

Orphan drugs in development for primary biliary cirrhosis: challenges and progress

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Abstract: Primary biliary cirrhosis (PBC) is a chronic progressive liver disease that often leads to fibrosis, cirrhosis, and end-stage liver disease. The diagnosis is made when there is evidence of cholestasis and reactivity to the antimitochondrial antibody. The etiology of PBC is poorly understood; however, several lines of evidence suggest an environmental factor that triggers a series of immune-mediated inflammatory reactions in the bile ducts in a genetically susceptible individual. Fatigue and pruritus are the most common symptoms of PBC; however, many patients are diagnosed with PBC only based on laboratory abnormalities. The only pharmacological treatment approved for PBC is ursodeoxycholic acid (UDCA). Several controlled studies have shown that UDCA improves liver biochemistries and prolongs transplant-free survival in PBC patients. Nearly 40% of PBC patients do not respond to UDCA, and those patients are at high risk of serious adverse events, such as the development of liver failure. Therefore, newer alternative therapeutic options for PBC are needed. Obeticholic acid is a first-in-class farnesoid X receptor agonist that has been recently evaluated in PBC patients with inadequate response to UDCA, and demonstrated beneficial results in improving liver biochemistries. Several other agents (fibrates and glucocorticoids) have been previously examined in PBC patients with inadequate response to UDCA, and preliminary results showed biochemical improvement. However, large-scale controlled clinical trials are needed to determine the long-term effects of fibrates and glucocorticoids on the clinical outcomes of PBC. Clinical trials of NGM282 (a fibroblast growth factor-19 analog) and Abatacept (a fusion protein composed of the Fc portion of immunoglobulin G1 fused to CTLA4) are currently underway.

Keywords: primary biliary cirrhosis, antimitochondrial antibody, farnesoid X receptor, fibrates, glucocorticoids

Introduction

Definition and significance of primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is a relatively rare but important cause of liver disease in the Western society. The disease affects <200,000 individuals in the USA and has been designated as an orphan disease by the US Food and Drug Administration.¹ Recent PBC epidemiological studies in North America, Europe, and Australia have reported a prevalence of 1.9–40.2 per 100,000 population and an incidence of 0.39–9.8 per 100,000 population.² Since it was first described in the year 1851 by Addison and Gull,³ significant progress has been made in the diagnosis and management of PBC.

PBC is an autoimmune disease characterized histologically by chronic inflammation and destruction of the interlobular bile ducts and affects women more commonly than men (ratio 10:1).⁴ The sera of nearly 95% of patients with PBC test positive

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for the antimitochondrial antibody (AMA), and the most common biochemical abnormality in PBC is an elevated serum alkaline phosphatase (ALP) level.⁵ The diagnosis of PBC can be made when two of the following criteria are met (provided that other causes of intra- and extrahepatic cholestasis have been excluded): 1) evidence of cholestasis based on serum ALP elevation, 2) the presence of AMA, and 3) histological evidence of PBC. These criteria are endorsed by the American Association for the Study of Liver Diseases and the European Association for the Study of the Liver.^{5,6}

AMA is an autoantibody found in the majority of patients with PBC (~95%) and targets a family of mitochondrial enzymes named the 2-oxo-acid dehydrogenase complexes.⁷⁻⁹ The levels of AMA may vary through the course of PBC, and there seems to be no relationship between the AMA level and the degree of severity or stage of PBC.⁵ Enzyme-linked immunosorbent assay, immunoblotting, and indirect immunofluorescence are the most common methods used for AMA detection.⁹ AMA is rarely found in healthy individuals, with a reported prevalence rate ranging from 0.5% to 2%.¹⁰⁻¹³

Presentation of and conditions associated with PBC

PBC is often diagnosed in a preclinical asymptomatic phase, after screening liver blood tests have been ordered by a primary care provider.¹⁴ Fatigue¹⁵⁻¹⁹ and pruritus²⁰⁻²³ are the most frequent symptoms of PBC, present in 60%–80% and 20%–70% of patients, respectively, and both have a marked negative impact on the quality of life of patients with PBC. Other manifestations of PBC include xanthomas and xanthelasmas (from underlying associated hyperlipidemia), vitamin D deficiency, osteopenia, and osteoporosis.¹⁴ When PBC has progressed to stage IV fibrosis (cirrhosis), portal hypertension may ensue with hepatomegaly, splenomegaly, ascites, varices, hepatic encephalopathy, and jaundice.⁵ Fatigue in PBC may lead to inability to work, depression, poor quality of life, more aggressive disease, and decreased survival.^{15-17,24-31} Pruritus has been associated with severe excoriations, skin bleeding, inability to sleep, and more aggressive disease.^{22,30,32-40} Severe pruritus can be very debilitating and is considered an acceptable indication for liver transplantation regardless of Child–Turcotte–Pugh or model for end-stage liver disease score by some centers.^{16,36,41} PBC is associated with other autoimmune intra- and extrahepatic conditions such as autoimmune hepatitis,⁴²⁻⁴⁵ Sjogren's syndrome, arthritis,⁴⁶ thyroid diseases, scleroderma, Raynaud's phenomenon, type I diabetes mellitus, systemic lupus erythematosus, and celiac disease.⁴⁷

