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Review Article

Understanding the Mechanism of Hepatic Fibrosis and Potential Therapeutic Approaches

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ABSTRACT

Hepatic fibrosis (HF) is a progressive condition with serious clinical complications arising from abnormal proliferation and amassing of tough fibrous scar tissue. This defiance of collagen fibers becomes fatal due to ultimate failure of liver functions. Participation of various cell types, interlinked cellular events, and large number of mediator molecules make the fibrotic process enormously complex and dynamic. However, with better appreciation of underlying cellular and molecular mechanisms of fibrosis, the assumption that HF cannot be cured is gradually changing. Recent findings have underlined the therapeutic potential of a number of synthetic compounds as well as plant derivatives for cessation or even the reversal of the processes that transforms the liver into fibrotic tissue. It is expected that future inputs will provide a conceptual framework to develop more specific strategies that would facilitate the assessment of risk factors, shortlist early diagnosis biomarkers, and eventually guide development of effective therapeutic alternatives.

Key Words: Antifibrotic strategies, biochemical abnormalities, cell types, gene therapy, hepatic fibrosis

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Hepatic fibrosis (HF) is a pathological condition resulting in abnormal proliferation and accumulation of tough fibrous connective tissue (scar tissue) in the liver. Although the formation of scar tissue is a normal body response to injury, in fibrosis, this healing process goes erroneous. Normal process of wound healing involves collagen deposition; however, the chronic activation of this healing mechanism leads to liver pathology. Among a variety of causes/factors or stimuli, which bring about this transformation are: chronic infection by hepatitis B, C viruses and parasite *Schistosoma*, chronic alcoholism and/or exposure to certain drugs and toxins, infections, non-alcoholic steatohepatitis (NASH), inherited metabolic diseases like hematochromatosis, Wilson's disease, α-1 antitrypsin deficiency, autoimmune diseases such as primary biliary cirrhosis, and auto-immune hepatitis.[1] Generally, HF begins with the stimulation of inflammatory immune cells to secrete cytokines, growth factors, and other activator molecules. These chemical messengers direct hepatic stellate cells (HSCs) to activate and synthesize collagen, glycoproteins (such as fibronectin), and proteoglycans. Deposition of the abnormal products of stellate cells, along with portal myofibroblasts, bone marrow-derived cells, and epithelial mesenchymal tissues (EMT), build-up of extracellular matrix (ECM, nonfunctional connective tissue) in the liver, and the impairment by collagenolysis are simultaneous processes.[2-5] In the long run, it may either lead to cirrhosis and related complications or may couple with carcinogenesis and ultimate death due to failure of normal liver functions.[6,7] Nearly 3 billion and 180 million people have been exposed to hepatitis B and C virus, respectively.[8,9] Epidemiological studies have shown that in China, hepatitis B viral (HBV) infection is the major cause of liver fibrosis, whereas in the United States, Europe, and Japan, hepatitis C viral (HCV) infection and alcohol are the main causes.[10,11] In the sub-Saharan African region, *Schistosoma mansoni* infection is reported to be the major cause of HF, resulting in almost 0.3 million deaths annually.[12] This review covers recent information on the general mechanisms of HF, its impact on some biochemical parameters, and therapeutic potential of certain antifibrotic agents. The discussion outlines possible strategies and applications of the published information while designing and formulating new treatment regimen.

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FUNCTIONAL CONSEQUENCES OF DIFFERENT CELL TYPES

HSCs

HSCs, also known as fat-storing cells or perisinusoidal lipocytes, represent almost 5–8% of all healthy liver cells. They are located near the hepatocyte laminated in the perisinusoidal space of Disse by means of star-like dendritic cytoplasmic processes extending along and around the hepatic endothelial cells and hypothesized of being mesenchymal in origin.[13] HSCs show two different phenotypes: quiescent in the normal liver and activated in the diseased. As a consequence of this transformation, diseased phenotype has altered functions. In the following, we summarize morphological and functional differentiation of the two phenotypes of HSCs.

