Liver fibrosis is a life-threatening disease, with challenging morbidity and mortality for healthcare systems worldwide. It imposes an enormous economic burden to societies, making continuous research and informational updates about its pathogenesis and treatment crucial. This review’s focus is on the current knowledge about the Wnt signaling pathway, serving as an important pathway in liver fibrosis development and activation of hepatic stellate cells (HSCs).

**Keywords:** Liver fibrosis; Wnt signaling pathway; Hepatic stellate cell; Therapeutic solutions.

**Abbreviations:** a-SM, alpha-smooth muscle actin; ADMA, asymmetric dimethylarginine; ALF, acute liver failure; ALT, alanine aminotransferase; APC, adenomatous polyposis coli; AST, aspartate aminotransferase; β-TCP, beta-transducin repeat containing protein; CamKII, calmodulin kinase II; CBP, cyclic-AMP response element-binding protein; CCl4, carbon tetrachloride; CK1α, casein kinase 1 alpha; COX-2, cyclooxygenase-2; CREB, cAMP-response element-binding protein; CRISPR-Cas9, clustered regularly interspaced short palindromic repeats-associated protein 9; CYP, cytochrome P450; DDAH1, dimethyl arginine dimethylaminohydrolase-1; DKK1, dickkopf-related protein 1; Dvl, Disheveled repeats-associated protein 9; DTT, dithiothreitol; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; Fz, Frizzled transmembrane receptor; GFAP, glial fibrillary acidic protein; GSK-3β, glycogen synthase kinase-3 beta; HBV, hepatitis B virus; HCV, hepatitis C virus; Hh, hedgehog; HSC, hepatic stellate cell; JNK, Jun N-terminal kinase; LRP, low-density lipoprotein receptor; MCF, monocyte chemoattractant protein-1; MMPs, matrix-degrading metalloproteinases; NASH, nonalcoholic fatty liver disease; NOR1, oxidized-nitro domain-containing protein 1; P38, p38 mitogen-activated protein kinase; PDGFR, platelet-derived growth factor receptor; PKC, protein kinase C; PLC, phospholipase C; PPAR, peroxisome proliferator-activated receptor; SB-216763, cyclic-AMP response element-binding protein (CBP) using PRI-724 and ICG-001, the lymphoid enhancer binding protein (LEF)/T cell-specific transcription factor (TCF) system as well as Wnt inhibitory factor 1 (WIF1) and miR-17-5p using pinoctinide hydrate (PSH). Significant progress has been made in inhibiting Wnt and thus stopping the progression of liver fibrosis by diminishing key components for its action. Comprehending the role of the Wnt signaling pathway in liver fibrosis may lead to discovery of novel targets in liver fibrosis therapeutic strategies’ development.

**Abstract**

Two types of Wnt pathways are distinguished, namely the β-catenin-dependent canonical and non-canonical Ca2+ or planar cell polarity (PCP)-dependent pathway. The dynamic balance of physiologically healthy liver and hepatocytes is disturbed by repeated liver injuries. Activation of the β-catenin Wnt pathway prevents the regeneration of hepatocytes by the replacement of extracellular matrix (ECM), leading to the appearance of scar tissue and the formation of regenerated nodular hepatocytes, lacking the original function of healthy hepatocytes. Therefore, liver function is reduced due to the severely advanced disease. Selective inhibition of β-catenin inhibits inflammatory processes (since chemokines and pro-inflammatory cytokines are produced during Wnt activation), reduces growth of activated HSCs and reduces collagen synthesis and angiogenesis, thereby reducing the progression of liver fibrosis in vivo. While the canonical Wnt pathway is usually inactive in a physiologically healthy liver, it shows activity during cell regeneration or renewal and in certain pathophysiological conditions, such as liver diseases and cancer. Targeted blocking of some of the basic components of the Wnt pathway is a therapeutic approach. These include the frizzled transmembrane receptor (Fz) receptors using the secreted frizzled-related protein family (FRP), Fz-co-receptors low-density LRP 5/6 through dickkopf-related protein 1 (DDK1) or niclosamide, glycogen kinase-3 beta (GSK-3β) using SB-216763, cyclic-AMP response element-binding protein (CBP) using PRI-724 and ICG-001, the lymphoid enhancer binding factor (LEF)/T cell-specific transcription factor (TCF) system as well as Wnt inhibitory factor 1 (WIF1) and miR-17-5p using pinoctinide hydrate (PSH). Significant progress has been made in inhibiting Wnt and thus stopping the progression of liver fibrosis by diminishing key components for its action. Comprehending the role of the Wnt signaling pathway in liver fibrosis may lead to discovery of novel targets in liver fibrosis therapeutic strategies’ development.
Liver fibrosis represents a significant global health problem. Due to the fact that fibrosis progression leads to the development of cirrhosis and liver cancer, worldwide mortality related to this condition is 1.5 million deaths per year. Numerous epidemiological studies have shown important clinical implications of fibrosis staging. Various clinical tools have also been developed in order to better distinguish stages of liver disease and predict mortality with cirrhosis, such as the Child-Pugh score and model for end-stage liver disease score. Etiology of liver fibrosis can be divided into the following two major types of injuries: cholestatic (reduction or obstruction of the gall flow in the liver) and hepatotoxic (hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, metabolic- and alcoholic-associated steatohepatitis). Common pathophysiological mechanisms involved in fibrosis development, regardless of the etiology, are cytokine release and chronic inflammation, hepatocyte death, HSC activation, and disruption of the endothelial or epithelial barrier. Endothelial cells, HSCs and Kupffer cells have various functions, with a particularly important role in fibrosis development. Kupffer cells are liable for the production of different proinflammatory cytokines, and also act as liver macrophages. As the headspring of transforming growth factor-beta (TGF-β) is proven to play a key role in fibrogenesis, Kupffer cells are also an important factor in the development of fibrosis. Accordingly, liver fibrogenesis represents a complex process which requires extracellular and cellular signaling.

