht gegeven van de effecten van verschillende geneesmiddelen die bij beide ziektebeelden in gerandomiseerde studies zijn bestudeerd. Vooral de behandeling van PBC met ursodeoxycholzuur (UDCA) leek destijds veelbelovend; er was echter geen effectieve medicamenteuze therapie voor PSC. Tenslotte worden de doelen van dit proefschrift uiteengezet.

Hoofdstuk 2
In 1997 werden de resultaten gepubliceerd van een studie, waarin de individuele data van 3 grote studies werden gecombineerd, die de gunstige effecten van UDCA op de transplantatie vrije overleving van patiënten met PBC bevestigden. Het was echter onduidelijk of prognostische factoren, die door het gebruik van UDCA in waarde veranderden (m.n. serum bilirubine), nog als zodanig mochten worden gebruikt. Na analyse van het cohort patiënten (n=203) dat werd gevolgd in het kader van de Nederlandse Multicentrische PBC studiegroep bleek dat bilirubine zowel voor aanvang van behandeling met UDCA als tijdens deze behandeling de sterkste onafhankelijke prognostische factor bleef.
Bovendien werd onderzocht of, naar analogie van auto-imuun hepatitis, biochemische remissie, gedefinieerd als het gelijktijdig optreden van een normaal bilirubine, aspartaat amino transaminase (AST) en alkalische fosfatase (APh) $\leq$ 1.5 x bovengrens van normaal (ULN), als prognostische factor zou kunnen dienen. Alhoewel biochemische remissie significant geassocieerd was met een betere prognose (univariaat) verdween deze associatie in een multivariate analyse.
Alle biochemische leverfunctietesten bleven op een stabiel verbeterd niveau, behalve serum bilirubine dat een lichte stijging vertoonde na 4 jaar behandeling met UDCA, hetgeen wijst op een langzame progressie van de ziekte.

Hoofdstuk 3
De dosering UDCA, die in verschillende studies naar de effecten van UDCA in PBC werd gebruikt loopt uiteen van 7.7 mg/kg/dag tot 15 mg/kg/dag. Het protocol van de Dutch Multi-Centre PBC Study Group schreef een dosering van 10 mg/kg/dag voor. Gepostuleerd werd dat een hogere dosering UDCA zou leiden tot een hogere concentratie in de gal, waardoor de effectiviteit zou toenemen. Daarom werden in een gerandomiseerde studie de effecten van 2 doseringen UDCA (10 mg/kg/dag versus 20 mg/kg/dag) gedurende 6 maanden met elkaar vergeleken. De hogere dosering werd goed verdragen. De patiënten die met de hogere dosering werden behandeld verkregen significante additionele biochemische verbeteringen. Het bilirubine bleef gelijk. Er was een significante stijging van het percentage UDCA in de gal. De optimale dosering voor UDCA lijkt 13-15 mg/kg/dag te zijn.

Hoofdstuk 4
Het voornaamste werkingsmechanisme van UDCA is het verminderen van leverschade die optreedt door cholestase. Het bij PBC aanwezige auto-immuun proces lijkt niet door UDCA te worden beïnvloed. Het toevoegen van immuunsuppressief geneesmiddel aan de behandeling met UDCA, zou dus kunnen leiden tot additionele gunstige effecten. In hoofdstuk 4 rapporteren wij de resultaten van een 1-jarige dubbelblinde gerandomiseerde studie bij 50 patiënten met PBC, die werden behandeld met prednison en azathioprine toegevoegd aan UDCA. Vergeleken met de placebo behandelde groep werden significante verbeteringen gevonden voor jeukscore en biochemische markers voor cholestase, levercelschade, immuunactiviteit en fibrogenese. Histologische scores voor ziekte activiteit en stadium toonden significante verbeteringen in de groep die werd behandeld met de combinatie van prednison en azathioprine en niet in de placebo behandelde groep. In een reeds eerder gepubliceerde studie (niet in dit proefschrift) werd het botbeschermende effect van behandeling met cyclisch etidronaat en calcium bij de patiënten die werden behandeld met prednison beschreven.

Hoofdstuk 5
De langetermijnseffecten op biochemische, histologische en cholangiographische kenmerken van PSC patiënten, die worden behandeld met UDCA waren bij aanvang van de studie, die wordt beschreven in hoofdstuk 6, niet uitvoerig bekend. Hoewel eerder onderzoek door onze groep had aangetoond dat de verrijking van de gal met UDCA en de biochemische effecten onafhankelijk waren van het doseringsschema, hadden anderen verondersteld dat dit wel het geval was. Na 2 jaar behandeling met UDCA van 48 PSC patiënten volgens het protocol van de Nederlandse Multicentrische PSC studiegroep werden geen veranderingen in klachten, histologische indices of bevindingen tijdens endoscopische retrograde cholangiopancreaticografie (ERCP) gevonden. Biochemische parameters voor cholestase en levercelverval verbeterden significant. Serum bilirubine bleef gelijk. UDCA werd goed verdragen. De 2-jaars transplantatievrije overleving was 91% en verschilde

niet significant van de voorspelde overleving voor onbehandelde patiënten (MAYO model). Er was geen enkel verschil in eindpunten (klachten, lever biochemie, bevindingen bij ERCP, histologie, ziekte progressie) tussen patiënten die de UDCA in een enkele dosis innamen of verdeeld over de dag (getest in een gerandomiseerde studieopzet).

