Review Article

Antihepatic Fibrosis Drugs in Clinical Trials

Yue-Cheng Guo[1,2] and Lun-Gen Lu*[1,2]

[1]Shanghai Key Laboratory of Pancreatic Diseases, Shanghai Jiao Tong University School of Medicine, Shanghai, China;
[2]Department of Gastroenterology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, China

Abstract

Liver fibrosis is not an independent disease. It refers to the abnormal proliferation of connective tissues in the liver caused by various pathogenic factors. Thus far, liver fibrosis has been considered to be associated with a set of factors, such as viral infection, alcohol abuse, non-alcoholic fatty liver disease, and autoimmune hepatitis, as well as genetic diseases. To date, clinical therapeutics for liver fibrosis still face challenges, as elimination of potential causes and conventional antifibrotic drugs cannot alleviate fibrosis in most patients. Recently, potential therapeutic targets of liver fibrosis, such as metabolism, inflammation, cell death and the extracellular matrix, have been explored through basic and clinical research. Therefore, it is extremely urgent to review the antihepatic fibrosis therapeutics for treatment of liver fibrosis in current clinical trials.

Citation of this article: Guo YC, Lu LG. Antihepatic fibrosis drugs in clinical trials. J Clin Transl Hepatol 2020;8(3):304–312. doi: 10.14218/JCTH.2020.00023.

Introduction

Hepatic fibrosis, a reversible response to various chronic liver injuries, may progress to cirrhosis, hepatocellular carcinoma and liver failure. Cirrhosis is the common end-stage of a series of chronic liver diseases and can be divided according to its compensated and decompensated states. A series of complications of cirrhosis (such as portal hypertension, infection, ascites, and esophageal bleeding) are associated with significant morbidity and mortality.

The mechanism involved in the progression and reversal of liver fibrosis is still not clear. Up to now, alcohol, hepatocyte lipid deposition, and insulin resistance (IR) are recognized to be the major risk factors in patients with chronic hepatitis.

Besides, intrahepatic oxidative stress, viral and schistosomiasis infection, hepatic sinus microcirculation disturbance, and microbiota dysbiosis also participate in the occurrence and development of liver fibrosis.[1]

Current therapies for liver fibrosis encompass two aspects: etiology treatments and antifibrotic therapeutics. Removal of underlying etiology of liver injury makes liver fibrosis reversible. However, up to now, there is lack of cause-specific treatment for certain liver diseases, such as non-alcoholic fatty liver disease (NAFLD), cholestatic liver diseases (CLDs), and some genetic liver diseases. Therefore, there remains a need to develop direct antifibrotic therapies for liver fibrosis. Over the past several decades, many researchers have proposed potential targets and alternative therapies for liver cirrhosis. Unfortunately, there is no effective antifibrotic drug approved for human use, up to now. This review will focus on the representative drugs for liver fibrosis in clinical trials.

Pathogenesis of hepatic fibrosis

Fibrosis in epithelial organs is produced by the reticular deposition of extracellular matrix (ECM) and chronic inflammation, accompanied by compromised immune systems and pathological angiogenesis. Liver fibrosis is an abnormal perpetuation of fibrogenesis due to various constant chronic liver injuries. Apoptosis of liver parenchymal cells and continuous accumulation of ECM gradually replace the liver parenchyma with scar tissue, eventually forming liver architectural distortion, cirrhosis, portal hypertension, liver cancer, and liver failure.[2]

As illustrated in Fig. 1, activation of hepatic stellate cells (HSCs) is well established as the central driver of hepatic fibrosis. HSCs are derived from embryonic mesothelial cells. Quiescent HSCs mainly store vitamin A and produce type IV collagen, while activated HSCs produce collagen type I, III and other proteins (fibronectin, elastin, laminin) after liver injuries. In addition, activated HSCs exhibit a developed proliferative phenotype and contractile function. Abundant ECM deposition and collagen production result in the increase of liver stiffness, thereby stimulating the activation of HSCs and forming a positive feedback loop to develop cirrhosis.[3,4]

Besides HSCs, other liver cells also contribute to matrix protein production. Hepatocytes, sinusoidal endothelial cells, and lymphocytes are involved in the development of liver fibrosis through releasing cell contents and cytokines.[1] Macrophages in the liver comprise Kupffer cells and monocyte-derived macrophages, and the latter stimulates HSCs to become collagen-producing myofibroblasts.[5]

Compelling evidence from animal models indicates that liver fibrosis is reversible. Firstly, modifications in ECM composition may regulate cellular functions partly through
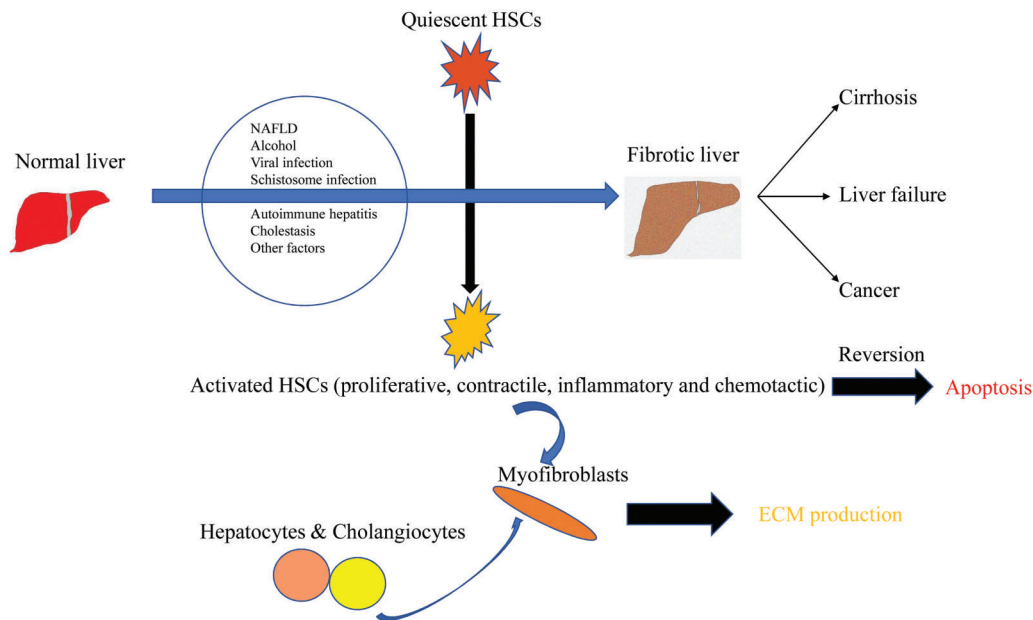
Fig. 1. HSCs are the key cells in the progression and regression of liver fibrosis.

cell adhesion molecules and participate in the regression of fibrosis.[1,6,7] Secondly, cell death not only induces inflammation and promotes fibrogenesis but may also contribute to fibrosis resolution through influencing the apoptosis and senescence of activated HSCs.[6] Lastly, infiltrating macrophages play a detrimental role in liver fibrosis. Infiltrating macrophages have shown profibrogenic and proinflammatory features in the progression of fibrosis. However, in a murine model of hepatic fibrosis, the increased restorative macrophages are associated with the accelerated resolution of fibrosis.[8] Overall, the resolution of liver fibrosis is also a complex process.