Recently, reactivation of several signaling pathways, such as Wnt, notch and hedgehog (Hh), has been related with liver injury and regeneration. The Wnt signaling pathway is a conserved signal transduction pathway included in the regulation of numerous cellular functions and controlling important aspects of development. Activation of this signaling pathway is associated to activation of HSCs and fibrogenesis, along with enhanced synthesis of ECM, transformation of epithelial cells or epithelial-to-mesenchymal transition (EMT) or interaction with other profibrotic mediators.

Through this critical review, we aimed to recapitulate current lore about the Wnt signaling pathway as a part of the underlying pathophysiological mechanism in liver fibrosis development. Also, emerging data about potential molecular and cellular treatment options targeting the Wnt signaling pathway are systematically presented.

Wnt signaling pathway

Among several intracellular signaling pathways included in the pathophysiology of liver fibrosis, the Wnt signaling pathway claims a growing share. During its activation, it shows fibrotic effects, while poor formation of structure as well as low processes of wound healing occur during its inactivation. As stated above, based on the involvement of β-catenin, the Wnt signaling pathway principally encompasses two classes: canonical and non-canonical. The correlation of these two pathways can also be expressed through the possibility that non-canonical Wnt ligands may have a negative effect on the canonical pathway.

Canonical pathway

The key component of the canonical Wnt signaling pathway is its downstream actions through β-catenin, acting as a protein with dual function (as a transcription factor and an adhesion molecule). It is important to emphasize that in healthy liver this protein is localized in the membrane of hepatocyte, but in injured liver this location changes to the cytoplasm. In the adhesion cell-cell processes, binding of adhesive and transmembrane component (such as E-cadherin to actin; basically adhesive component-protein) within the cytoskeleton is enabled when β-catenin is bound to the plasma membrane; the catenin acts as a bridge between cadherin and actin, as shown in Figure 1. Meanwhile, catenin is the Wnt pathway’s major transducer when localized in the cytoplasm. Due to the fact that β-catenin cannot bind directly to DNA in order to make contact with target genes, it relies on coactivators and various transcription factors. Its own transcription factor function is mostly regulated by canonical Wnt proteins (e.g., Wnt1, Wnt3a, Wnt8b), which are mainly located in the extracellular space. As extracellular signaling molecules, they bind and initiate signaling processes, leading to β-catenin’s cascade reaction.

When Wnt signaling is off, β-catenin is located in the cytoplasm in the low regime, where its stability is controlled by a destruction complex composed of protein axin, adenomatous polyposis coli (APC), GSK-3β and casein kinase 1 alpha (CK1α). β-catenin is phosphorylated by CK1 and GSK-3β and afterwards ubiquitinated by beta-transducin repeat containing protein (β-TrCP). Final degradation by the proteasome results in insufficient levels of β-catenin to activate the transcription process. Binding of canonical Wnt proteins to Fz and LRP 5/6 activates the canonical pathway. Relocation of Axin to LRP 5/6 due to the Fz/Disheveled (Dvl) complex leads to phosphorylation of LRP 5/6. As a result, GSK-3β is inactivated and the destruction complex dissociated, with absence of β-catenin phosphorylation. Subsequently, the proportion of unphosphorylated β-catenin increases, followed by its translocation to the nucleus. Although the mechanism of β-catenin’s translocation to nucleus is yet unknown, the main action is to become bound to lymphoid enhancer binding factor (LEF)/T cell-specific transcription factor (TCF) in order to initiate targeted genes’ transcription. On the molecular level, it requires engagement with either one of the two transcriptional coactivators: cAMP-response element-binding protein (CREB) or p300. Recently, Yu et al. demonstrated a suppressive effect on Wnt by acting through the clustered regularly interspaced short palindromic repeats-associated protein 9 (CRISPR-Cas9) system as an editing system to reduce the effect of LRP 6 gene in mice with alcohol-induced liver injury.