Etiology and pathogenesis of PBC

The cause of PBC remains unidentified. There is increasing evidence that PBC is caused by an environmental factor that triggers a series of inflammatory reactions in a genetically susceptible host, leading to PBC.^{48,49} Cigarette smoking, infections (particularly urinary tract and *Helicobacter pylori* infections), the use of hormone replacement therapies, and frequent use of nail polish have been found to be associated with an increased risk of PBC.⁵⁰⁻⁵³ The strong spatial variation of risk and clustering of PBC cases in certain geographical areas around the world (around superfund toxic waste sites in New York City,⁵⁴ near the Revelin reservoir in England,⁵⁵ the Tyneside region in Northeast England,⁴⁹ and in Hiroshima among the survivors of the atomic bomb⁵⁶) suggest that one or more environmental risk factors are implicated in the pathogenesis of PBC, and that toxin exposure may be responsible for the clustering of PBC cases. In PBC, humoral and cellular immune responses are exaggerated, likely due to the loss of tolerance to self-antigens.^{57,58} PBC patients frequently exhibit high levels of serum autoantibodies (immunoglobulin [Ig] M, IgG, and IgA) and significantly increased numbers of cytotoxic T (CD8⁺) and helper T (CD4⁺) lymphocytes compared to control subjects.⁵⁷⁻⁶³ Helper T (CD4⁺) lymphocytes release cytokines (such as interleukin [IL]-1, IL-2, IL-4, IL-6, interferon- γ) that activate cytotoxic T (CD8⁺) lymphocytes, which in turn directly destroy the hepatocytes and cholangiocytes.⁶⁴⁻⁶⁶ In addition, the cytokines released by the helper T (CD4⁺) lymphocytes recruit natural killer cells, which contribute to the destruction of biliary epithelium.^{57,64} The overexpression of the major histocompatibility complex (MHC)-I and MHC-II is believed to contribute to the local liver tissue damage that occurs in PBC, as they enable the recognition of the self-antigens by the activated lymphocytes, leading to further destruction of the portal tracts and surrounding hepatic tissue.^{57,58}

It is strongly believed that accumulation of the toxic hydrophobic bile acids in the liver tissue contributes to the liver damage that occurs in PBC.⁶⁷⁻⁶⁹ Therefore, modulation of the bile acid pool or removal of the toxic hydrophobic bile acids would theoretically have beneficial effects in patients with PBC. Acting through this mechanism, it is believed that ursodeoxycholic acid (UDCA, a naturally occurring hydrophilic bile acid, and the only agent approved by the US Food and Drug Administration for the treatment of PBC) enriches the total bile acid pool by replacing the unwanted toxic bile acids,⁷⁰ which can have deleterious effects on the liver tissue. For example, in mice, lithocholic acid has been found to promote destruction of the portal tracts and bile ducts.⁷¹

An Australian group⁷² monitored 12 patients with PBC and six patients with primary sclerosing cholangitis over a 4-year period. They found that patients with a serum total chenodeoxycholic acid (CDCA) concentration at study entry that exceeded 15 $\mu\text{mol/L}$ were 10 times more likely to die from liver disease or need a liver transplant in the following 4 years than patients with CDCA levels $<15 \mu\text{mol/L}$.

Genetics of PBC

In addition to environmental factors, genetic predisposition is believed to play an important role in the development of PBC.⁴⁸ Data from genetics-based studies have shown that first-degree family members of PBC patients are at high risk of PBC. In one study, the prevalence of PBC has been reported to be 720 per 100,000 and 1,200 per 100,000 in first-degree relatives and offspring of affected individuals, respectively.⁷³ In addition, the prevalence of positive AMA (without clinical or biochemical evidence of PBC) is high among first-degree relatives of PBC patients; one study found that 20% of sisters, 15% of mothers, and 10% of daughters of PBC patients were seropositive for AMA.⁷⁴ The clinical significance of AMA seropositivity in first-degree relatives of PBC patients remains unclear. The genome-wide associated studies have identified important gene alleles believed to be related to PBC.^{73,75–80}

Natural history and outcomes of PBC

The progression and outcome of PBC depend on various clinical and biochemical factors at the time of diagnosis. Asymptomatic PBC patients have a better survival compared to patients who have symptoms at the time of diagnosis of PBC, and approximately two-third of asymptomatic PBC patients develop symptoms over a median time interval of 4.2–5.3 years of follow-up.^{81–86} PBC patients have worse survival than the general healthy population, irrespective of the presence or absence of symptoms at the time of diagnosis.⁵ Risk factors that have been associated with poor prognosis of PBC are male sex, the presence of symptoms at diagnosis of PBC, elevated serum bilirubin, elevated serum ALP, the presence of anti-Sp100 and anti-gp210 autoantibodies, prolonged prothrombin time, development of esophageal varices, inadequate response to UDCA, and advanced histological stage.^{83,87–94} Despite its rarity, PBC remains an important cause of morbidity in the Western world. Many patients with PBC progress to cirrhosis and end-stage liver disease, requiring liver transplantation.⁹⁵ PBC is one of the leading indications for liver transplantation.^{41,95,96} Moreover, PBC has been identified as an important risk factor for hepatocellular carcinoma.^{93,97–102}