Quiescent HSCs

Quiescent HSCs have a star-like shape and their cytoplasm encloses numerous lipid droplets, which contain retinoids, triglycerides, cholesterol, and free fatty acids.[14] Storage and limited release of retinoids is a major function of HSC in the healthy liver. An essential and prominent structural feature of HSCs is the presence of microfilament bundles of actin and intermediate filaments such as desmin, vimentin, and synemin.[15] HSCs also express the LIM-homeodomain protein Lhx2, a transcription factor responsible for maintaining their quiescence.[16] Quiescent HSCs express peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor considered as fundamental transcriptional regulator for adipogenesis that also displays antifibrogenic effects by inhibiting type I collagen expression at the transcriptional level.[17] Quiescent HSCs also regulate the expression of hepatocyte growth factor (HGF), TGF-β, insulin-like growth factor-I (IGF-I), and other cytokines in an auto- and paracrine manner [Figure 1].[18-20] However, the phenotype lacks expression of fatty acid synthase (FAS) receptor CD95 (a cellular surface protein with a molecular weight of 42-52 kDa that promotes apoptosis).[20] Reportedly, HSCs have a role in the expression of some other important neural proteins like glial fibrillary acidic proteins, neuronal growth factor, synaptophysin, RhoN, glutamine synthetase, and neurotrophin receptors.[21]

Activated HSCs

Activation of hepatic stellate cells depends on a number of factors discussed under "Introduction," which are either directly or indirectly involved in progression of the HF.[1] Activated HSCs develop into myofibroblast-like cell types, which are differentiated by the loss of lipid droplets, lack of glial fibrillary acidic proteins, and increased cell proliferation. Consequently, excessive synthesis of ECM components occurs, causing increased expression of α-smooth muscle actin (α-SMA) and changes in the expression of L-type voltage-operated Ca^{2+} channels, which are known to mediate Ca^{2+} influx and regulate cellular contraction.[22] The activation of HSCs is controlled by the gene expression, which is itself regulated by various transcription factors briefly described below.

FoxO

Forkhead box gene group O. Its functions and its intracellular localization are regulated by growth factor (mainly PDGF) activated kinases, especially phosphoinositide 3-kinase and protein kinase-B (PKB), through phosphorylation.[23,24] Phosphorylation suppresses transactivation and promotes the translocation of FoxO proteins from the nucleus to the cytosol, reducing the expression of their target genes. The transcription factor FoxO is a key player in controlling the trans-differentiation and proliferation of HSCs that leads to liver fibrosis *in vivo*.[25]

ILK

Integrin-linked kinase, plays a crucial role in HSC activation, fibrogenesis, and transducing signals from the ECM or from two known growth factors, TGF-β and ET-1, to the cytoplasm.[26] ILK couples the integrins and growth factors to downstream signaling pathways, which favor the suppression of apoptosis and promote cell cycle progression. The increased expression of ILK is unregulated during HF.[26]

PPAR-γ

Peroxisome proliferator-activated receptor-γ, a nuclear receptor transcriptional factor considered to be the fundamental transcriptional regulator for adipogenesis (anabolic pathways) and also reported to have antifibrogenic activity, is expressed by quiescent HSCs. Reduced activity of PPAR-γ, results in increased HSC activation and proliferation.[17]

Kupffer Cells in HF

Kupffer cells are highly phagocytic tissue macrophages of the liver, responsible for the removal of circulating microorganisms, immune complexes, and debris from the blood stream as also detoxicating endotoxins. They constitute about 15% of the total liver cell population. Moreover, these Kupffer cells take up different substances from the circulation via receptor-mediated endocytosis. Being a part of the innate immune system, these cells play an important role in the regulation of inflammatory processes in liver by secreting cytokines such as TNF-α, IL-1, IL-6, and reactive oxygen species (ROS), which promote chemotaxis, phagocytosis, and ROS production by other inflammatory cells.[27]

Kupffer cells, when treated with gadolinium chloride, produce interstitial collagenase MMP-13, which reduces