Non-canonical pathway

Non-canonical signaling pathways are β-catenin-independent. They encompass non-the canonical Wnt/Ca2+ pathway and PCP pathway, as shown on Figure 2. In the Wnt/Ca2+ signaling pathway, binding of a non-canonical Wnt protein (e.g., Wnt5a) results in activation of the cytoplasmic protein Dvl, which increases the concentration of cytoplasmatic Ca2+ and subsequently activates the protein kinase C (PKC) and calcium sensitive enzymes calmodulin kinase II (CaMkII). It is also important to emphasize its role in increasing the activity of phospholipase C (PLC) and the nuclear factor related to T cells’ activation in transcri-
Additionally, a study from Sen et al. suggested that the non-canonical Wnt signaling pathway contributes to transcriptional activation of NF-κB responsive genes, responsible for various proinflammatory cytokines’ and chemokines’ expression. The second non-canonical Wnt signaling pathway is the PCP; in the literature, it is also commonly referred to as the Wnt/c-Jun N-terminal kinase (JNK) pathway, being important in cytoskeletal organization. The Wnt/PCP ligands (i.e. Wnt5a, Wnt7, and Wnt11) bind to the Fz receptor encompassing Dvl-mediated stimulation of the Rho and Rac (which are small GTPases). Subsequently, activation of a kinase (such as ROK and JNK) is stimulated, which, in the end, are comprehensively involved in cellular proliferation and differentiation (including transcription). Additionally, a study from Sen et al. suggested that the non-canonical Wnt signaling pathway contributes to transcriptional activation of NF-κB responsive genes, responsible for various proinflammatory cytokines’ and chemokines’ expression. The second non-canonical Wnt signaling pathway is the PCP; in the literature, it is also commonly referred to as the Wnt/c-Jun N-terminal kinase (JNK) pathway, being important in cytoskeletal organization. The Wnt/PCP ligands (i.e. Wnt5a, Wnt7, and Wnt11) bind to the Fz receptor encompassing Dvl-mediated stimulation of the Rho and Rac (which are small GTPases). Subsequently, activation of a kinase (such as ROK and JNK) is stimulated, which, in the end, are comprehensively involved in cellular proliferation and differentiation (including

![Fig. 1. Activated and inactivated canonical Wnt signaling.](image-url)

In an inactivated state, β-catenin in the hepatocyte membrane forms a bridge between actin and E-cadherin. When Wnt signaling is off, β-catenin (in a multiprotein complex with GSK-3β, axin, CK1α, β-TrCP and APC) is phosphorylated by GSK-3β and CK1α and ubiquitinated by β-TrCP. In the end, β-catenin is degraded by the proteasome. When Wnt signaling is on, Wnt-Fz and LRP coordinate the activation of Dvl, leading to dissociation of the multiprotein complex and resulting in the inactivation of GSK-3β (no phosphorylation anymore). Excessive free β-catenin translocates to the nucleus and binds to TCF/LEF transcription factors, resulting in a transcriptional activation of Wnt target genes. GSK-3β, glycogen synthase kinase-3 beta; CK1α, casein kinase 1 alpha; β-TrCP, beta-transducin repeat containing protein; Wnt-Fz, Wnt-Frizzled transmembrane receptor; LRP, low-density lipoprotein receptor-related protein; Dvl, Disheveled gene; TCF/LEF, T cell-specific transcription factor/lymphoid enhancer binding factor.
Liver fibrosis

HSCs

HSCs belong to the group of specialized liver pericytes located in the space of Disse, between endothelial sinusoidal cells and hepatocytes. Their major role relates to molecular mechanisms of transdifferentiation, itself representing a key role in liver fibrosis. Numerous investigations have attempted to discover the key role of the Wnt signaling pathway in HSCs and liver fibrosis. Nevertheless, its role in HSC biology still remains to be fully elucidated.

In the physiological condition, HSCs are in a quiescent state and store retinoids. Also, in the inactive stage, they

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Fig. 2. The non-canonical Wnt signaling pathways include the Wnt/Ca$^{2+}$ pathway and PCP pathway. In the Wnt/Ca$^{2+}$ signaling pathway, activated Dvl increases the concentration of cytoplasmatic Ca$^{2+}$, leading to activation of the Ca$^{2+}$-sensitive enzymes CamKII and PKC, and NF-$\kappa$B, resulting in transcriptional activation of target genes. In the PCP pathway, Wnt proteins activate Dvl, which activates Rac and Roc and subsequently activates ROK and JNK kinase. Dvl, Disheveled gene; CamKII, calmodulin kinase II protein-1; PKC, protein kinase C; PCP, planar cell polarity; JNK, Jun N-terminal kinase.
Pathophysiological mechanism of fibrosis