Current pharmacological treatment of PBC

UDCA is the only drug approved by the US Food and Drug Administration for the treatment of PBC. Several controlled and uncontrolled clinical trials have shown that UDCA improves liver biochemistries, delays histological progression, delays development of esophageal varices, and improves the transplant-free survival in patients with PBC.^{103–111} UDCA is 3 α ,7 β -dihydroxy-5 β -cholanoic acid, a bile acid with two hydroxy groups (–OH) at positions 3 and 7 in the cholane ring structure, with an α - and β -orientation, respectively.¹¹² The C-7 β -orientation confers the molecule a far higher hydrophilicity than that of its structural analog with an α -orientation, CDCA.¹¹²

The exact mechanism of action of UDCA in PBC has not been established yet. However, there are a number of proposed mechanisms by which UDCA is thought to exert its beneficial effects in PBC. First, UDCA changes the hydrophobicity index of the endogenous bile acid pool and replaces the potentially toxic hydrophobic bile acids by enriching the endogenous bile acid pool.^{70,112} UDCA comprises no more than 4% of the total endogenous bile acid pool in healthy individuals, whereas this percentage is increased to 40%–60% in individuals taking conventional UDCA doses of 13–15 mg/kg body weight per day.¹¹³ Depending on the dose used, UDCA enriches the bile acid pool, accounting for 19%–64% of the total biliary bile acids.¹¹⁴ These effects are thought to protect the liver tissue against the deleterious effects of detergent bile acids. Second, UDCA protects hepatocytes and cholangiocytes against cell death induced by the cytotoxic bile acids by counteracting the extrinsic and intrinsic apoptotic pathways responsible for the hepatocyte and cholangiocyte damage in PBC.¹¹² Third, UDCA decreases the intracellular concentration of bile acids and favors their elimination through the urinary route by preventing the uptake of bile acids by hepatocytes (by downregulating the organic anion-transporting polypeptide 1, a basolateral transporter involved in bile acid hepatocyte uptake),¹¹⁵ enhancing the excretion of conjugated bile acids into the blood (by upregulating the basolateral export pumps multidrug resistance-associated protein [Mrp] 3 and Mrp4),^{116–118} repressing bile acid synthesis,¹¹⁹ and eliminating the toxic bile acids through the kidneys (by upregulating the renal apical bile acid export pumps Mrp2 and Mrp4).^{116,120–122} Fourth, UDCA reduces portal inflammation and ductular proliferation and preserves bile duct integrity by stimulating HCO_3^- secretion by the cholangiocytes.^{123–125} HCO_3^- secretion is thought to improve the bile flow through the affected

bile ducts, thus ameliorating the deleterious effects of toxic bile acids on the liver tissue.¹²⁶ Fifth, UDCA exerts immunomodulatory and anti-inflammatory effects on the humoral and cellular immune responses by suppressing the production of autoantibodies,¹²⁷ suppressing the cytotoxic T (CD8⁺) and helper T (CD4⁺) lymphocytes,^{127,128} inhibiting the release of cytokines produced by immune cells (IL-2, IL-4, interferon- γ , and tumor necrosis factor- α),^{127,129–132} and inhibiting the overexpression of cell surface molecules such as MHC-I, MHC-II, intercellular adhesion molecule-1, and lymphocyte function-associated antigen-1.^{133–135}

Progress in the treatment of PBC

Despite the proven efficacy of UDCA in PBC, nearly 40% of PBC patients do not respond adequately to treatment with UDCA.¹³⁶ There are several criteria proposed to define biochemical response and nonresponse to UDCA therapy:^{89,136–140} the Mayo Clinic criteria¹³⁷ (serum ALP decrease to <2 times the upper limit of normal [ULN] at 6 months of UDCA treatment), Barcelona criteria¹³⁶ (serum ALP decrease to >40% from baseline or to normal value after 12 months of UDCA treatment), Paris criteria⁸⁹ (decrease in serum ALP to ≤ 3 times ULN, decrease in serum aspartate aminotransferase to ≤ 2 times ULN, and normal serum bilirubin after 12 months of UDCA treatment), Toronto criteria¹⁴¹ (decrease in serum ALP to ≤ 1.67 times ULN after 12 months of UDCA treatment), and the Rotterdam criteria¹³⁹ (normalization of serum bilirubin and albumin after 12 months of UDCA treatment when one or both parameters were abnormal before treatment, or normalization of serum bilirubin or albumin after 12 months of UDCA treatment when both were abnormal before treatment). These criteria of biochemical response to UDCA have now become surrogate markers of therapeutic efficacy in PBC. PBC patients who have suboptimal biochemical response to UDCA treatment are at risk of late and serious complications, such as development of liver cirrhosis, hepatocellular carcinoma, and signs of portal hypertension,¹⁴ and therefore, newer treatments for PBC are needed, as discussed later.