Cause-specific treatments for liver fibrosis

Currently, the most important treatment is controlling the underlying cause of the liver diseases. These include effective suppression or elimination of hepatitis virus replication (hepatitis B virus and hepatitis C virus), drug eradication of schistosomiasis, relieving cholestasis, reduction of body weight in NAFLD, improvement of associated metabolic disorders, cessation of alcohol use in patients with alcoholic liver disease (ALD), phlebotomy in hemochromatosis, and use of corticosteroids/immunosuppressants for autoimmune liver disease. All these therapies can reduce persistent hepatic injury, thereby inhibiting liver fibrogenesis and/or promoting fibrolysis.

Many researchers demonstrated that effective inhibition of hepatitis B virus or hepatitis C virus replication significantly improved the fibrosis stage in patients with hepatitis B or C, and that liver fibrosis can be reversed in some participants.[9–11] However, there are still some patients with virological and biochemical attenuation, whose liver fibrosis still exists and even progresses, eventually into liver cancer.[12] Additionally, it takes a prolonged period of time to promote the resolution of liver fibrosis after viral elimination. Antiviral

benefits may be offset by the increased rates of drug resistance over time.[11]

Treatment based on the etiology may not completely attenuate all fibrosis patients, as there are currently no effective managements for eliminating the cause of certain liver diseases, such as autoimmune hepatitis.[12] Thus, direct antifibrotic therapies targeting ECM metabolism and HSC activation are indispensable.

Antifibrotic therapies

Liver fibrosis plays an important role in liver tissue repair in the early stage of injury. However, the management of liver fibrosis is required in significant or advanced fibrosis and cirrhosis. At present, there is no recognized and effective antifibrotic agent. Chronic inflammatory response is the premise of fibrogenesis and the driving force of fibrotic progression. Antiinflammation, hepatocyte protection and antioxidation are important methods for hepatic fibrosis. In the following sections, we mainly review representative drugs based on therapeutic targets. Table 1 displays phase II/III/IV clinical trials for current therapies.

Bile acid homeostasis and energy metabolism

Ursodeoxycholic acid

CLDs represent a series of hepatobiliary diseases accompanied by disorders of bile formation, secretion, and excretion. The CLDs mainly include primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC), both having a risk of progression to liver fibrosis. Ursodeoxycholic acid (UDCA), a physiologic hydrophilic dihydroxy bile acid, is the primary drug for treating CLD. Its antifibrotic property may be associated with the stimulation of bile secretion and the suppression of inflammation.[13] Parés *et al.*[14] pointed out that UDCA (15 mg/kg daily, 1.5-14 years) improved survival

Table 1. Clinical trials of potential antifibrotic therapies in chronic liver disease (https://clinicaltrials.gov)

NCT	Phase	Status	Drug	Target	Population (*n*)	Primary endpoints
NCT01510860	Phase IV	Completed	UDCA	Bile acid	PBC (65)	Biochemical response
NCT03345589	Phase IV	Unknown	UDCA	Bile acid	refractory PBC (40)	Biochemical response
NCT00550862	Phase II	Completed	INT-747	FXR agonist	PBC (165)	Biochemical response
NCT01473524	Phase III	Completed	Obeticholic acid	FXR agonist	PBC (217)	Biochemical response
NCT00570765	Phase II	Completed	INT747	FXR agonist	PBC (59)	Biochemical response
NCT02516605	Phase II	Completed	Tropifexor	FXR agonist	PBC (61)	Biochemical response
NCT04065841	Phase II	Suspended (COVID-19 pandemic)	Tropifexor	FXR agonist	NASH (210)	Histological improvements
NCT03517540	Phase II	Recruiting	Tropifexor	FXR agonist	NASH (200)	Adverse events
NCT02943460	Phase II	Completed	Cilofexor	FXR agonist	PSC (52)	Adverse events
NCT02854605	Phase II	Completed	Cilofexor	FXR agonist	NASH (140)	Adverse events
NCT01654731	Phase III	Completed	Bezafibrate	PPAR agonist	Refractory PBC (100)	Biochemical response
NCT02937012	Phase III	Unknown	Bezafibrate	PPAR agonist	Refractory PBC (34)	Biochemical response
NCT02823353	Phase III	Unknown	Fenofibrate	PPAR agonist	PBC (200)	Biochemical response
NCT02955602	Phase II	Unknown	Seladelpar	PPAR agonist	Refractory PBC (116)	Biochemical response and adverse events
NCT02217475	Phase II	Completed	Cenicriviroc	Dual antagonists of chemokine receptors CCR2 and CCR5	NASH (289)	Histological improvements
NCT02462967	Phase II	Completed	GR-MD-02	Galectin-3 inhibitor	NASH (162)	HVPG
NCT02443116	Phase II	Active, not recruiting	NGM282	FGF19 analogs	NASH (250)	Liver fat content
NCT02413372	Phase II	Completed	BMS-986036	FGF21 analogs	NASH (184)	Liver fat content and adverse events
NCT03486912	Phase II	Active, not recruiting	BMS-986036	FGF21 analogs	NASH and liver fibrosis (152)	Histological improvements

(*continued*)