Pathophysiology of liver fibrosis involves a complex interplay of many mechanisms, including the intracellular signaling pathways of Wnt/β-catenin, GAS6/Axl and TGF-β/Smad, and other preserved morphogenic developmental signaling pathways, such as of notch and Hh. Repeated liver injuries, massive accumulation of ECM leading to cell stiffening, and the appearance of scar tissue disrupts liver homeostasis. In the absence of hepatocyte regeneration, the cells are replaced by ECM and the formation of regenerating nodular hepatocytes. The Wnt pathway has been shown to be activated in the process of liver fibrosis involving β-catenin, Fz receptors and LRP coreceptor upregulation. Downregulation or selective inhibition of the Wnt/β-catenin pathway significantly inhibits activation of HSCs, contractility and migration of HSCs in vitro, also processes of fibrosis, inflammation and angiogenesis are attenuated in vivo. Selective inhibition of the Wnt/β-catenin pathway using ICG-001 inhibits HSCs activation, differentiation, contractility and migration in vitro, as well as collagen deposition and processes of fibrosis, inflammation and angiogenesis in vivo. It is known that activation of HSCs also results in the secretion of CXCL12, which stimulates macrophage infiltration of the liver and HSCs' activation, thus promoting fibrosis, inflammation and angiogenesis. When inhibiting the Wnt/β-catenin pathway, production of CXCL12 and the processes of fibrosis, inflammation and angiogenesis are attenuated in the liver.

Studies have shown the connection between Wnt5a and TGF. When Wnt5a expression was studied in fibrotic livers of mouse and human, upregulation of the Wnt5a gene and protein was found in comparison to that in healthy livers, and the level of Wnt5a was also found to have decreased after therapeutic intervention in mice. In vitro studies have shown that expression of Wnt5a and Wnt receptors Fz2 and Fz8 were significantly enhanced by TGF. Levels of collagen type I and fibronectin in TGF-stimulated myofibroblasts are increased, along with Wnt, and decreased when Wnt5a is suppressed by anti-fibrotic cytokine in vitro and in vivo. In addition, suppression of Wnt5a in activated LX2 cells decreases production of both collagen type I and TGF-β. A recent study showed that establishment of fibrosis is affected by the induction of EndMT, which is crucial for the production of myofibroblasts in fibrous organs and tissues. Increased TGF-β expression increases asymmetric dimethylarginine (ADMA) and factors of inflammation, while a decrease is expected in nitric oxide secretion and nitric oxide synthase activity, as well as in dimethyl arginine dimethylaminohydrase-1 (DDAH1), Nrf2 and VE-cadherin; all, together are defined as factors for fibrosis improvement through EndMT. Levels of Wnt5a in serum follow hepatosteatosis, nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). It is supposed that suppression of Wnt5a, as it has pro-inflamatory effects, can reduce NASH; based on that hypothesis, studies using celecoxib, an inhibitor of cyclooxygenase-2 (COX-2), were performed. In a rat model of NASH, the expression of Wnt5a, COX-2, JNK1 and NF-kB p65 were higher, as observed by levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and histologically; however, the administration of celecoxib suppressed their expression and inflammation in liver. It was concluded by the authors that it is possible to ameliorate NASH by suppressing the Wnt5a/JNK1 pathway. In another study, pharmacological inhibition of Wnt5a and β-catenin, along with suppressed Wnt5a, produced an anti-inflammatory effect in hepatosteatosis, NAFLD, and NASH.

Hepatic fibrosis used to be considered an irreversible process, taking into account that hepatic parenchyma is destroyed and replaced with fibrotic tissue; however, laboratory and clinical studies have revealed possible reversibility of progressive Wnt signaling pathology-related liver fibrosis. Studies performed in vitro and in vivo have also lent support to the hypothesis that β-catenin protects hepatocytes through the inhibition of apoptosis associated with FoxO3; importantly, such findings can be relevant for future therapeutic interventions in the field of liver injury protection, repair, and regeneration. A recent study confirmed that increased EMT due to Dact2 (an antagonist of β-catenin) deficiency helps to promote healing processes in the liver. Dact2 inhibits binding of LEF1 to β-catenin and promotes degradation of Dvl; by establishing re-expression of Dact2, T-cell factor 4 (Its transcriptional activity) and downstream signaling of Wnt are stimulated, which is known to play a role in gene suppression.

Therapeutic solutions

Scientists have been steadily working for years on elucidat-
being the underlying molecular mechanisms responsible for liver fibrosis development and to develop adequate therapeu tic strategies (which have to be validated in preclinical and clinical trials).67–72 Alleviation of the Wnt signaling pathway is possible, due to its members Wnt1, Wnt3a and Wnt10b, as well as Fz1 and 5; WIF is influential, in that it joins to either its ligand, sFRP family or antagonist to prevent association between the LRP coreceptor and Fz. Agents that exert inhibitory effects on the Wnt signaling pathway are being considered to have preventive or therapeutic effects in liver fibrosis; these are DKK1, niclosamide, sFRP5, SB-216763, and pinostilbene hydrate (PSH), as shown in Figure 3.