Hoofdstuk 6

Bij patiënten met PBC is er een overlapbeeld met auto-immuun hepatitis (AIH) beschreven. Ook bij PSC lijkt een dergelijk beeld te bestaan. In hoofdstuk 6 beschrijven wij de biochemische, histologische en cholangiographische kenmerken van 8 patiënten, bij wie zowel de diagnose PSC als AIH gesteld kon worden. Alle patiënten reageerden initieel goed op therapie met corticosteroïden. Twee patiënten echter, ondergingen een levertransplantatie. Het overlapbeeld van PSC en AIH is een reële klinische variant waarvan de ware incidentie nog onbekend is, maar die minder zeldzaam lijkt dan tot nu toe werd aangenomen.

Hoofdstuk 7

Na de hoopgevende resultaten, die werden bereikt met corticosteroïden toegevoegd aan UDCA bij patiënten met PBC, werd een pilot-study met een dergelijk behandelmethode bij patiënten met PSC uitgevoerd. In hoofdstuk 7 worden de resultaten gerapporteerd van een 8-weekse dubbelblinde gerandomiseerde studie met prednison (10 mg/dag) of budesonide (3 mg/dag of 9 mg/dag) toegevoegd aan UDCA (12 mg/kg/dag) bij 18 patiënten met PSC. Behandeling met budesonide had geen effect. De jeukscore, het alkalische fosfatase en immunoglobuline G (IgG) daalden significant in de groep die werd behandeld met prednison. Na het staken van de therapie trad er een ernstige bijwerking in de vorm van AIH op bij één van de patiënten die werd behandeld met 9 mg budesonide. Terughoudendheid in het gebruik van corticosteroïden bij PSC lijkt derhalve op zijn plaats.

Hoofdstuk 8

Na de scherpere vaststelling van de effecten van UDCA in de behandeling van cholestatische leverziekten is nu duidelijk dat aanvullende therapeutische mogelijkheden noodzakelijk zijn. In hoofdstuk 8 wordt een overzicht gegeven van de studies die verricht zijn met verschillende therapeutische combinaties. Bij PBC lijkt een combinatie van immuunsuppressiva met UDCA veelbelovend. Bij PSC zijn de medicamenteuze opties beperkter maar lijkt endoscopische therapie in combinatie met UDCA een belangrijke aanwinst. Tenslotte worden verschillende prognostische factoren, m.n. biochemische remissie besproken.

DANKWOORD

Het schrijven van een proefschrift lijkt vaak uit 3 fases te bestaan. Het eerste half jaar heb je geen idee waar je aan begonnen ben en daar maakte ik me vreselijk ongerust over. Als de projecten eenmaal gaande zijn, breekt een drukke tijd aan van het verzamelen van data en lijkt het daadwerkelijk schrijven van het proefschrift nog comfortabel ver weg. Het laatste half jaar sloeg de paniek toe en lijk je de manuscripten nooit op tijd klaar te krijgen. De zucht van verlichting, die ik slaakte toen dit boekje was geboren, moet van verre te horen zijn geweest. Echter, zonder de hulp van velen had het "hora est" nooit kunnen gaan klinken. Een aantal personen wil ik bij naam noemen.

De hooggeleerde prof dr Solko W. Schalm, mijn promotor, is de vader van dit proefschrift. Zijn inbreng heeft dit proefschrift tot iets moois gemaakt. Mijn 2e promotor, prof dr Gerard P. van Berge Henegouwen, dank ik voor de vriendelijke en enthousiasmerende woorden als ik de voltooiing van mijn proefschrift in het geheel niet meer zag zitten.

Henk van Buuren, study-supervisor, alhoewel je een hekel aan dit woord en alle vormen van decorum had, wil ik heel bijzonder danken. Hij moest als roker en iemand die graag een biertje drinkt, 3 jaar lang een kamer delen met mij, een niet-roker, die van alcohol alleen maar misselijk wordt. Ik zal je altijd dankbaar blijven voor je brede rug waarachter ik mocht schuilen, je luisterend oor, en voor je eindeloze geduld in het corrigeren van mijn werk.

Samen met Frank Vleggaar heb ik het laatste jaar zorggedragen voor de studies en hij zal de vaandel verder dragen. Ik denk met veel plezier terug aan onze lange autoritten, soms zeer vroeg in de morgen (of eigenlijk nacht) op weg naar weer een ziekenhuis voor patiënt-inclusie en dataverzameling. Het door ons samen voor alle collegae bereide kerstdiner was een groot succes.