Table 1. *(continued)*

NCT	Phase	Status	Drug	Target	Population (*n*)	Primary endpoints
NCT03486899	Phase II	Active, not recruiting	BMS-986036	FGF21 analogs	NASH (160)	Histological improvements
NCT03053063	Phase III	Terminated	Selonsertib	Apoptosis signal-regulating kinase 1 inhibitor	NASH (883)	Histological improvements and Event-Free Survival
NCT02230670	Phase II	Completed	Emricasan	Inhibitor of pan-caspase	Cirrhosis with MELD between 11-18 (87)	Change in cCK18/M30
NCT03205345	Phase II	Active, not recruiting	Emricasan	Inhibitor of pan-caspase	NASH-related cirrhosis (210)	Event-free survival
NCT02161952	Phase II	Completed	Pirfenidone	Inhibitor of TGF-β	Chronic hepatitis C (150)	Histological improvements
NCT04099407	Phase II	Recruiting	Pirfenidone	Inhibitor of TGF-β	Advanced cirrhosis (100)	Histological improvements
NCT02499562	Phase II	Unknown	Hydronidone	Inhibitor of TGF-β	Chronic hepatitis B (240)	Histological improvements
NCT01672866	Phase II	Terminated	Simtuzumab	LOXL2 antibody	NASH-related cirrhosis (222)	Histological improvements
NCT01672879	Phase II	Terminated	Simtuzumab	LOXL2 antibody	NASH-related cirrhosis (259)	HVPG and Event-Free Survival
NCT01707472	Phase II	Completed	Simtuzumab	LOXL2 antibody	HIV-hepatitis C virus coinfected cirrhosis (18)	Adverse events
NCT01672853	Phase II	Completed	Simtuzumab	LOXL2 antibody	PSC (235)	Histological improvements
NCT00990639	Phase II	Completed	Candesartan	Angiotensin receptor blocker	Alcoholic liver fibrosis (85)	Histological improvements
NCT03770936	Phase III	Recruiting	Candesartan and ramipril	Angiotensin receptor blocker and angiotensin-converting enzyme inhibitor	Chronic hepatitis C (45)	FibroScan or APRI score

Abbreviations: APRI, aspartate aminotransferase-to-platelet ratio index; HVPG, hepatic venous pressure gradient; PPAR, peroxisome proliferators-activated receptor.

and delayed the histologic stage progression of PBC patients. UDCA use can significantly reduce alkaline phosphatase (ALP; an essential indicator of CLD activity) levels in patients. The most frequent adverse event in clinical trials is diarrhea, which rarely leads to discontinuation of therapy.

However, only 20-30% of PBC patients had a complete response to UDCA, and UDCA discontinuation deteriorates liver biochemical indicators and is associated with pruritus.[15] UDCA has an optimal dose of 13 to 15 mg/kg/d. Lindor *et al.*[16] found that high-dose UDCA (28-30 mg/kg daily, 5 years) increased the risk of advanced cirrhosis, esophageal varices, and bile duct cancer. A new phase IV clinical trial (NCT03345589) has been proposed by West China Hospital, Chengdu, China. This trial aims to define whether an alternative dose (18-22 mg/kg daily) of UDCA is effective in refractory PBC.

For PSC, the American Association for the Study of Liver Diseases recommends not to use UDCA as a treatment, as PSC patients did not exhibit significant improvements after a long-term UDCA therapy.[17]

Farnesoid X receptor agonist

Farnesoid X nuclear receptor (FXR), also known as bile acid receptor, is involved in the synthesis, secretion and reabsorption of bile acids. FXR activation effectively improves lipid metabolism through improving insulin sensitivity, decreasing hepatic gluconeogenesis, and reducing circulating triglycerides.[18] Thus, FXR is a potential target for non-alcoholic steatohepatitis (NASH) and CLD-related liver fibrosis.

Obeticholic acid (OCA), an agonist of FXR, decreases bile acids synthesis and exerts antiinflammatory and antifibrotic effects. In a multicenter, randomized, placebo-controlled trial,[19] patients with NASH exhibited improvements of liver histological features after treatment with OCA (25 mg, 72 weeks). However, OCA therapy was accompanied by a deterioration involving dyslipidemia and IR, indicating an increased risk of atherogenesis. Given these adverse events, OCA treatment for NASH should be further evaluated in long-term prospective trials.

OCA has also been widely used in clinical trials for PBC. Nevens *et al.*[20] conducted a phase III clinical trial in PBC patients with inadequate response to conventional drugs. After 12 months of therapy, the OCA had significantly reduced ALP, high-density lipoprotein, and total bilirubin levels. These effects were sustained for 2 years. Although improvements were observed at the lower dose (5 mg) in some patients, incremental benefits occurred with adjustment to the higher dosage (10 mg). Another double-blind phase II trial,[21] including 165 patients with inadequate response to UDCA, showed that OCA resulted in a clinically significant reduction in ALP at all doses (10, 25, and 50 mg). There were also significant improvements in gamma-glutamyltransferase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and conjugated bilirubin levels. In 2016, OCA was approved by the Food and Drug Administration for patients intolerant or with a null response to UDCA.

Dose-dependent pruritus was the only clinical adverse event that differed between OCA treatment and placebo.[21,22] Pruritus can be controlled by antipruritic agents or temporary OCA interruption, but high OCA doses may result in the discontinuation due to pruritus. The mechanism of cholestatic pruritus may be related to the activation of TGR5,[23] as OCA is a weak TGR5 agonist.

Non-bile acid FXR agonists have also been developed and are expected to cause less pruritus than OCA.[24] Tropifexor (also known as LJN-452), a well-tolerated FXR agonist, is suitable for once-daily dosing[25] and has been used in phase II clinical trials for PBC and NASH (NCT02516605, NCT04065841, and NCT03517540). Similarly, cilofexor (otherwise known as GS-9674) is also currently undergoing phase II trials for NASH and CLD. Cilofexor therapy (30 mg or 100 mg daily, 12 weeks) reduced ALP levels in a dose-dependent manner in patients with PSC, while adverse events (pruritus) were similar among groups.[26] In another trial,[27] cilofexor (30 mg or 100 mg daily, 24 weeks) significantly improved hepatic steatosis and liver biochemistry in patients with NASH. However, no significant improvements in liver stiffness were observed with magnetic resonance imaging-proton density fat fraction and magnetic resonance elastography. Pruritus was more common in patients receiving cilofexor at 100 mg than those receiving cilofexor at 30 mg or placebo. Additional studies of cilofexor in NASH with a longer duration are warranted.