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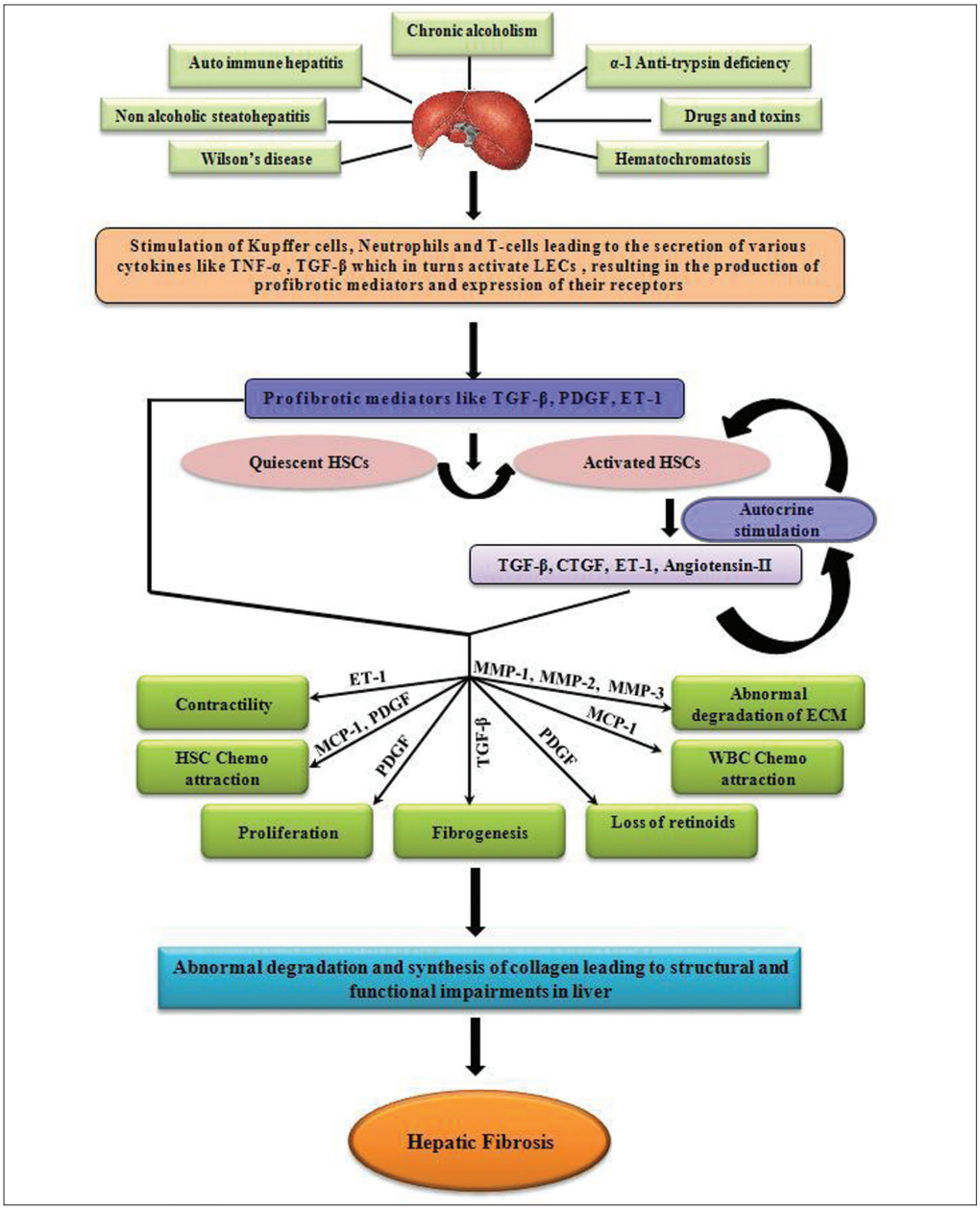

Figure 1: Early causes and mechanisms of matrix degradation and progression of hepatic fibrosis. Stimulation of Kupffer cells, neutrophils, and T-cells cause secretion of various cytokines and profibrotic mediator to convert quiescent to activated hepatic stellate cells (HSCs). HSCs are associated with matrix degradation due to increased production of membrane type matrix metalloproteinase-1 (MMP-1), matrix metalloproteinase-2, -3 (MMP-2, -3), and tissue inhibitors of metalloproteinases (TIMPs), leading to amassing of scar tissue

ECM deposition during experimental fibrosis.[28] In addition, activated Kupffer cells can effectively kill HSC by a caspase 9-dependent mechanism via the involvement of TNF-related apoptosis-inducing ligand (TRAIL).[29,30] Their antifibrotic effect is evident by the presence of IL-10, another important cytokine that has anti-inflammatory and antifibrotic effects, especially in the early stages of fibrosis and during acute liver injury.[31] While decreasing collagen production, IL-10 up-regulates collagenase secretion, resulting in a reduction of collagen deposition.