**DKK1**

DDK1 is among the best-characterized inhibitors of the Wnt/β-catenin signaling pathway. It prevents binding between Wnt and the LRPs/6/6 component of the receptor complex,\(^{73}\) resulting in a disruption of Fz-LRP6 dimerization.\(^{74}\) DKK1 was used to prove the role of the Wnt canonical pathway in activation of HSCs as well as their quiescence and renewal of regulation (adipogenesis).\(^{72}\) In activated HSCs, the expression of Wnt3a and 10b (as canonical) and Wnt4 and 5a (as non-canonical) forms of Wnt, receptors Fz1 and 2, and LRPs and Ryk (coreceptors) is induced, as shown by the TCF promoter-luciferase gene becoming activated. However, administration of DKK1 inhibits Wnt signaling and results in both decreasing TCF and increasing peroxisome proliferator-activated receptor (PPAR) y-trigger activity of the PPAR response element, a crucial transcriptional parameter (adipogenic).\(^{75}\) As demonstrated in earlier studies, PPAR is one of the major transcriptional factors in adipocyte differentiation and for the maintenance of HSC quiescence in vitro; the expression of transcriptional adipogenic factors is substantial according to its activation being associated with forfeiture of transcriptional adipogenic regulation.\(^{76}\) The anti-adipogenic effect of DKK1 on HSCs was shown in vitro by detection of increasing levels of PPARγ mRNA.\(^{77}\) By extending these findings from an animal model of cholestatic liver fibrosis, an antifibrotic effect of DKK1 has been proven.\(^{75}\) DKK1 can abolish epigenetic repression, return PPARγ activity and reduce fibrosis due to its inhibitory effect on Wnt signaling inhibition.\(^{42}\) DKK1 also showed a protective role/growth antagonist to prevent association between the LRP coreceptor and Fz. Agents that exert inhibitory effects on the Wnt signaling pathway are being considered to have preventive or therapeutic effects in liver fibrosis; these are DKK1, niclosamide, sFRP5, SB-216763, and pinostilbene hydrate (PSH), as shown in Figure 3.

**sFRP5**

sFRP5 is declared as an adipocytokine with anti-inflammatory and anti-fibrotic effects, that plays a regulatory role in metabolic homeostasis. In mouse models, it has been shown that sFRP5 can inhibit effects of Wnt5a/Fz2 on proliferation and migration of HSCs, and therefore ameliorate liver fibrosis.\(^{54}\) Lower level of sFRP5 in serum is associated with obesity, diabetes, and NASH.\(^{54}\) In studies with sFRP5 knockout mice, development of adipose inflammation and obesity is noted, while the high levels in breast cancer tissue were found to be associated with a better outcome for patients.\(^{90}\) Beneficial effects of administration of recombinant sFRP5 has been shown in methionine- and choline-deprived diet-induced NASH,\(^{86}\) including lowered serum transaminases, reduced steatosis (intrahepatic) and inflammation scores, and inhibited activation of Kupffer cells and inflammatory factor expression of adipokines in hepatic tissues (also shown in a different NASH model).\(^{87}\) Interaction with recombinant sFRP5 significantly lowered levels of IL6, monocyte chemoattractant protein-1 (MCP-1), TNFα and IL-1β in hepatic tissue.\(^{87}\) The recombinant sFRP protein has so far been used in vitro and in animal models, in all showing a positive effect on fibrosis. To date, according to our knowledge, there are no literature data about the use of recombinant sFRP in humans or its side effects. However, in a recently published study, serum levels of circulating sFRP were measured in women with breast cancer. A favorable predicted survival was found, while the high levels in breast cancer tissue were found to be associated with a better outcome for patients.\(^{59}\)

It is notable that even restored sFRP5 can also inhibit β-catenin and the Wnt pathway by downregulation of cyclin D1 and c-myc genes.\(^{87}\) The observed significant effects of sFRPs in liver fibrosis could be related to its ability to bind the cysteine domain of proteins in the Wnt pathway, but the underlying molecular mechanisms are yet unknown.\(^{51}\)

**Niclosamide**

Antifibrotic effect of the Food and Drug Administration-approved anthelmintic niclosamide was recently confirmed by El Ashmawy et al.\(^{56}\) in carbon tetrachloride (CCL4)-induced fibrosis in mice. Niclosamide suppressed expression of TGF-β1 and the Dvl2 genes\(^{56,79}\) and activity of β-catenin.\(^{56,80}\) Serum levels of ALT, AST, L-hydroxyproline and L-glutaminase activity and total bilirubin were significantly reduced by niclosamide and CCL4 compared to a mouse group treated with CCL4 only.\(^{56}\) Significant reduction of α-SMA expression, which confirmed niclosamide’s inhibitory effect of S100A4\(^{81}\) and was responsible for induction of α-SMA,\(^{82}\) was also proven. Niclosamide achieved a weakened effect of autophagy-induced apoptosis,\(^{83,84}\) which can be explained by the fact that apoptosis may be one of the main mechanisms of liver fibrosis resolution, despite its association with exacerbated stages of fibrosis.\(^{56}\) Niclosamide could be established as a future antifibrotic therapeutic solution, since recently reported adverse effects of niclosamide seem to be neglectable. The latest review concluded that several shortcomings, including certain cytotoxicity, limited aqueous solubility and partial absorption from the intestinal tract, could be overcome using nano-based formulations.\(^{85}\)