Farnesoid X receptor agonists and PBC

Farnesoid X receptors (FXRs)¹⁴² are nuclear hormone receptors expressed in high quantities in tissues that are involved in bile acid, carbohydrate, and lipid metabolism, such as the liver and intestines.¹⁴³ Manipulation of FXR is a breakthrough that has significantly improved our understanding of bile acid metabolism, and it also opened new therapeutic avenues for many liver disorders such

as nonalcoholic fatty liver disease and cholestatic liver diseases.

Rationale for use

FXRs have been found to be important, key regulators of bile acid metabolism in humans.¹⁴³ When activated, FXR modulates the bile acid size and pool composition by reducing the production of endogenous bile acids through suppression of the gene encoding the enzyme cholesterol 7 α -hydroxylase, the rate-limiting step in the biosynthesis of endogenous bile acids.¹⁴⁴ The reduction of the total bile acid pool decreases the bile acid workload on the liver, with beneficial effects on liver health and regeneration capacity.¹⁴⁵ Bile acids have been found to be natural ligands of FXR.¹⁴⁴ In addition to directly suppressing the gene encoding the enzyme cholesterol 7 α -hydroxylase, FXR activation has been found to indirectly suppress cholesterol 7 α -hydroxylase through regulating the expression of an intestinal growth factor named fibroblast growth factor-19 (FGF-19).^{146,147} FGF-19 has been found to suppress the expression of cholesterol 7 α -hydroxylase in human hepatocytes through a c-Jun N-terminal kinase-dependent pathway.¹⁴⁸ FXR also plays a key role in the enterohepatic circulation, as activation of FXR results in expression of the cytosolic intestinal bile acid-binding protein.¹⁴⁹ This protein facilitates the movement of bile acids from the intestines through the enterocytes into the portal circulation.¹⁵⁰

Preclinical experiments have shown that FXR activation protects against bile acid-related injury to the liver, prevents development of liver fibrosis, and exerts immunoregulatory effects on cells of innate immunity.^{151,152} Collectively, these effects could be of therapeutic benefit to patients with cholestatic liver diseases. Obeticholic acid (OCA), also known as INT-747, is a first-in-class selective FXR agonist. It is a 6 α -ethyl derivative of CDCA and selectively binds to FXR, with ~100-fold greater binding affinity to FXR than to CDCA.¹⁵²

Clinical experience

OCA has been recently investigated in 165 patients with PBC who had an inadequate response to UDCA.¹⁵³ In this Phase II clinical trial, patients with PBC and inadequate response to UDCA were initially randomized to either placebo or one of the three OCA treatment groups (10 mg, 25 mg, and 50 mg per day) for 3 months. All patients continued UDCA treatment on a stable dose throughout the study period. After completion of the initial 3 months of treatment (the randomized placebo-controlled phase), 78 patients continued treatment

with OCA in an open-label extension clinical trial for 12 more months.¹⁵³ The primary endpoint was the percentage change in serum ALP from baseline value (on day 0) to the end of treatment in the randomized placebo-controlled phase (day 85). Patients in the OCA treatment groups experienced significant reductions in serum ALP, γ -glutamyl transpeptidase (GGTP), alanine aminotransferase, aspartate aminotransferase, and bilirubin levels, compared to patients in the placebo group.¹⁵³ Specifically, mean serum ALP reduced from baseline values by 24%, 25%, and 21% in the OCA 10 mg, OCA 25 mg, and OCA 50 mg per day groups, respectively, compared to only a 3% reduction in serum ALP in the placebo group. Statistically significant change in serum ALP values in the OCA groups was observed as early as 2 weeks of treatment.¹⁵³ Only 7% (7/99) of patients in the OCA groups experienced normalization of serum ALP compared to none in the placebo group. Pruritus was the principal side effect in the OCA treatment groups: 92/127 (72.4%) compared to 19/38 (50%) in the placebo group.¹⁵³ The incidence and severity of pruritus were worse in the intermediate- and high-dose OCA treatment groups. The incidence of pruritus in the open-label extension trial phase was 87% (68/78), and 13% (10/78) discontinued OCA due to severe pruritus. Patients who were enrolled in the open-label extension clinical trial maintained biochemical response throughout the 12-month treatment period.

Patients in the OCA treatment groups (low, intermediate, and high dose) experienced significant reductions in serum ALP by the end of treatment, and this is important because ALP is a prognostic marker in PBC patients. In a recent meta-analysis involving 4,845 PBC patients from 15 North American and European clinical centers,⁸⁷ ALP was a strong predictor of clinical outcomes (liver transplantation or death), and PBC patients who experienced ALP reduction had better survival than those who had persistently elevated serum ALP levels.⁸⁷ Although the mean serum bilirubin levels were normal for patients enrolled in this study, PBC patients in the intermediate-dose and high-dose OCA treatment groups experienced statistically significant reductions in their serum bilirubin by the end of treatment. This is another potentially important finding, because elevated bilirubin is the strongest predictor of clinical outcomes and survival in patients with PBC.¹⁵⁴ Mean serum ALP levels continued to further decrease in the group of patients who were enrolled in the open-label extension trial phase (285 \pm 15 U/L at baseline versus 210 \pm 12 U/L at 3 months versus 202 \pm 11 U/L at 12 months of OCA treatment), suggesting that OCA treatment in patients with PBC should be continued for at least 1 year, if

not indefinitely, to observe the desired effect of OCA on the long-term outcomes of PBC.