PROFIBROTIC MEDIATORS

The activated HSCs produce a number of cytokines and active peptides that promote their constrictive, proliferative, and transformative properties in an autocrine manner, promoting the development of liver fibrosis.[32-35] Among fibrotic mediators, TGF-β is certainly one of the most important polypeptides along with PDGF, which is regarded as a potent stellate cell mitogen.[36] Activated HSCs are known to up-regulate and enhance the autocrine effect

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of receptors for IL-10, FAS (CD95), PDGF, FGF, VEGF, TGF-β1, and p75 (a member of the TNF receptor super family).[20,32,35,37,38] At the same time, these HSCs also down-regulate the expression of IGF-I receptor, three TGF-β type receptors, and signaling mediators commonly named "Smad proteins."[20,39]

In HSCs, TGF-β participate in intracellular signaling cascade and transcriptional regulation of the genes: Ras, Raf-1, MEK, and MAPK.[40] Though the main source of TGF-β in the fibrotic liver is activated HSCs, liver endothelial cells and Kupffer cells also contribute to synthesis of this growth factor.[41]

Cholestasis (decreased excretion of bile) may result in accumulation of bile acids in liver, which encourages biliary epithelial cells to secrete ET-1, TNF-α, and PDGF. A 21-amino acid peptide, ET-1, induces HSC activation, invasiveness, and fibrogenesis by up-regulating type-I collagen gene expression and procollagen α-1 during the early phase.[42] The up-regulation of ET-1 is catalyzed by endothelin-converting enzyme-1 that continuously converts precursor ET-1 into mature ET-1 under favorable conditions.[42]

MOLECULAR BASIS OF HF

HF involves the activation of HSCs and over-expression and over-secretion of collagens, resulting in the excessive accumulation of ECM proteins. Broadly, development of the fibrosis has two main phases: inflammation and fibrogenesis. Initially, various hepatotoxic factors induce synthesis of mediators, which cause inflammatory reactions within hepatic cells. Mediators of inflammation phase bring about the strenuous phenotypic change from quiescent HSCs to activated HSCs, which have altered ECM composition. In the course of activation process, transformed HSCs become proliferative and acquire characteristic features. Morphologically, they appear myofibroblast-like, which are devoid of lipid droplets, lack glial fibrillary-acidic protein, have excess production of ECM components, and exhibit increased expression of α-smooth muscle actin (α-SMA) fibers. Stimulated by TGF-β released initially from Kupffer cells, activated HSCs also start to synthesize markedly increased amounts of ECM proteins; specifically type-I and type-III collagen.

Activated HSCs show chemotactic response and migrate towards regions of injury and start accumulating around damaged tissue. The same cytokines, which are mitogens for HSCs, play the role of chemo-attractants for these cells. Convincing evidence supports the secretion of chemo-attractants (for instance, monocyte chemotactic protein, MCP-1), which not only activate HSCs but also attract other activated HSCs. The cytokines promote the recruitment of monocytes and leukocytes.[43] Activated HSCs express the cytoskeleton protein, α-smooth muscle actin (α-SMA), equipping the cells with a contractile apparatus and various connective tissue proteins including collagen types I, III, and IV.[38,44,45] HSCs are thus capable of constricting individual sinusoids as well as the entire fibrotic liver. A balance between ET-1 and NO is assumed to regulate the contractile activities of HSC, wherein ET-1 is the key contractile stimulus of HSC while NO an antagonist of ET-1 produced by HSC, Kupffer cells, and liver endothelial cells.[42]

During fibrosis, the low-density matrix that is normal for healthy liver is degraded and replaced by an excess of non-functional collagenous ECM tissue. Calcium-dependent enzymes and matrix metalloproteinases (MMP) disrupt both collagen and non-collagenous compounds of ECM.[46] Activated HSCs also secrete MMP-1 (interstitial collagenase), MMP-2 (Gelatinase A), and MMP-3 (Stromelysin 1), which degrades the main components of ECM such as type IV collagen and laminin. In a way, the lytic activities indirectly perpetuate the deposition of collagen type I and III. Moreover, in fibrotic liver, markedly increased levels of tissue inhibitors of matrix metalloproteinases (TIMPs) have been recorded. They are capable of inhibiting the action of MMP-1, thereby causing the accumulation of collagen fibers [Figure 1].[47,48]