According to 2021 published results of a phase Ib trial regarding prostate cancer treatment, reformulated orally-bioavailable niclosamide nanoparticles had similar and improved pharmacokinetic results, the most common side effect.\(^{86}\) Only few patients experienced grade 3 adverse effects, including fatigue (n=1), abdominal pain (n=1), anemia (n=1), hypoalbuminemia (n=1), and hyperglycemia (n=1).\(^{80}\)
3-yl)-1H-pyrrole-2,5-dione) is a selectable and potent GSK-3β inhibitor, capable of exerting an effective role in therapy of many diseases and currently under investigation. GSK-3β has a known regulatory role in the inflammatory response and cytokine production, as well as in cellular proliferation. Over the years, GSK-3β has gained importance among researchers, for its role in the Wnt/β-catenin signaling pathway. Studies have shown that inhibition of GSK-3β...
results in anti-inflammatory cytokine production. Inhibition of GSK-3β has been studied on pulmonary fibrosis, for which administration of SB-216763 has produced beneficial effects on the inflammatory and profibrotic milieu by inhibiting the production of MCP-1 and TNFα in pulmonary macrophages; significant reduction in bleomycin-induced apoptosis of alveolar epithelial cells has also been noted. In vivo administration of SB-216763 to mice has been proven as safe for preventing bleomycin-induced respiratory distress syndrome, as there were no toxic effects on heart, liver or kidney, and in improving survival of treated mice. These results positioned GSK-3β as a potential molecular target in pulmonary fibrosis treatment, and it has been further investigated in diabetes mellitus, bipolar disorder, Huntington’s disease, Alzheimer’s disease, Parkinson’s disease and septic shock.53

It is well known that liver dysfunction can occur in cases of sepsis. Studies suggest that activation of GSK-3β is involved in apoptosis and excessive inflammation in acute liver failure (ALF). Zhang et al.94 investigated the inhibitory effects of GSK-3β on polymicrobial sepsis in a liver injury model (induced by cecal ligation and puncture). Inhibition of GSK-3β in an animal model, by means of SB-216763 administration, resulted in reduction of mortality, amelioration of liver injury and suppression of hepatic apoptosis. On the molecular level, decreases in leukocyte infiltration, expression and release of inflammatory cytokines in the liver were noted. NF-κB transcriptional activity was suppressed, while CREB transcriptional activity was enhanced.94 The authors concluded that inhibition of GSK-3β reduces inflammatory cytokine production through modulation of the NF-κB and CREB signaling pathways in macrophages simulated by lipopolysaccharide (LPS).94 As the studies have shown, GSK-3β is activated during ALF progression, and its inhibition mitigated inflammation of the liver and ameliorated ALF in the mouse model. In the study by Ren et al.,95 the co-injection of D-galactosamine and LPS was used to induce ALF. Inhibition of GSK-3β by administration of SB-216763 resulted in increased autophagy and decreased liver inflammation. GSK-3β inhibition by SB-216763 protected against aldosterone-induced renal and cardiac injuries by activating autophagy.96 However, for cholestatic liver disease in a mouse model, a study by Zhuang et al.97 demonstrated that SB-216763 therapy aggravated liver fibrosis.

ICG-001

ICG-001 is characterized as a selective first-generation CBP inhibitor, capable of disrupting β-catenin interaction with CBP and thereby reducing the mRNA and protein expression of survivin significantly; survivin is one of the inhibitors of cyclin D1 and apoptosis.28 This therapeutic solution was primarily designed for targeting colon carcinoma cells, in order to induce apoptosis; although, in physiologically normal (epithelial) colon cells, the effect was not noticed.28 There are reports on the beneficial and inhibitory effects of ICG-001 in fibrosis; meanwhile, another report indicated that mRNA levels of collagen I, Wnt3a, αSMA, LRP6 and Wnt10b stimulated with TGF-β in mouse fibroblasts and human HSCs were inhibited with this CBP inhibitor.28 Attenuation of lung fibrosis (as induced by bleomycin) in mice was observed with ICG-001 treatment, as was prevention of fibrosis when simultaneously administering bleomycin and ICG-001, which subsequently reversed the pathogenic effect at later stages of the disease and positively impacted survival rate.28 A similar outcome was achieved with renal fibrosis in mice, through suppressive effects on collagens I and III, α-SMA, fibronectin plasminogen activator inhibitor-1, Snail 1 and 2 and fibroblast-specific protein-1; this benefit also occurred with treatment applied in the late stage of fibrosis.28 Akcera et al.55 reported an inhibitory effect of ICG-001 on deposition of collagen and HSC activation, contraction and migration in in vitro models, and also attenuation of angiogenesis, fibrogenesis and inflammation in in vivo models. ICG-001 can be an inhibitor of PDGFβR, α-SMA, vimentin and collagen 1; these effects were suggested by down-regulated expression of the corresponding genes in in vitro models of TGF-β-induced LX2 cells (HSCs).55 Furthermore, due to HSC migration during fibrogenesis and differentiation into myofibroblasts (contractile), scientists discovered an inhibitory effect with exactly 5 µM of ICG-001 in 24 h (then 48 h and maximum inhibition in 72 h).55 The almarBlue cell viability assay (ThermoFisher, Waltham, MA, USA) was used to confirm the absence of a relationship between migration of cells and proliferation differences.55 Confirmatory investigations of the findings from the previous study on LX2 cells (all parameters included, except vimentin) resulted in the same outcome on primary human HSCs, and ICG-001 was shown to inhibit expression of peroxisin.55 ICG-001-related downregulation of Axin-2 and β-catenin was also noted in a mouse liver injury model induced by CCl4.55 The beneficial effects on fibrosis by ICG-001 were enhanced when it was used as a prototype in the development of PRI-724,58 a novel promising and effective therapeutic solution for liver fibrosis.28