There are several questions that remain to be answered: 1) What is the effect of the combination therapy of OCA and UDCA on the clinical course and long-term outcomes of PBC? 2) What are the long-term side effects and adverse events related to OCA alone, and to the combination therapy of OCA and UDCA, if any? 3) What is the effect of OCA monotherapy on short- and long-term outcomes in PBC patients? Clearly, to answer these questions, large-scale and long-term controlled clinical trials are needed.

Fibrates and PBC

Fibrates (fenofibrate and bezafibrate) are fibric acid derivatives that are widely used for the treatment of hyperlipidemia and hypertriglyceridemia. Fibrates act mainly by suppressing acetyl coenzyme A carboxylase.¹⁵⁵ The peroxisome proliferator-activated receptors (PPARs) are the main molecular targets of fibrates.¹⁵⁶ There are three distinct isoforms of PPARs in humans that are encoded by distinct genes: PPAR- α , PPAR- δ , and PPAR- γ .¹⁵⁷ PPAR- α is highly expressed in tissues that participate in lipid metabolism, such as the liver, kidney, heart, and skeletal muscle, and activation of PPAR- α results in β -oxidative degradation of fatty acids and regulation of transcription of genes involved in lipid metabolism.^{158,159} Fibrates exhibit different potencies to all three human isoforms of PPARs.¹⁵⁷

Rationale for use

In 1999, Iwasaki et al noted that patients with hypercholesterolemia experienced significant reductions in serum ALP, GGTP, and IgM when they were started on bezafibrate.¹⁶⁰ Based on these events, they suggested that treatment with fibrates might be of therapeutic benefit in patients suffering from cholestatic liver disease. Since then, significant biochemical improvement in patients with PBC receiving fibrates (bezafibrate and fenofibrate) has been reported across several pilot studies, and Phase III clinical trials are needed.¹⁶¹

Although the exact mechanism of action of fibrates in cholestatic liver diseases remains unclear, several hypotheses have been proposed. PPAR- α activation results in downregulation of bile acid synthesis by inhibiting cholesterol 7 α -hydroxylase and sterol 27-hydroxylase¹⁶² and regulation of bile acid detoxification by upregulation of uridine 5'-diphospho-glucuronosyltransferase 2B4, cytochrome P450 3A4, apical sodium-dependent bile acid transporter (ASBT), and sulfotransferase 2A1.^{159,163–165}

These induced changes in bile acid metabolism are thought to be beneficial in patients with cholestatic liver diseases. Fibrates have been found to directly enhance biliary excretion of phosphatidylcholine by upregulation of the Mrp3 through stimulation of PPAR- α .¹⁶⁶ This activity is thought to be important because it aids in the excretion of the toxic hydrophobic bile acids by forming hydrophilic compounds and micelles. In animal models, fibrates minimize the degree of injury induced by cholestasis by upregulation of the Mrp4 and Mrp3.¹⁶⁷ These proteins are normally expressed in the basolateral surfaces of the hepatocytes and play a crucial role in the efflux of bile acids into the systemic circulation.¹⁶⁸ In cholestasis, their upregulation is therapeutically important because it is believed to be a defensive mechanism by which the liver minimizes the tissue damage caused by cholestasis.¹⁶⁹

Nitric oxide (NO) production pathway is an attractive therapeutic target in many diseases. NO production is catalyzed by the enzyme nitric oxidase synthase.¹⁷⁰ In inflammatory and autoimmune states, NO contributes to the tissue inflammation by damaging the mitochondria and inducing proinflammatory cytokines.¹⁷⁰ Increased levels of NO in the sera and increased expression of nitric oxidase synthase at the site of damaged biliary epithelial cells have been reported in PBC.^{171–173} It has been proposed that fibrates ameliorate portal tract inflammation and bile duct injury in PBC by downregulation of NO production.¹⁷⁴

Fibrates have also been found to inhibit migration of immune cells to the liver in PBC by decreasing the expression of bile acid-induced regulated upon activation normal T-cell expressed and secreted (RANTES) and by inhibiting DNA-binding activity and transcriptional activity of nuclear factor kappa B (NF- κ B).¹⁷⁵ In addition, fibrates may also have immunoregulatory effects in PBC such as inhibiting CD4⁺CD25⁺ regulatory T-cells (Tregs) apoptosis induced by B-cell activating factor.¹⁷⁶ Tregs play a critical role in controlling the production of inflammatory cytokines by the activated immune cells in autoimmune conditions such as PBC.¹⁷⁶

In patients with PBC, the addition of bezafibrate to UDCA improved the serum hepatic fibrosis parameters,¹⁷⁷ suggesting that fibrates not only act as inflammatory agents in PBC but also act as antifibrotic agents. A recent study has shown that fibrates act as both PPAR- α and pregnane X receptor (PXR) agonists.¹⁷⁸ Collectively, these data suggest that fibrates might be of therapeutic benefit in patients with cholestatic liver diseases.