PPAR agonist

PPAR is a key regulator of lipid metabolism in the liver and has been approved by the Food and Drug Administration as a molecular target for dyslipidemia. As distinct PPAR isoforms, PPARα regulates cholesterol and bile acid homeostasis,[28] while PPARγ contributes to inhibiting the activation of HSCs and reducing collagen production.[29] Hence, PPAR is a potential target for hepatic fibrosis.

Bezafibrate, an agonist of PPAR, is a widely used antihyperlipidemic agent. In a phase III clinical trial,[30] bezafibrate treatment (400 mg, 24 months) in addition to UDCA contributed to a significant biochemical attenuation in PBC patients with an inadequate response to UDCA. The bezafibrate group also exhibited decreased liver stiffness and improved fibrosis scores compared with placebo. Another prospective study[31] has compared the long-term efficacy between combination therapy (UDCA + bezafibrate) and UDCA monotherapy for PBC patients. The combination therapy significantly decreased ALP levels and Mayo risk scores but did not improve the survival rate. Bezafibrate may result in dose reduction or discontinuation due to the renal dysfunction or muscle pain.

Other PPAR agonists such as fenofibrate and seladelpar have also been used in phase II/III clinical trials (Table 1). According to a meta-analysis,[32] combination therapy of UDCA and fenofibrate had a superior efficacy to UDCA monotherapy in reducing ALP levels, whereas no improvements were observed in clinical symptoms. No significant differences were observed in the incidence of adverse events. However, all trials included in this meta-analysis had a small sample size and no adequate histological results were reported. In prior studies, several serious adverse events were found after fibrates therapy, including hepatotoxicity, elevated creatinine, and increased creatinine kinase.[33] Hence, larger controlled multicenter studies are required to confirm the long-term efficacy and safety of PPAR agonists.

Hepatic inflammation

Cenicriviroc

Approaches directly targeting the inflammatory recruitment are also important. CCR2 and its ligand are significantly increased in liver fibrosis, and fibrosis is significantly ameliorated in CCR2-deficient mice,[34] suggesting that CCR2 is involved in liver fibrosis. CCR5 is also associated with the accumulation of collagen and ECM.[35] Therefore, inhibition of CCRs may be a novel approach for liver fibrosis.

Cenicriviroc (CVC), an oral antagonist of the dual CCR2/CCR5 receptor, is safe, well-tolerated and has been used in phase II clinical trials in NASH patients with liver fibrosis. CVC (150 mg daily, 2 years) can significantly promote NASH regression and decrease the collagen area (as detected by morphometry on liver biopsy). Treatment benefits were more significant in patients with higher disease activity and fibrosis stage at baseline. Safety and tolerability of CVC were comparable to placebo, and adverse events (fatigue, diarrhea) had a mild or moderate severity.[36] Another phase III trial (NCT03028740) has been posted which investigates the long-term efficacy and safety of CVC in advanced cirrhosis patients. This trial mainly aims to evaluate liver histological improvements based on a larger sample. Overall, CVC has a favorable prospect in the application for liver fibrosis.

Galectin-3 inhibitor

Galectins are secreted proteins that bind to terminal galactose residues in glycoproteins on components of the ECM. Galectin-3 is highly expressed on Kupffer cells and plays a vital role in cell adhesion, inflammation, and fibrogenesis.[37] In animal models, glectin-3 inhibitors significantly reduced fibrosis stages and portal pressure.[38,39] GR-MD-02, a galectin-3 inhibitor, was safe and well-tolerated in subjects who had a definite histological diagnosis of NASH with advanced fibrosis.[40] FibroScan test showed the potential benefits of GR-MD-02 therapy (8 mg/kg daily) on liver stiffness.[40] Recently, Chalasani *et al.*[41] conducted a phase II clinical trial of GR-MD-02 in 162 patients with NASH, cirrhosis and portal hypertension. Though improvements of fibrosis or NAFLD activity scores did not differ significantly among groups, a subgroup analysis showed that GR-MD-02 therapy (2 mg/kg biweekly, 52 weeks) did reduce hepatic venous pressure gradient and development of varices in patients without esophageal varices. Spasmodic cough was the only adverse event related to the study drug. A phase III trial has been initiated to evaluate to safety and efficacy of GR-MD-02 in NASH cirrhosis patients without esophageal varices (NCT04365868).

Human fibroblast growth factor analogs

The fibroblast growth factor (FGF) family of hormones regulate metabolic functions and participate in tissue repair and regeneration.[42] Among them, FGF19 and FGF21 are considered to be closely related to NAFLD. NGM282, an engineered FGF19 analogue, has been used in phase II clinical trial for NASH.[43] NGM282 therapy (3 or 6 mg daily, 12 weeks) can significantly reduce liver fat content and decrease ALT and AST levels without severe adverse event. Similarly, pegbelfermin (BMS-986036), a pegylated human FGF21 analogue, was well-tolerated and significantly reduced hepatic fat fraction in patients with NASH.[44] No severe adverse events or drug-related discontinuations were reported. However, whether FGF analogs contribute to improving liver fibrosis histology is still unknown. It's worthy of further discussion in the follow-up clinical trials. Currently, two active phase II clinical trials (NCT03486912, NCT03486899) are investigating the efficacy and safety of pegbelfermin in patients with NASH-related fibrosis as determined by liver biopsy.

Cell death

Apoptosis signal-regulating kinase 1 inhibitor

Continuous cell death of hepatocytes is a critical trigger for liver fibrosis. The apoptosis signal-regulating kinase 1 (ASK1) regulates intracellular pathways of cell death, and its activation results in a deterioration in hepatic inflammation, apoptosis, and fibrosis. ASK1 inhibitor treatment protects against liver injury through decreasing oxidative stress reaction and the expression of pro-inflammatory cytokines.[45] As an ASK1 inhibitor, selonsertib (6 or 18 mg daily, 24 weeks) decreased collagen content and reduced lobular inflammation on liver biopsy in patients with NASH and stage 2-3 fibrosis, accompanied by improvements in liver stiffness on magnetic resonance elastography.[46] However, the majority of patients receiving selonsertib experienced at least one adverse event. Side effects, such as numbness of upper extremities and elevated liver enzymes, resulted in the discontinuation in several patients. A recent phase III clinical trial (NCT03053063) investigated the long-term (240 weeks) efficacy and safety of selonsertib in liver cirrhosis due to NASH. However, this trial was terminated early due to lack of efficacy based on the results of the week 48 analysis.