PRI-724

PRI-724 is selective CBP second-generation inhibitor and influences β-catenin interaction. It acts through HSC inhibition and disruption of the macrophage-inflammation system, exerting antifibrotic effects that have been confirmed in murine models of fibrosis.49 In a murine model, CCL4-induced liver fibrosis, administration of PRI-724 showed accelerating effects on the resolution of fibrosis in liver followed by an increasing effect on MMPs in intrahepatic leukocytes. It is significant that this inhibitor reduced CCL4-induced liver fibrosis in mice without affecting levels of serum ALT and proved incoherence with reparation of hepatic fibrosis.49 Effects of C-82, an active PRI-724 metabolite, was demonstrated through a mRNA-mediated suppression of α-SMA, TIMP-1 and collagen I, as well as down-regulation of proteins such as Ki67, α-SMA and cyclin D.28 Gene expressions in activated HSCs were abrogated by PRI-724 treatment, whereas CCL4 was inhibited, which led to antifibrotic effects.28 HCV transgenic mice are another model system used to determine effects of PRI-724 on liver fibrosis, collagen production, and levels of α-SMA.99 A study using this model showed attenuation of the previous increased collagen.99 Levels of α-SMA, reflecting HSC activation were reported to be higher in the transgenic mice in comparison with controls and the administration of PRI-724 attenuated this effect.99 Another study showed decreased levels of TIMP-1 and elevated levels of MMP-8 as another line of evidence supporting PRI-724’s antifibrotic effect.99

A NASH mouse model was used to investigate the antifibrotic effects of PRI-724; the administration of PRI-724 inhibited hepatocyte apoptosis and hepatic fibrosis.28 PRI-724 was also used in clinical trials that included advanced stages of myeloid malignancies, pancreatic cancer and solid tumors, and according to the available literature there has been a phase I clinical trial (single-center, open-label) on the tolerance and safety of PRI-724 in HCV cirrhosis patients, in which it showed good tolerability.28 Reduction of hepatic lobule fibrosis by PRI-724 improved liver histology (in a dose-dependent manner) as well as Child-Pugh scores (in some individuals).28 In a pre-clinical study of PRI-724 administration to dogs for 28 days at the dosage of 120 mg/
kg/day showed that this therapeutic solution did not yield any adverse effect; in addition, when given to 18 participants with solid tumors (dose range: 40–1,280 mg/m²/day, any adverse effect; in addition, when given to 18 participants with solid tumors (dose range: 40–1,280 mg/m²/day), no adverse effects were reported. Taking into account a small sample of patients (n=14 in total) in one study, the following side effects were noted: fatigue (n=3) and nausea (n=4), vomiting (n=2), constipation (n=2), and reaction at the injection site (n=4). Other less common side effects included pruritus, rash, headache, fever, vertigo, anaphylactic shock, and reduced biochemical results showed the most common side effect to be elevations in total bilirubin and thrombocytopenia. Two other serious adverse events not directly related to the study were reported, namely bacilluria due to injection site infection and hemorrhage due to liver biopsy. However, a phase Ia/Ib clinical trial (NCT01302405) was discontinued due to low patient response. Currently, PRI-714 (new drug name: OP-724) is in a phase I clinical trial (NCT04688034) for patients with liver cirrhosis caused by human immunodeficiency virus/HCV co-infection with hemophilia.

Of note, the previous clinical studies investigating PRI-724 have included a small number of patients. As such, further and more detailed studies with larger samples are needed to gain a clearer understanding of its safety and efficacy. We hope that one of the aforementioned clinical trials will yield significant results.

**PSH**

PSH, a methylated derivative of resveratrol, has been previously evidenced to have antioxidant, anti-inflammatory and antiangiogenic effects, and has been observed to act reducing HSC activity. The inhibitory effect was achieved by suppressing the Wnt/β-catenin pathway via miR-17-5p (acting as an oncogene) and also that it could enhance WIF1 expression. Since activation of HSCs is at the core of liver fibrosis development, it is rational to develop a therapeutic strategy that would block or limit their activation. A study by Yu et al. showed how resveratrol can reduce the progression of liver fibrosis, while one by Chao et al. demonstrated that the PSH, as a methylated form of resveratrol, is more stable than other forms. As previously mentioned, the imbalance between ECM synthesis and its degradation is at the core of the liver fibrosis process. The presence of myofibroblasts derived from HSCs form the basis for the production of ECM that contributes to the development of liver fibrosis. PSH’s beneficial effects include reducing cell proliferation, collagen production and α-SMA expression, and partially inhibiting HSC activation. The inability of β-catenin translocation to the nucleus and reduction of TCF activity is due to PSH along with an increased amount of APC, phosphorylated-β-catenin, GSK-3β and WIF1 (acting as Wnt antagonist) that inhibit the Wnt β-catenin-dependent pathway. This therapeutic solution has shown potential in reducing the expression of PSH, as a methylated form of resveratrol, is more stable than other forms. It has been suggested that the PSH, as a methylated form of resveratrol, is more stable than other forms. As previously mentioned, the imbalance between ECM synthesis and its degradation is at the core of the liver fibrosis process. The presence of myofibroblasts derived from HSCs form the basis for the production of ECM that contributes to the development of liver fibrosis.