Clinical experience

Several pilot studies have consistently shown that fibrates might be of therapeutic benefits in patients with cholestatic liver diseases, particularly PBC.^{160,179–192} The use of fibrates in PBC has demonstrated satisfactory outcomes with an excellent safety profile, and although results are preliminary, data suggest that fibrates are beneficial in PBC. However, large-scale multicenter clinical trials are awaited. Most of the studies reported the use of fibrates in addition to UDCA in PBC patients with insufficient response to UDCA, and fibrates are not approved by the federal health agencies for the treatment of PBC. Only one pilot study reported the use of bezafibrate alone compared to UDCA in PBC patients.¹⁸² In this clinical trial, patients in the bezafibrate group showed more significant improvement in liver biochemistries compared to patients in the UDCA group.¹⁸²

Fibrate use results in significant reduction in cholestatic parameters (serum ALP and GGTP), transaminases, and IgM levels in PBC.^{160,179,182–184,189–192} Effects of fibrates on the biochemical parameters can be observed as early as 1 month of the beginning of therapy, and most patients sustained the biochemical response as long as they were on fibrates. Discontinuation of fibrates results in rebound elevation of biochemical indices in patients with PBC, and an improvement in the biochemical indices is almost always observed after treatment with fibrates is reinstituted, further supporting the potential therapeutic benefit in PBC. The effects of fibrates on the histological progression of PBC have been reported from only two studies; one study showed amelioration of portal tract inflammation and cholangitis in two out of three cases of PBC,¹⁸⁷ and another study showed no significant change in follow-up histological evaluation in one case and histological progression in the other case of PBC.¹⁸¹

Almost all studies have shown a remarkable reduction in serum IgM when measured, and whether this change is of prognostic importance in PBC patients receiving fibrates is unclear. In addition to the biochemical improvement, significant pruritus relief has been observed in patients with PBC with inadequate response to UDCA following institution of fibrate therapy.¹⁸³ Patients experienced worsening of pruritus when bezafibrate was discontinued, and pruritus improved or completely disappeared after bezafibrate was reinstituted.¹⁸³ These data lend support to the use of fibrates in PBC patients, and suggest that fibrates could be used in the management of pruritus.

Data from a preliminary Spanish study examining the effects of bezafibrate on symptoms and hepatic biochemical

indices in PBC patients with inadequate response to UDCA suggest that patients with early-stage PBC benefit more from the combination therapy than patients with advanced-stage PBC.¹⁸³ These results need to be verified in a large-scale, long-term clinical trial.

The use of fibrates in PBC patients (alone or in combination with UDCA) has been shown to be generally safe and well tolerated. Heartburn and nausea are the most commonly reported side effects related to fibrates therapy in PBC, with a reported incidence of 25% and 15%, respectively.¹⁹⁰ In the pilot study reported from the US, one out of 20 PBC patients developed ulcerative esophagitis, possibly related to treatment with fenofibrate.¹⁹⁰ Elevated serum transaminases (2–5 times the ULN) have been reported (~10%), but these events are usually transient, lasting a few weeks, and rarely lead to discontinuation of the drug.¹⁹⁰

The long-term use (~9 years) of combination therapy of UDCA and bezafibrate in PBC patients with inadequate response to UDCA has been associated with increased serum creatinine levels,¹⁹³ raising concerns for potential kidney injury due to fibrates. Although these studies have provided useful preliminary information with regard to the use of fibrates in PBC, they are criticized for small sample sizes, short-term duration of therapy, scarcity of data collected, and more importantly, lack of control groups.

Glucocorticoids and PBC

Glucocorticoids (GCs) have long been used in the treatment of numerous inflammatory and autoimmune clinical conditions such as asthma, Crohn's disease, and autoimmune hepatitis. In this review, we discuss the role of GCs, particularly budesonide, in the treatment of PBC.

Rationale for use

GCs are known to suppress the inflammation by various ways. GCs act mainly via a cytosolic GC receptor (cGCR) that binds to specific DNA-binding sites resulting in induced synthesis of anti-inflammatory molecules such as lipocortin 1 and IκB, and suppression of transcription of inflammatory genes such as IL-1, IL-2, tumor necrosis factor-α, and interferon-γ.¹⁹⁴ Moreover, the GC/cGCR complex interacts physically with NF-κB to block its transcriptional activity within the cell.¹⁹⁵ NF-κB carries out key functions in the induction and perpetuation of inflammation such as stimulation of transcription of chemokines, inflammatory cytokines, complement proteins, cell adhesion molecules, and receptors for these molecules.¹⁹⁶ NF-κB also induces transcription of cyclooxygenase 2, an enzyme essential for

prostaglandin production.¹⁹⁶ GCs suppress the dendritic cell activity, decrease the number of B-cells, attenuate B-cell progenitor proliferation, and suppress antibody production by B-cells.¹⁹⁴ GCs inhibit helper and cytotoxic T-cell activity, and suppress production of cytokines by T-cells.¹⁹⁴ More recently, GCs have been found to improve bile flow in cholestatic conditions by upregulation of the anion exchanger 2 protein,¹⁹⁷ which may play an important role in biliary excretion of bicarbonate.