Pan-caspase inhibitor

Apoptosis-mediated inflammation and immune response also play a crucial role in the process of fibrosis. Caspases are intracellular cysteine proteases that participate in the process of apoptosis and inflammation through proinflammatory cytokines. Emricasan is a well-tolerated pan-caspase inhibitor. Emricasan (25 mg twice daily, 28 days) can effectively decrease ALT levels, reduce hepatic venous pressure gradient, and improve intrahepatic inflammation in patients with compensated cirrhosis.[47] Most reported adverse events were not serious and the frequent side effect was fatigue. Despite improvements in liver enzymes and hepatic inflammation, it is unlikely that Emricasan exerted an antifibrotic effect in 28 days.[47] Although improvements of hepatitis may occur relatively rapidly, attenuation of fibrosis will take longer. A recent phase II trial[48] demonstrated that Emricasan (25 mg twice daily, 3 months) improved liver function and reduced Child-Pugh scores in patients with advanced cirrhosis. Incidence of adverse events was similar between Emricasan and placebo groups. However, it is worth noting that the multiple etiologies of cirrhosis may obscure the actual efficacy when setting clinical scores as endpoints. Optimal doses and long-term efficacy of Emricasan will be explored in an active phase II trial (NCT03205345) in NASH patients with cirrhosis.

ECM and fibrogenesis

Transforming growth factor-β inhibitor

Transforming growth factor-β (TGF-β) is considered to be the central factor participating in liver fibrosis. Various strategies have been developed to inhibit the TGF-β pathway. Pirfenidone, an inhibitor of TGF-β, can ameliorate fibrogenesis through inhibiting the activation of HSCs and reducing collagen synthesis *in vitro*.[49] Borunda *et al.*[50] found that pirfenidone was well-tolerated and attenuated histological injuries in patients with established advanced cirrhosis after 12 months of treatment. Only 15% of patients developed adverse events, and these included photosensitivity, rash, itching, and nausea. A phase II clinical trial[51] showed that pirfenidone (1200 mg daily, 24 months) improved inflammation, fibrosis, and steatosis in patients with hepatitis C virus-related cirrhosis. All patients on pirfenidone displayed improvements of life quality and Child-Pugh scores. Side effects such as gastritis and nausea were observed. As viral clearance is indispensable to resolve liver damage, a combination therapy of pirfenidone and antiviral agents may be a new approach for viral fibrosis.

Similarly, based on structural modification of pirfenidone, hydronidone is a novel antifibrotic agent for hepatic fibrosis.[52] An open-label and randomized clinical trial showed that hydronidone was well tolerated and rapidly absorbed in young, healthy volunteers.[52] A phase II clinical trial (NCT02499562) has explored the effective dose and safety of hydronidone capsules in patients with liver fibrosis induced by hepatitis B virus infection in Shanghai General Hospital, Shanghai, China. However, related results have not been posted.

Lysyl oxidase-like protein 2 antibody

The lysyl oxidase (LOX) family is crosslinking enzymes over-expressed in liver cirrhosis and contributes to fibrogenesis by catalyzing cross-linkage of collagen and increases the stability of fibrosis in chronic hepatitis settings.[53] Among the five isoforms, LOXL2 is overexpressed by activated HSCs in liver fibrosis. Selective inhibition of LOXL2 suppresses hepatic fibrosis progression and accelerates its reversal in animal fibrosis models.[54]

However, simtuzumab, a humanized monoclonal antibody directed against LOXL2, did not show a promising benefit in clinical trials. In two phase IIb trials, simtuzumab alone (subcutaneous injections: 75 or 125 mg; intravenous infusions: 200 or 700 mg, 96 weeks) was ineffective in decreasing hepatic collagen content or fibrosis stage in patients with bridging fibrosis and hepatitis C virus infection.[55] In another 6-month open-label safety trial, though treatment was well-tolerated, no significant improvements were observed in hepatic venous pressure gradient or liver biopsy after simtuzumab therapy (700 mg, intravenous infusion every 2 weeks for 22 weeks).[56] Also, in patients with PSC, simtuzumab therapy (75 or 125 mg daily, 96 weeks) did not reduce liver collagen content or Ishak fibrosis stage.[57]

Renin-angiotensin system inhibitor

The renin-angiotensin system is a crucial regulator of liver fibrosis and portal hypertension.[58] Activated HSCs can secrete angiotensin II that promotes liver fibrosis through angiotensin receptors. Yoshiji *et al.*[59] demonstrated that angiotensin-converting enzyme inhibitor (ACEI) combination (perindopril: 4 mg daily, 12 months) with interferon decreased serum fibrosis markers (hyaluronic acid, 7-S-collagen, P-III-P, TGF-β) in patients with chronic refractory hepatitis C. Another clinical trial[60] demonstrated that ACEI (captopril: 25-75 mg daily, 3 months) effectively reduced the portal pressure and prevented variceal bleeding in portal hypertensive patients. The adverse effects of captopril (orthostatic hypotension and dry cough) were not severe enough to stop medication. However, these studies are limited by their lack of histological and immunohistochemical examinations.

Similarly, angiotensin receptor blockades may also attenuate liver fibrosis. Losartan (50 mg daily, 48weeks) can decrease serum aminotransferase levels and promote histological improvements in NASH with no adverse events.[61] In a randomized open-label controlled study, a combination therapy of candesartan (8 mg daily) and UDCA (600 mg daily) for 6 months also suppressed the expression of fibrosis biomarkers and decreased arterial blood pressure in alcoholic liver fibrosis with no significant complications or side effects.[62] Recently, a phase III trial (NCT03770936) has been posted to compare the efficacy of candesartan and ramipril in hepatitis C virus-related liver fibrosis.

Challenges

At present, due to the complex mechanisms involved in hepatic fibrosis, antihepatic fibrosis therapies still face many problems, as outlined here:[12,63] 1) Hepatic fibrosis has a long disease course. The most reliable method for evaluating the efficacy of treatments is to observe the histopathological changes. In contrast, it is not appropriate to use the clinical serum or radiologic measurements directly as the primary endpoints. 2) Currently, the mechanism involved in liver fibrosis has not been elucidated thoroughly. Murine models may not accurately mimic human liver diseases. 3) Up to now, many antifibrotic trials still assess the efficacy by liver biopsy, which is costly and can be affected by sampling. The invasive inspection may result in complications. 4) Though many clinical trials confirm the efficacy and safety of combination therapy, the role of each component is not defined. 5) Many scholars consider stem cells as a potential option. However, the cellular and molecular basis of liver regeneration in liver fibrosis have not been elucidated totally.