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At a dose of 100 µM, resveratrol reduces the regulation of vascular endothelial growth factor and inhibits the formation of major blood vessels in zebrafish; in this model, it also reduces survival and hatching rate of eggs, and causes teratogenic deformities and cardiac edema. Administration of resveratrol to rats at doses of 300/1000/3000 mg/kg/day led to hepatic impairment (confirmed by aberrant liver gene expression); at a dose of 1000 mg/kg/day, it increased bilirubin levels. A dose of 1000–3000 mg/kg/day, administered for 4 weeks, induced renal toxicity. Guha et al. showed that resveratrol can delay the healing of gastric ulcers in mice. At very high doses, resveratrol has been shown to cause severe adverse effects. Lethal outcomes have been reported in rats due to acute inflammation of the pelvic region, renal tubular dilatation, papillary necrosis, severe nephropathy, and cardiac inflammation. In addition, increased levels of liver enzymes as well as of blood urea nitrogen and creatinine can be a significant problem.

It is important to emphasize that resveratrol can act as a substrate for tyrosinase (an enzyme essential for the production of melanin) for the production of toxic α-quinones, which cause cytotoxicity of melanocytes (thiol protein production) and may have an adverse effect on the skin. The adverse effect on the skin can be explained by the fact that resveratrol is very similar to rhododendron, which is used for whitening and lightening in cosmetics and is basically also a tyrosinase inhibitor that can increase the incidence of leukoderma skin toxicity, so a similar effect of resveratrol on the skin should be assumed. It is known to exert pleiotropic effects on humans, and so far adverse effects involving nephrotoxicity and gastrointestinal problems have been reported. Although there is little information on human clinical trials (several are awaiting publication), it is related that resveratrol can cause an increase in plasma ALT and decreases in white blood cell count and plasma IL-6 and TNF.

Resveratrol at a dose of 1000 mg/day or higher can cause significant interactions with other drugs because it activates the cytochrome P450 (CYP) isoform CYP1A2 and inactivates CYP3A4, CYP2C9 and CYP2D6; at the same dose, an increase in markers of cardiovascular disease has been observed. Administration of resveratrol in high doses (2000–5000 mg/day) may cause nausea, hypersensitivity, anal pruritus, and episodes of diarrhea (light and mild), which is a more important cause than for healthy ones. The aforementioned has been confirmed by a phase II clinical trial in humans with refractory multiple myeloma, in whom 5000 mg of resveratrol was administered; one patient had lethal outcome, probably due to the adverse effects (renal toxicity, fatigue, nausea, diarrhea).

The agents discussed above have evidenced potential therapeutic effects in liver fibrosis, acting through numerous molecular mechanisms and signaling cascades, including inflammation and fibrogenesis.

**Conclusions**

Liver fibrosis is a significant global health problem and economic burden, with potential to progress to liver cirrhosis and cancer. Its worldwide mortality is 1.5 million deaths per year. Regardless of etiology, the pathophysiology of fibrosis includes chronic inflammation, hepatocyte death, HSC activation, and endothelial or epithelial barrier disruption, all mediated by various proinflammatory cytokines and other chemokines, and including several signaling pathways, such as those of Wnt, notch and Hh. The Wnt signaling pathway is activated in the process of ongoing liver fibrosis. It acts through canonical and non-canonical pathways. Several agents that have shown inhibitory effects on Wnt signaling are being considered to impart preventive or therapeutic effects on liver fibrosis. These are DKK1, niclosamide, sFPR5, SB216763, ICG-001, PRI-724 and PSH. Their validation and utility with regards to potential adverse effects has yet to be proven, but the future remains promising. In this review, the current lore about the Wnt signaling pathway,
Duspara K. et al; Drug targets of Wnt signaling in liver fibrosis which notably participates in liver fibrosis development, was gathered and summarized. Considering the critical points of the Wnt signaling pathway in liver fibrosis, the findings on potential therapeutic targets in liver fibrosis and agents that are being considered for preventive or therapeutic effects in liver fibrosis were presented.

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Conflict of interest

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

Author contributions

Conceived of and designed the article, and critically revised the manuscript (MS, KB), obtained funding and provided administrative, technical and material support (MS), performed literature searches and wrote the manuscript (KD, DK, TOK, JIP), updated the text of the manuscript (KB, MG), generated administrative, technical and material support (MS), performed

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