Clinical experience

GCs have been evaluated in patients with PBC. Prednisolone use in PBC improves liver biochemistries, but its extensive systemic side effects hamper its long-term use.^{198,199} Budesonide has gained more attention recently due to its high binding affinity to GC receptors and relatively low bioavailability.²⁰⁰

Two randomized clinical trials have shown that the combination therapy of UDCA and budesonide was superior to UDCA alone in PBC.^{201,202} In particular, the combination therapy of UDCA and budesonide improved liver biochemistries and histological abnormalities compared to UDCA alone.^{201,202} The reduction in liver chemistries was significantly more pronounced in the combination therapy (UDCA plus budesonide) groups.^{201,202} Side effects reported were acne, skin bruises, hirsutism, nausea, and weight gain.^{201,202}

In a German study,²⁰¹ the reported changes in the bone mineral density (BMD) after 2 years of treatment were not significant between the two treatment groups (–1.74% in the UDCA plus budesonide group versus –0.98% in the UDCA monotherapy group). In the Finnish study,²⁰² the reported change in BMD after 3 years of treatment was not significant between the two groups (UDCA plus budesonide versus UDCA alone). In the UDCA plus budesonide group, the BMD in femoral neck and lumbar spine was decreased by 3.6% and 2.8%, respectively, from the baseline.²⁰³ In the UDCA monotherapy group, the corresponding decreases were 1.9% and 0.7% from baseline.²⁰³

In a 1 year open-label study²⁰⁴ of 22 PBC patients with inadequate response to UDCA, the addition of budesonide resulted in marginal improvement of serum ALP and bilirubin. In this study, treatment with the combination therapy was associated with significant bone loss in the lumbar spine.²⁰⁴ This study²⁰⁴ included a small number of patients (n=22) compared to the German (n=40)²⁰¹ and Finnish (n=69)²⁰² clinical trials, and had no comparator group. These data suggest that combination therapy of UDCA and

budesonide is potentially safe and effective in patients with early-stage PBC, and long-term clinical trials are warranted. BMD should be regularly monitored in PBC patients on budesonide therapy.^{201–204} The use of budesonide in patients with advanced-stage PBC, however, has been associated with serious adverse events (mainly portal vein thrombosis).²⁰⁵

Combination therapy with UDCA (13–15 mg/kg body weight per day), budesonide (6 mg per day), and mycophenolate mofetil (MMF, 1 g per day) has been shown in a pilot study to improve the liver biochemistries and histological abnormalities in 13 out of 15 patients with non-cirrhotic PBC with significant interface hepatitis who had suboptimal response to UDCA alone.²⁰⁶ Taken together, these studies suggest that the UDCA–budesonide combination therapy, with or without MMF, could be considered in PBC patients with suboptimal response to UDCA alone. Patients with pre-cirrhotic-stage PBC are more likely to benefit from GC therapy than patients with cirrhotic-stage PBC.¹⁹⁹ The UDCA–budesonide combination therapy is currently being evaluated in a randomized clinical trial in Europe.

Other treatments in PBC

NGM282 is an FGF-19 analog and downregulates bile acid synthesis by decreasing the expression of cholesterol 7 α -hydroxylase. NGM282 is currently being investigated in a Phase II clinical trial in patients with PBC.

The CD28/CTLA4:B7 costimulatory pathway is a crucial step in T-cell-dependent B-cell activation and is characterized by binding of CD28 and CTLA4 on T-cells to B7-1 and B7-2 on activated antigen-presenting cells (B-cells, macrophages, and dendritic cells).²⁰⁷ Binding of CD28 to B7-1 and B7-2 results in T-cell activation, proliferation, differentiation, and release of inflammatory cytokines.²⁰⁷ On the other hand, binding of CTLA4 to B7-1 and B7-2 results in inhibition of T-cell activation, proliferation, and differentiation. Abatacept,²⁰⁷ a fusion protein composed of the Fc portion of IgG1 fused to CTLA4, is currently being investigated in PBC patients with inadequate response to UDCA.

The ASBT (or *SLC10A2*), localized at the apical membrane of the cholangiocytes, ileum, and renal proximal tubules, plays an important role in maintaining the enterohepatic circulation of bile salts.²⁰⁸ Uptake of bile salts across the apical membrane of enterocytes is mediated by the ASBT.²⁰⁸ Inhibition of ASBT results in disruption of the enterohepatic circulation and increased fecal loss of bile salts.²⁰⁹ The resultant inhibition of uptake of bile salts at the intestinal level reduces the amount of bile salts circulating back to the liver, and this effect is thought to be of therapeutic benefit in

patients suffering from cholestatic liver diseases. LUM001, a novel ASBT inhibitor, is currently being evaluated in a Phase II clinical trial in patients with PBC.

The TGR5 receptor, the first known G-protein-coupled receptor specific for bile acids,²¹⁰ is distributed throughout the body organs and tissues, including the liver, and is found in Kupffer cells, liver sinusoidal endothelial cells, biliary tree, and gall bladder epithelial cells.²¹¹ Experimental studies have shown that TGR5 receptor activation results in downregulation of inflammatory responses, and stimulation of NO production,²¹⁴ and bicarbonate secretion by the biliary epithelium.²¹⁵ These physiological effects might have important therapeutic implications in hepatic diseases, including PBC. Several TGR5 receptor agonists are currently being developed. Clinical trials are needed to examine whether TGR5 receptor agonists have clinical efficacy in the treatment of PBC.