Conclusions

Cirrhosis is a severe form of chronic liver disease and is the typical outcome of various etiologies-induced liver injuries. In this article, we reviewed current therapies in clinical trials. Safe and effective therapies may delay the development of decompensated cirrhosis and even reverse fibrosis stage. Apart from conventional cause-specific therapies, the most promising treatments for liver fibrosis are those that target cellular and molecular mechanisms involved in the reversal of fibrosis. As we continue to explore the mechanisms and targets of hepatic fibrosis, effective antifibrotic therapies will be a reality in the future.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Drafting of the manuscript and creation of figure and table (YCG), critically revising the document for important intellectual content (LGL), approval of the final version of this manuscript to be published (YCG, LGL).

References

[1] Trautwein C, Friedman SL, Schuppan D, Pinzani M. Hepatic fibrosis: Concept to treatment. J Hepatol 2015;62:S15–S24. doi: 10.1016/j.jhep.2015.02.039.

[2] Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749–1761. doi: 10.1016/S0140-6736(14)60121-5.

[3] Friedman SL. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. Physiol Rev 2008;88:125–172. doi: 10.1152/physrev.00013.2007.

[4] Zhao G, Cui J, Qin Q, Zhang J, Liu L, Deng S, *et al*. Mechanical stiffness of liver tissues in relation to integrin β1 expression may influence the development of hepatic cirrhosis and hepatocellular carcinoma. J Surg Oncol 2010;102:482–489. doi: 10.1002/jso.21613.

[5] Krenkel O, Puengel T, Govaere O, Abdallah AT, Mossanen JC, Kohlhepp M, *et al*. Therapeutic inhibition of inflammatory monocyte recruitment reduces steatohepatitis and liver fibrosis. Hepatology 2018;67:1270–1283. doi: 10.1002/hep.29544.

[6] Lee YA, Wallace MC, Friedman SL. Pathobiology of liver fibrosis: a translational success story. Gut 2015;64:830–841. doi: 10.1136/gutjnl-2014-306842.

[7] Campana L, Iredale JP. Regression of liver fibrosis. Semin Liver Dis 2017;37:1–10. doi: 10.1055/s-0036-1597816.

[8] Ramachandran P, Pellicoro A, Vernon MA, Boulter L, Aucott RL, Ali A, *et al*. Differential Ly-6C expression identifies the recruited macrophage phenotype, which orchestrates the regression of murine liver fibrosis. Proc Natl Acad Sci U S A 2012;109:E3186–E3195. doi: 10.1073/pnas.1119964109.

[9] Chang TT, Liaw YF, Wu SS, Schiff E, Han KH, Lai CL, *et al*. Long-term entecavir therapy results in the reversal of fibrosis/cirrhosis and continued histological improvement in patients with chronic hepatitis B. Hepatology 2010;52:886–893. doi: 10.1002/hep.23785.

[10] Curry MP, O'Leary JG, Bzowej N, Muir AJ, Korenblat KM, Fenkel JM, *et al*. Sofosbuvir and velpatasvir for HCV in patients with decompensated cirrhosis. N Engl J Med 2015;373:2618–2628. doi: 10.1056/NEJMoa1512614.

[11] Marcellin P, Gane E, Buti M, Afdhal N, Sievert W, Jacobson IM, *et al*. Regression of cirrhosis during treatment with tenofovir disoproxil fumarate for chronic hepatitis B: a 5-year open-label follow-up study. Lancet 2013;381:468–475. doi: 10.1016/S0140-6736(12)61425-1.

[12] Chinese Society of Hepatology Chinese Medical Association; Chinese Society of Gastroenterology Chinese Medical Association; Chinese Society of Infectious Diseases, Chinese Medical Association. Consensus on the diagnosis and therapy of hepatic fibrosis in. Zhonghua Gan Zang Bing Za Zhi 2019;27:657–667. doi: 10.3760/cma.j.issn.1007-3418.2019.09.001.

[13] Goel A, Kim WR. Natural history of primary biliary cholangitis in the ursodeoxycholic acid era: Role of scoring systems. Clin Liver Dis 2018;22:563–578. doi: 10.1016/j.cld.2018.03.007.

[14] Parés A, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. Gastroenterology 2006;130:715–720. doi: 10.1053/j.gastro.2005.12.029.

[15] Wunsch E, Trottier J, Milkiewicz M, Raszeja-Wyszomirska J, Hirschfield GM, Barbier O, *et al*. Prospective evaluation of ursodeoxycholic acid withdrawal in patients with primary sclerosing cholangitis. Hepatology 2014;60:931–940. doi: 10.1002/hep.27074.

[16] Lindor KD, Kowdley KV, Luketic VA, Harrison ME, McCashland T, Befeler AS, *et al*. High-dose ursodeoxycholic acid for the treatment of primary sclerosing cholangitis. Hepatology 2009;50:808–814. doi: 10.1002/hep.23082.

[17] Chapman R, Fevery J, Kalloo A, Nagorney DM, Boberg KM, Shneider B, *et al*. Diagnosis and management of primary sclerosing cholangitis. Hepatology 2010;51:660–678. doi: 10.1002/hep.23294.

[18] Porez G, Prawitt J, Gross B, Staels B. Bile acid receptors as targets for the treatment of dyslipidemia and cardiovascular disease. J Lipid Res 2012;53:1723–1737. doi: 10.1194/jlr.R024794.

[19] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, *et al*. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015;385:956–965. doi: 10.1016/S0140-6736(14)61933-4.

[20] Nevens F, Andreone P, Mazzella G, Strasser SI, Bowlus C, Invernizzi P, *et al*. A placebo-controlled trial of obeticholic acid in primary biliary cholangitis. N Engl J Med 2016;375:631–643. doi: 10.1056/NEJMoa1509840.

[21] Hirschfield GM, Mason A, Luketic V, Lindor K, Gordon SC, Mayo M, *et al*. Efficacy of obeticholic acid in patients with primary biliary cirrhosis and inadequate response to ursodeoxycholic acid. Gastroenterology 2015;148:751–761.e8. doi: 10.1053/j.gastro.2014.12.005.