De dames van het trialsecretariaat dank ik bijzonder voor de adequate en precieze wijze waarop allerlei formulieren werden ontwikkeld, waarna de verzamelde informatie feilloos in de computer werd ingevoerd. Marcelle Rensink was in het bijzonder bij de studies betrokken en nam steeds meer administratief werk over, waarvoor mijn hartelijke dank. Sylvia de Vlaming droeg zorg voor de uiteindelijke correctie en lay-out van de manuscripten en was altijd bereid tot ver na kantoortijd mij terzijde te staan. Jan Boot zorgde dat alle computersystemen goed werkten en eventuele problemen snel werden opgelost. De computer werd mede door hem tot een belangrijk "maatje" in het schrijven van dit proefschrift maar heeft niet echt geleid tot een "paperless office".

De statistici Wim Hop en Bettine Hansen wil ik bijzonder danken voor hun engelengeduld bij de statistische begeleiding van de verschillende projecten en hun niet aflatende moeite om mij te behoeden voor de immer aanwezige statistische valkuilen. Tijdens het "peer-review" proces van onze manuscripten is nooit commentaar geweest op de gevolgde statistische methoden!

Ik ben van mening dat een belangrijk deel van mijn plezier in mijn werk tot stand komt door het contact met collegae, hetgeen de afgelopen periode keer op keer werd bevestigd. Jullie zijn allemaal vrienden geworden, waarvoor mijn dank.
De lekkere hapjes, die Türkan Terkivatan mee nam tijdens het werk aan ons manuscript hebben zeker bijgedragen aan de totstandkoming hiervan. Kom nog een langs!
De inzet van dr. Johan Nix, expert op het gebied van cholangiografie, om de beoordeling van de ERCP's voor de PSC -studie tot een goed einde te brengen, was van uitzonderlijk belang voor het welslagen van de studie. Helaas is hij veel te vroeg overleden en mag hij de totstandkoming van dit proefschrift niet meer meemaken.
Prof dr Fibo JW ten Kate en zijn medewerkers, in het bijzonder Hilde Kuiper, dank ik voor al hun inspanningen om een groot aantal leverbiopten op tijd te beoordelen.
De leden van de "kleine" en "grote" promotie commissie dank ik hartelijk voor hun tijd en moeite om mijn promotie mogelijk te maken.
De firma's (in alfabetische volgorde) Falk Pharma, Tramedico en Zambon Nederland hebben ruimschoots bijgedragen aan de totstandkoming van dit boekje. Hiervoor en voor alle andere hulp tijdens het werk aan het proefschrift mijn oprechte dank. Ook de overige sponsors dank ik voor hun bijdrage.
Dit proefschrift is opgedragen aan mijn grootmoeder, geboren in 1905. Haar steun aan ons gezin tijdens mijn langdurige opname in het Sophia Kinderziekenhuis is van onschatbare waarde geweest. Tijdens haar bezoekjes in het ziekenhuis waarin ze heel rustig over de dagelijkse dingen praatte en wat in de krant las, is onze band gegroeid. Ik ben ervan overtuigd dat mijn ouders, broers en ik mede door haar steun zo goed door die tijd zijn heen gekomen, waardoor uiteindelijk mijn studie geneeskunde mogelijk werd en dit proefschrift kon worden geschreven.

SPONSORING

Mijn dank gaat uit naar de diverse firma's (in alfabetische volgorde) die hebben bijgedragen aan de kosten van dit proefschrift.

Hoofdsponsors:
Falk Pharma GmbH
Tramedico B.V.
Zambon Nederland B.V.

Overige sponsors:
Boehringer Ingelheim B.V.
Merck Sharp & Dohme B.V.
Norgine B.V.
Pentax Benelux B.V.
Parke-Davis B.V.
Roche Nederland B.V.
Servier Nederland B.V.

CURRICULUM VITAE

De auteur van dit proefschrift werd op 14 februari 1967 geboren te Rotterdam. Zijn gymnasium-ß diploma behaalde hij in 1985 aan Coriovallum College in Heerlen, waarna hij startte met de studie geneeskunde aan de Erasmus Universiteit Rotterdam. De Hippocratische eed legde hij af in 1993. Naast zijn studie was hij actief als medewerker in het studenten-verpleegteam van het Sophia Kinderziekenhuis en was hij bestuurslid van diverse jongerenorganisaties. Alhoewel hij tijdens zijn studie enkele malen in de tropen verbleef (Zaïre, Brazilië en Ghana), koos hij voor een loopbaan in Nederland, die startte als assistent-geneeskundige in het Reinier de Graaf Gasthuis te Delft. Vanaf december 1994 was hij gedurende 3 jaar verbonden als arts-onderzoeker aan de sectie hepatologie van de afdeling maag-, darm- en leverziekten van het Academisch Ziekenhuis Rotterdam. Hij was verantwoordelijk voor de studies die werden verricht in het kader van de Nederlandse Multicentrische PBC Studie Groep en Belgisch Nederlandse Multicentrische PSC Studie Groep. De wetenschappelijke resultaten van dit werk worden weergegeven in dit proefschrift dat tot stand kwam onder leiding van prof dr SW Schalm, prof dr GP van Berge Henegouwen en drs HR van Buuren.
Sinds maart 1998 is hij in opleiding tot internist in het Albert Schweitzer Ziekenhuis, locatie Dordwijk te Dordrecht (opleider dr J van der Meulen).