Table 1: Changes in the levels of amino acid and proteins noted during the progression of hepatic fibrosis

Category	Biomolecules	Source	Status in HF and remarks	References
Amino acid	Hydroxyproline	Serum	Significant increase in patients with HF of alcoholic origin	[57]
Proteins	Total proteins	Urine	Significant increase in urinary Excretion due to an increased catabolism of proteins	[58]
	Collagen	Liver	Owing to the enhanced expression of TGF-β, there is an increased collagen synthesis in fibrotic liver	[59-61]
	Albumin	Serum	Marked decrease of serum albumin level has been reported	[62,63]
	Haptoglobins	Serum	Because of the enhanced expression of TGF-β there is a significant decrease in the levels of haptoglobins	[64,65]
	α-2 Macroglobulin	Serum	Increased synthesis of α-2 macroglobulin enhance fibrosis by inhibiting the catabolism of other ECM proteins	[65,66]
	Apolipoprotein A-1	Serum	Decreased levels of serum Apo A-1 has been observed in liver fibrosis	[67]

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In the sub-Saharan African region, *Schistosoma mansoni* infection is the major cause of HF, affecting more than 200 million people of tropical countries, and is accepted to be highly endemic, specifically in agricultural regions of Egypt and Sudan.[49] Fibrosis caused by schistosomes is characteristically periportal type and can be subdivided into four stages: fibroblasts recruitment, followed by HSC differentiation, their proliferation, and subsequent secretion and remodeling of ECM.[50,51] In schistosomiasis, there is granuloma formation followed by inflammation that in subsequent steps causes portal hypertension, collagen deposition, and gastrointestinal bleeding mediated by T_H2 cytokines (i.e., IL-4, IL-5, IL-10) and TGF-β.[52,53] Literature shows that high levels of IL-5 and IL-13 are found in subjects with parasite-induced liver fibrosis.[54] Contrary to a general mechanism, some interesting work published in the recent past also showed inhibitory roles of TNF-α, IL-12, INF-γ, and NO, associated with the T_H1 response in schistosomiasis.[53]

Table 2: Adjustments in the levels of different enzymes from sera and liver tissue during progression of hepatic fibrosis

Enzymes			References
LDH			
TLDH	Serum	Significant increase in total LDH activity in the serum	[68]
LDH_1, LDH_2, LDH_3	Serum, Liver	Unaltered activity of these isoenzymes of LDH	[69,70]
LDH_4	Serum, Liver	Increased activity of LDH_4	[70]
LDH_5	Serum, Liver	The appearance of LDH_5 in the serum indicates hepatocellular damage. Increased activity of LDH_5	[69,70]
ALP	Serum	Increased activity	[70]
GOT	Serum	Increased activity	[70]
GPT	Serum	Increased activity	[70]
G6Pase	Serum	Increased activity	[71]
GGT	Serum	Increased activity	[65]
Hyaluronidase	Serum	Decreased activity	[72]
β-glucuronidase	Liver	Increased activity	[73]
Urea cycle enzymes	Serum	Unaffected	[74]

LDH: Lactate dehydrogenase, ALP: Alkaline phosphatase, GOT: Glutamic oxaloacetic transaminase, GPT: Glutamate pyruvate transaminase, G6Pase: Glucose 6 phosphatase: GGT: Gamma glutamyl transferase

BIOCHEMICAL ABNORMALITIES AND HISTOLOGICAL CHANGES OBSERVED DURING PROGRESSION OF HF

Hepatic toxicity is always accompanied by impaired hepatocyte metabolism and deposition of connective tissue components in the liver.[55,56] Update of literature reveals that biochemical abnormalities are mainly associated with disruption in the levels of intermediate metabolites or their end products in experimentally induced as well as naturally occurring HF. Moreover, enzymes like GOT, ALP, LDH, and many others have been reported to vary during the progression of HF. The summary of these abnormalities in either of the above conditions is listed in Tables 1-3.[57-79]

Like vitamins, minerals and metabolites also play important roles in the living systems. The minerals are components of metalloproteins and metalloenzymes, apart from acting as enzyme cofactors. As these compounds are metabolized mainly in the liver, functional impairment of hepatic tissue in fibrosis and cirrhosis alters their levels as well. A compilation of levels of important vitamins, minerals, and metabolites altered during HF is given in Table 4.[80-91]