[22] Kowdley KV, Luketic V, Chapman R, Hirschfield GM, Poupon R, Schramm C, *et al*. A randomized trial of obeticholic acid monotherapy in patients with primary biliary cholangitis. Hepatology 2018;67:1890–1902. doi: 10.1002/hep.29569.

[23] Dawson PA, Karpen SJ. Bile acids reach out to the spinal cord: new insights to the pathogenesis of itch and analgesia in cholestatic liver disease. Hepatology 2014;59:1638–1641. doi: 10.1002/hep.26786.

[24] Silveira MG, Lindor KD. Investigational drugs in phase II clinical trials for primary biliary cholangitis. Expert Opin Investig Drugs 2017;26:1115–1121. doi: 10.1080/13543784.2017.1371135.

[25] Badman MK, Chen J, Desai S, Vaidya S, Neelakantham S, Zhang J, *et al*. Safety, tolerability, pharmacokinetics, and pharmacodynamics of the novel non-bile acid FXR agonist tropifexor (LJN452) in healthy volunteers. Clin Pharmacol Drug Dev 2020;9:395–410. doi: 10.1002/cpdd.762.

[26] Trauner M, Gulamhusein A, Hameed B, Caldwell S, Shiffman ML, Landis C, *et al*. The nonsteroidal farnesoid X receptor agonist cilofexor (GS-9674) improves markers of cholestasis and liver injury in patients with primary sclerosing cholangitis. Hepatology 2019;70:788–801. doi: 10.1002/hep.30509.

[27] Patel K, Harrison SA, Elkhashab M, Trotter JF, Herring R, Rojter SE, *et al*. Cilofexor, a nonsteroidal FXR agonist, in patients with noncirrhotic NASH: A phase 2 randomized controlled trial. Hepatology 2020;72:58–71. doi: 10.1002/hep.31205.

[28] Ghonem NS, Assis DN, Boyer JL. Fibrates and cholestasis. Hepatology 2015;62:635–643. doi: 10.1002/hep.27744.

[29] Miyahara T, Schrum L, Rippe R, Xiong S, Yee HF Jr, Motomura K, *et al*. Peroxisome proliferator-activated receptors and hepatic stellate cell activation. J Biol Chem 2000;275:35715–35722. doi: 10.1074/jbc.M006577200.

[30] Corpechot C, Chazouillères O, Rousseau A, Le Gruyer A, Habersetzer F, Mathurin P, *et al*. A placebo-controlled trial of bezafibrate in primary biliary cholangitis. N Engl J Med 2018;378:2171–2181. doi: 10.1056/NEJMoa1714519.

[31] Hosonuma K, Sato K, Yamazaki Y, Yanagisawa M, Hashizume H, Horiguchi N, *et al*. A prospective randomized controlled study of long-term combination therapy using ursodeoxycholic acid and bezafibrate in patients with primary biliary cirrhosis and dyslipidemia. Am J Gastroenterol 2015;110:423–431. doi: 10.1038/ajg.2015.20.

[32] Zhang Y, Li S, He L, Wang F, Chen K, Li J, *et al*. Combination therapy of fenofibrate and ursodeoxycholic acid in patients with primary biliary cirrhosis who respond incompletely to UDCA monotherapy: a meta-analysis. Drug Des Devel Ther 2015;9:2757–2766. doi: 10.2147/DDDT.S79837.

[33] Davidson MH, Armani A, McKenney JM, Jacobson TA. Safety considerations with fibrate therapy. Am J Cardiol 2007;99:3C–18C. doi: 10.1016/j.amjcard.2006.11.016.

[34] Mitchell C, Couton D, Couty JP, Anson M, Crain AM, Bizet V, *et al*. Dual role of CCR2 in the constitution and the resolution of liver fibrosis in mice. Am J Pathol 2009; 174:1766–1775. doi: 10.2353/ajpath.2009.080632.

[35] Coppola N, Perna A, Lucariello A, Martini S, Macera M, Carleo MA, *et al*. Effects of treatment with Maraviroc a CCR5 inhibitor on a human hepatic stellate cell line. J Cell Physiol 2018;233:6224–6231. doi: 10.1002/jcp.26485.

[36] Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, *et al*. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology 2018;67:1754–1767. doi: 10.1002/hep.29477.

[37] de Oliveira FL, Carneiro K, Brito JM, Cabanel M, Pereira JX, Paiva LA, *et al*. Galectin-3, histone deacetylases, and Hedgehog signaling: Possible

convergent targets in schistosomiasis-induced liver fibrosis. PLoS Negl Trop Dis 2017;11:e0005137. doi: 10.1371/journal.pntd.0005137.

[38] Traber PG, Chou H, Zomer E, Hong F, Klyosov A, Fiel MI, *et al*. Regression of fibrosis and reversal of cirrhosis in rats by galectin inhibitors in thioaceta-mide-induced liver disease. PLoS One 2013;8:e75361. doi: 10.1371/journal.pone.0075361.

[39] Traber PG, Zomer E. Therapy of experimental NASH and fibrosis with galectin inhibitors. PLoS One 2013;8:e83481. doi: 10.1371/journal.pone.0083481.

[40] Harrison SA, Marri SR, Chalasani N, Kohli R, Aronstein W, Thompson GA, *et al*. Randomised clinical study: GR-MD-02, a galectin-3 inhibitor, vs. placebo in patients having non-alcoholic steatohepatitis with advanced fibrosis. Aliment Pharmacol Ther 2016;44:1183–1198. doi: 10.1111/apt.13816.

[41] Chalasani N, Abdelmalek MF, Garcia-Tsao G, Vuppalanchi R, Alkhouri N, Rinella M, *et al*. Effects of belapectin, an inhibitor of galectin-3, in patients with nonalcoholic steatohepatitis with cirrhosis and portal hypertension. Gastroenterology 2020;158:1334–1345.e5. doi: 10.1053/j.gastro.2019.11.296.

[42] Ornitz DM, Itoh N. The Fibroblast Growth Factor signaling pathway. Wiley Interdiscip Rev Dev Biol 2015;4:215–266. doi: 10.1002/wdev.176.

[43] Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, *et al*. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174–1185. doi: 10.1016/S0140-6736(18)30474-4.