The nuclear receptors, constitutive androstane receptor (CAR) and PXR, participate in the regulation of genes involved in the detoxification and transportation of bile acids and bilirubin.²¹⁶ Preclinical studies have shown that activation of CAR and PXR through ligands increases the expression of hepatic export systems (Mrp2, Mrp3, and Mrp4) for bile acids and bilirubin, enhances the detoxification of biliary compounds, and reduces serum levels of bilirubin and bile acids.²¹⁷ The hepatic expression of the Mrp2 and Mrp3 has been shown to be enhanced only in the early stages of PBC compared to the late stages of PBC, suggesting that the lack of upregulation of these proteins contributes to the progression of PBC.²¹⁸ Clearly, clinical trials are needed to test the role of CAR and PXR agonists in the treatment of PBC.

Nor-UDCA, a novel C23 homolog of UDCA, has been shown to have potent choleretic and antifibrotic effects in mouse models of liver disease.^{219–222} These findings might be of therapeutic significance in PBC. Currently, a clinical trial of *nor*-UDCA in primary sclerosing cholangitis patients is underway.

Challenges in the treatment of PBC

There are several challenges in the development and evaluation of new treatments for PBC. First, PBC is an uncommon disease and is designated as an orphan disease by the US Food and Drug Administration. The relative rarity of PBC does not easily allow enrollment of the number of study subjects needed to detect a statistically significant difference when comparing a candidate agent against a comparator (such as placebo). Consequently, the results of clinical trials are frequently based on a small group of patients, and strong

conclusions regarding preliminary safety and efficacy data are often difficult to make. Even Phase III clinical trials in PBC are often criticized by the small number of patients enrolled for the study. For example, the Canadian multicenter randomized clinical trial,¹⁰⁶ one of the largest trials to date in PBC, enrolled only 54.4% (222/408) of the planned number of study subjects.

PBC is a chronic liver disease with a variable course that often slowly progresses to fibrosis, cirrhosis, and end-stage liver disease. Natural history studies of PBC have shown that the estimated median survival of PBC is 10–15 years.^{83,86} In reality, PBC patients enrolled in clinical trials of candidate agents should be followed for at least 10 years (after an enrollment period of ~3–5 years) to determine the true effect of the new candidate agent on the clinical outcomes of the disease. This is impractical because of the difficulty in enrolling the required number of patients in a short period of time and the difficulty in retaining the study subjects for such a long period of time.^{223,224}

Another major challenge in developing an effective treatment for PBC is the lack of surrogate endpoints.²²⁵ There has been an intense search to identify accurate noninvasive markers that could serve as surrogate endpoints in clinical trials of PBC.⁸⁷ For a surrogate endpoint to be an effective substitute for the clinical outcome of a specific disease or condition, the effects of the intervention (drug, biological agent, device, procedure, etc) on the surrogate endpoint must reliably predict the overall effect on the clinical outcome.²²⁶ Generally, surrogate endpoints can be useful in Phase II clinical trials for identifying if a new intervention is promising enough to be evaluated in a Phase III clinical trial.²²⁶ In Phase III clinical trials, the endpoint ideally should be a clinical event relevant to the patient.²²⁶ In PBC, of all the noninvasive markers, serum bilirubin is the strongest predictor of clinical outcome.^{92,154} Serum bilirubin levels in PBC patients tend to increase as the disease progresses.²²⁷ Thus, one might expect a favorable outcome if a new treatment results in a decrease in serum bilirubin level to the normal range and maintenance of serum bilirubin level within the normal range over a long period of time. In PBC, serum bilirubin levels tend to increase in the late stages.²²⁷ Therefore, serum bilirubin could serve as a surrogate endpoint only in patients with advanced-stage PBC. ALP as discussed previously is currently the most frequently used surrogate measure in clinical trials in PBC. The use of liver stiffness measurements (measured by transient elastography, TE) as a surrogate endpoint in clinical trials of PBC has been recently evaluated. TE has been shown to have high

performance (diagnostic performance of 0.92 for fibrosis stage ≥ 3 , and 0.99 for fibrosis stage =4)²²⁸ and to perform better than noninvasive markers²²⁸ in identifying any grade of fibrosis or cirrhosis in PBC.^{228–230} Long-term studies are needed for confirmation.

Conclusion

PBC is an autoimmune disease of the liver that results in destruction of the interlobular hepatic bile ducts. Currently, UDCA is the only drug approved for the treatment of PBC. UDCA improves the liver biochemistries, delays histological progression, and prolongs survival free of liver transplantation. Approximately 40% of PBC patients do not respond to UDCA; these patients are at high risk of serious adverse events. There are several new drugs that are currently being investigated as alternative therapeutic options in patients with PBC who fail UDCA therapy, and preliminary results from these clinical trials are promising. OCA is a first-in-class FXR agonist that showed encouraging results in a Phase III clinical trial in PBC patients who had inadequate response to UDCA. Larger controlled clinical trials are needed to determine the long-term effects of fibrates and GCs on the clinical outcomes of PBC. Studies to identify reliable surrogate endpoints are needed.

Disclosure

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