Table 3: Status of various biologically important molecules during liver fibrosis

Category	Biomolecules	Source	Status in HF	References
Lipid	Lipid Peroxides	Serum	Lipid peroxidation lead to decrease in fluidity of the lipid phase of biomembrane and have important consequences in relation to many of the major metabolic functions dependent on membrane structure and integrity. Increased oxidative stress and lipid peroxidation has been reported in HF	[75]
	Triglycerides	Liver	Significantly increased levels	[71]
	Malondialdehyde (MDA)	Liver	Significantly increased levels	[71]
	Cholesterol	Serum	Total serum cholesterol levels are markedly depressed in HF. The decreased serum cholesterol may be due to the reduction in the packed cell volume associated with chronic liver diseases. However, decreased serum cholesterol level does not have much clinical significance in the physiologic system	[76]
GAG	Hyaluronan	Serum	Marked increase in tissue bound and circulating Hyaluronan has been reported	[71,77]
Hormone	Insulin	Plasma	Hyperglycemia and impaired glucose tolerance have also been observed in patients with established HF	[78]

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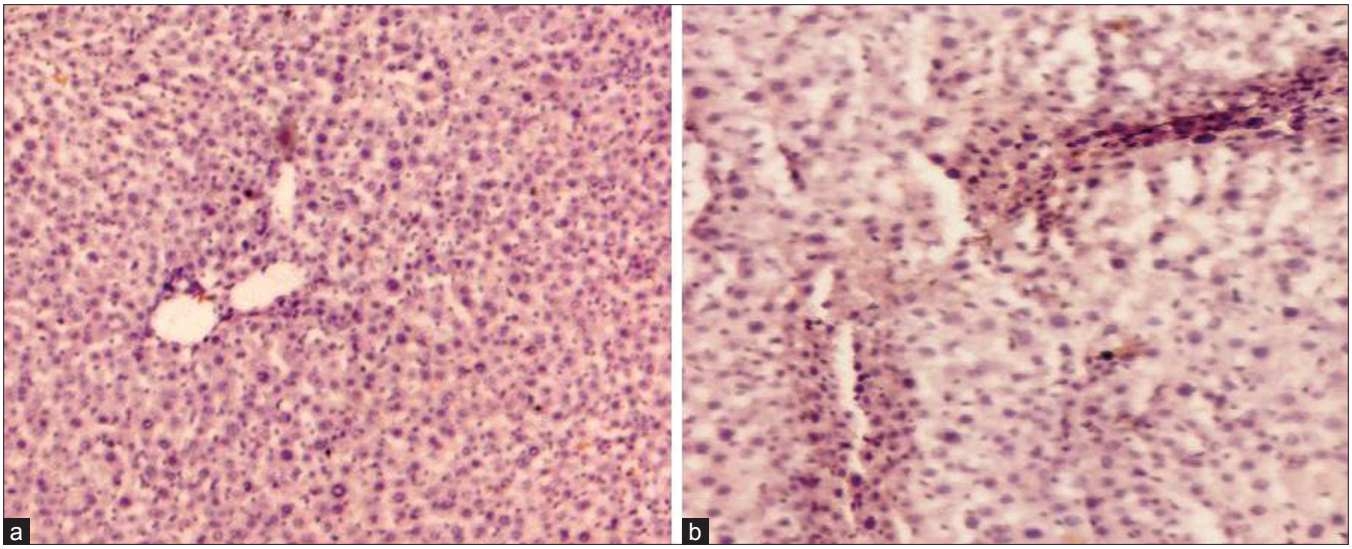

Figure 2: Immunohistochemical staining of α-smooth muscle actin (α-SMA) showing activated hepatic stellate cells during the progression of *N'*-Nitrosodimethyl amine (NDMA)-induced hepatic fibrosis in rats. (a) Control liver (×100) demonstrates absence of α-SMA staining and, (b) NDMA treatment day 21 (×100) exhibiting significant staining of α-SMA with enormous number of activated stellate cells in fibrotic zone

Table 4: Variations in the levels of vitamins, minerals, and other indispensable metabolites in the fibrotic liver