[44] Sanyal A, Charles ED, Neuschwander-Tetri BA, Loomba R, Harrison SA, Abdelmalek MF, *et al*. Pegbelfermin (BMS-986036), a PEGylated fibroblast growth factor 21 analogue, in patients with non-alcoholic steatohepatitis: a randomised, double-blind, placebo-controlled, phase 2a trial. Lancet 2019;392:2705–2717. doi: 10.1016/S0140-6736(18)31785-9.

[45] Xie Y, Ramachandran A, Breckenridge DG, Liles JT, Lebofsky M, Farhood A, *et al*. Inhibitor of apoptosis signal-regulating kinase 1 protects against acet-aminophen-induced liver injury. Toxicol Appl Pharmacol 2015;286:1–9. doi: 10.1016/j.taap.2015.03.019.

[46] Loomba R, Lawitz E, Mantry PS, Jayakumar S, Caldwell SH, Arnold H, *et al*. The ASK1 inhibitor selonsertib in patients with nonalcoholic steatohepatitis: A randomized, phase 2 trial. Hepatology 2018;67:549–559. doi: 10.1002/hep.29514.

[47] Garcia-Tsao G, Fuchs M, Shiffman M, Borg BB, Pyrsopoulos N, Shetty K, *et al*. Emricasan (IDN-6556) lowers portal pressure in patients with compensated cirrhosis and severe portal hypertension. Hepatology 2019;69:717–728. doi: 10.1002/hep.30199.

[48] Frenette CT, Morelli G, Shiffman ML, Frederick RT, Rubin RA, Fallon MB, *et al*. Emricasan improves liver function in patients with cirrhosis and high model for end-stage liver disease scores compared with placebo. Clin Gastroenterol Hepatol 2019;17:774–783.e4. doi: 10.1016/j.cgh.2018.06.012.

[49] Zhao XY, Zeng X, Li XM, Wang TL, Wang BE. Pirfenidone inhibits carbon tetrachloride- and albumin complex-induced liver fibrosis in rodents by preventing activation of hepatic stellate cells. Clin Exp Pharmacol Physiol 2009;36:963–968. doi: 10.1111/j.1440-1681.2009.05194.x.

[50] Armendáriz-Borunda J, Islas-Carbajal MC, Meza-García E, Rincón AR, Lucano S, Sandoval AS, *et al*. A pilot study in patients with established advanced liver fibrosis using pirfenidone. Gut 2006;55:1663–1665. doi: 10.1136/gut.2006.107136.

[51] Flores-Contreras L, Sandoval-Rodríguez AS, Mena-Enriquez MG, Lucano-Landeros S, Arellano-Olivera I, Alvarez-Álvarez A, *et al*. Treatment with pirfenidone for two years decreases fibrosis, cytokine levels and enhances CB2 gene expression in patients with chronic hepatitis C. BMC Gastroenterol 2014;14:131. doi: 10.1186/1471-230X-14-131.

[52] Liu Y, Wu J, Li Z, Luo Y, Zeng F, Shi S. Tolerability and pharmacokinetics of hydronidone, an antifibrotic agent for hepatic fibrosis, after single and multiple doses in healthy subjects: an open-label, randomized, dose-escalating, first-in-human study. Eur J Drug Metab Pharmacokinet 2017;42:37–48. doi: 10.1007/s13318-015-0316-z.

[53] Barry-Hamilton V, Spangler R, Marshall D, McCauley S, Rodriguez HM, Oyasu M, *et al*. Allosteric inhibition of lysyl oxidase-like-2 impedes the development of a pathologic microenvironment. Nat Med 2010;16:1009–1017. doi: 10.1038/nm.2208.

[54] Ikenaga N, Peng ZW, Vaid KA, Liu SB, Yoshida S, Sverdlov DY, *et al*. Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. Gut 2017;66:1697–1708. doi: 10.1136/gutjnl-2016-312473.

[55] Harrison SA, Abdelmalek MF, Caldwell S, Shiffman ML, Diehl AM, Ghalib R, *et al*. Simtuzumab is ineffective for patients with bridging fibrosis or compensated cirrhosis caused by nonalcoholic steatohepatitis. Gastroenterology 2018;155:1140–1153. doi: 10.1053/j.gastro.2018.07.006.

[56] Meissner EG, McLaughlin M, Matthews L, Gharib AM, Wood BJ, Levy E, *et al*. Simtuzumab treatment of advanced liver fibrosis in HIV and HCV-infected adults: results of a 6-month open-label safety trial. Liver Int 2016;36:1783–1792. doi: 10.1111/liv.13177.

[57] Muir AJ, Levy C, Janssen HLA, Montano-Loza AJ, Shiffman ML, Caldwell S, *et al*. Simtuzumab for primary sclerosing cholangitis: Phase 2 study results with insights on the natural history of the disease. Hepatology 2019;69:684–698. doi: 10.1002/hep.30237.

[58] Shim KY, Eom YW, Kim MY, Kang SH, Baik SK. Role of the renin-angiotensin system in hepatic fibrosis and portal hypertension. Korean J Intern Med 2018;33:453–461. doi: 10.3904/kjim.2017.317.

[59] Yoshiji H, Noguchi R, Kojima H, Ikenaka Y, Kitade M, Kaji K, *et al*. Interferon augments the anti-fibrotic activity of an angiotensin-converting enzyme inhibitor in patients with refractory chronic hepatitis C. World J Gastroenterol 2006;12:6786–6791. doi: 10.3748/wjg.v12.i42.6786.

[60] Baik SK, Park DH, Kim MY, Choi YJ, Kim HS, Lee DK, *et al*. Captopril reduces portal pressure effectively in portal hypertensive patients with low portal venous velocity. J Gastroenterol 2003;38:1150–1154. doi: 10.1007/s00535-003-1222-8.

[61] Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, *et al*. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with non-alcoholic steatohepatitis. Hepatology 2004;40:1222–1225. doi: 10.1002/hep.20420.

[62] Kim MY, Cho MY, Baik SK, Jeong PH, Suk KT, Jang YO, *et al*. Beneficial effects of candesartan, an angiotensin-blocking agent, on compensated alcoholic liver fibrosis - a randomized open-label controlled study. Liver Int 2012;32:977–987. doi: 10.1111/j.1478-3231.2012.02774.x.

[63] Bansal MB, Chamroonkul N. Antifibrotics in liver disease: are we getting closer to clinical use? Hepatol Int 2019;13:25–39. doi: 10.1007/s12072-018-9897-3.