Category	Biomolecules	Source	Status in HF	References
Vitamins	Ascorbic acid	Liver, Serum	Significant decrease	[80]
	1α-25 Dihydroxyvitamin D	Plasma	Significant decrease in the plasma level of 1α-25 Dihydroxyvitamin D resulting in the retention and resorption of calcium ions in kidney tubules	[81,82]
Minerals	Sodium	Serum	Significant decrease	[83,84]
	Potassium	Serum, Liver	Significant decrease	[63,85]
	Calcium	Serum	Significant decrease	[86]
	Magnesium	Plasma, Serum	Significant decrease	[87,88]
	Phosphorus	Liver, Serum	Significant decrease	[76]
Metabolites	Creatinine	Serum, Urine	An abnormally low serum creatinine concentration has been observed in patients with severe hepatic disease. This decrease is due to decreased synthesis of creatinine from diminished muscle mass and inadequate production of creatine, a creatinine precursor, by the fibrotic liver	[89,90]
	Bilirubin	Serum	Increased levels	[70,71]
	Uric acid	Serum, Urine	Significantly elevated uric acid levels were observed in serum and urine samples because uric acid is the end product of purine catabolism, an elevated plasma level and urinary excretion of uric acid indicates increased degradation of nucleic acids in HF	[76,82]
	Urea	Blood	Normal blood urea levels were observed in hepatic fibrosis indicating the normal renal functions	[91]

Substantial literature is available on the histological changes occurring during HF.[58,70] These reports invariably demonstrate significant decrease in "liver weight" and decreased "liver to body weight ratio" in *N'*-Nitrosodimethyl amine (NDMA)-induced HF.[59,70] Decreased protein synthesis, cell necrosis, and collapse of liver parenchyma in late stages of HF are among suggested reasons. As a reference, in this section of review, we have taken up the changes, which occur in the liver only due to NDMA administration in mammalian model [Figure 2]. Immuno-histochemical (IHC) staining using monoclonal antibodies showed the presence of α-SMA in NDMA-treated liver sections [Figure 2b]. The presence of α-SMA convincingly showed that it was synthesized by activated HSCs during the course of HF. Normal liver specimens do not stain for α-SMA [Figure 2a]. IHC staining clearly demonstrated much higher number of positive stained cells in the fibrotic area when compared with normal areas of the liver sections.[70] In a recent study, peculiar changes in the RBC rheology along with the changes in other blood-related parameters and enzymes in a mammalian fibrotic model have also been demonstrated.[79]

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Table 5: Existing antifibrotic strategies, target molecules, and their mechanism of action in obstructing or regressing hepatic fibrosis

Antifibrotic strategy	Compound	Mechanism of action	References
TGF-β inhibition strategies	N-acetyl-L-cysteine	Block TGF- β -dependent Smad pathway signaling in HSCs, oppose the effect of ROS, induces cell cycle arrest, favor redox-mediated extracellular proteolysis of Platelet-derived growth factor (PDGF) receptor type β	[92,93]
	Prostacyclin	Suppress collagen production by inhibiting CTGF activity	[94]
	IL-10	Potent anti-TGF-β, suppress the activation of NF-kB as well as messenger RNA expression of TNF-α and macrophage inflammatory protein- 2 (MIP-2)	[19,95]
	IFN- γ	Inhibits the TGF-β -induced phosphorylation of Smad3. Induces the expression of Smad7 (an effective inhibitor of TGF-β-induced fibrogenesis)	[39,96]
	Prostaglandin E2 (PGE2)	Suppress TGF-β1-mediated induction of collagen by HSCs	[97]
PPAR-γ stimulation strategies	Curcumin	Interrupt the PDGF and EGF (Epithelial growth factor) signaling pathway resulting in the induction of gene expression of endogenous PPAR-γ gene causing the suppression of the expression of TGF-β, α-SMA and MCP-1 genes	[98]
	Thiazolidinediones	Reduce TGF-β mRNA expression; inhibit TGF-β induced human type I procollagen promoter activity in human HSCs by interrupting the inhibition of PPAR-γ transcriptional activity	[99,100]
	15-deoxy-delta12,14-prostaglandin J (2)	These PPAR-γ agonists markedly inhibit TGF-β1-induced CTGF expression in HSCs, inhibit cell growth both through cell cycle arrest and an increase in apoptosis.	[101]
Apoptosis stimulation strategies	Adiponectin	An adipokine released by HSCs, critical in maintaining the HSC quiescent phenotype or in reversing hepatic fibrosis by induction of activated HSC apoptosis	[102]
	IGF-1	Treatment of activated HSCs with IGF-I is also able to induce apoptosis. Over expression of IGF-I by activated HSCs restrict their activation, attenuate fibrogenesis and accelerate liver regeneration.	[20,103]
	Gliotoxin	Induce HSC apoptosis and attenuate the liver fibrosis through inhibition of NF-kB.	[104]
	Sulfasalazine	Inhibit the autophosphorylation of IKK-α and IKK-β and the subsequent activation of the IKK (Inhibitor kappa kinase) complex. Thus it helps in the rapid clearance of α-SMA positive fibroblast, decreases hepatic expression of type 1 procollagen and TIMP-1 increases the hepatic MMP activity and accelerate the resolution of hepatic fibrosis.	[105]
Suppression/ inhibition of HSCs proliferation and activation	Brain natiuretic peptide	Suppress HSCs proliferation	[106]
	Retinoic acid	Suppress HSCs proliferation	[107]
	L-Cysteine	Suppress HSCs proliferation and activation	[108]
	Serine protease inhibitor-Camostat mesilate	Inhibit HSCs activation	[108]
	Dilinoleoyl phosphatidyl colchicines	Inhibit HSCs activation	[109]
	PPAR-γ antagonist rosiglitazone	Inhibit HSCs activation	[109]

ANTIFIBROTIC STRATEGIES

Recent research in molecular biology has helped to discover and better characterize several elements that are perfectly and dynamically involved in making the liver fibrotic. This deeper molecular understanding of the pathogenesis of liver fibrosis has opened up opportunities for novel and exciting complementary therapeutic approaches. A remarkable achievement has been the demonstration of reversibility of advanced fibrosis and even cirrhosis after administering certain anti-fibrotic agents.[70,71,79]

Antifibrotic compounds are classified in accordance with their mechanism of action [Table 5].[92-110] For instance, compounds such as antioxidants and those with properties of reducing inflammation, promoting ECM degradation, inhibiting HSC activation and proliferation, reducing ECM production by HSC, neutralizing HSC contractile response, and stimulation of HSC apoptosis.

Participants like ECM proteins, some receptors mediating cell–ECM interactions, cytokines, integrins, growth factors (principally TGF-β and PDGF) and post-receptor signal regulators (i.e., Smads), and transcription factors (principally a decreased expression of PPAR-γ) can be the focus of an antifibrogenic therapeutic strategy.[39,98,99,101,109] Even though a large number of drugs have also been developed for antifibrotic effects, they have their own limitations and side

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effects. Interestingly, some recent studies have highlighted remarkable therapeutic effects of plant derivatives and the whole phytoextracts in reversing HF [Table 5].

Besides, few other compounds have been demonstrated to possess antifibrotic properties, as given below briefly.

Relaxin

A peptide hormone, has been shown to be antifibrotic that works by decreasing TIMP-1 and TIMP-2 expression in HSCs, which enhances matrix degradation and reduces interstitial collagen deposition.[111,112] When added to cultured TGF-β1-stimulated rat HSCs, relaxin displays anti-TGF-β effects by inhibiting collagen over-expression *in vitro* and reducing the level of α-SMA.[111]

Imatinib

A drug used for the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, inhibits PDGF-β receptor kinase.[113] This is a potentially useful property for attenuating liver fibrosis. Despite proven antifibrotic effects in cultured HSCs as well as in animal models of liver fibrosis, this drug has a limitation due to its cardiotoxic side effects.[114]

Angiotensin II receptor antagonist

Circulating levels of angiotensin II (ANGII), a powerful vasoconstrictor factor, frequently increase in chronic liver diseases. In these conditions, HSCs proliferate, acquire contractile properties, and excessively synthesize endothelin, PDGF, and chemokines.[33,115] *In vitro* and *in vivo* studies on

Table 6: Known phytoextracts and their derivatives with probable therapeutic action used in traditional medicine to treat hepatic fibrosis

Extracts (source)	Therapeutic action	References
Turbud, *Operculina turpethum* (white roots)	Inhibit HF by reducing the expression of α- SMA in HSCs	[70]
Curcumin, *Curcuma longa* (roots)	Inhibit HF by suppressing the activation of HSCs, activate PPAR-γ to reduce cell proliferation, induce apoptosis and suppress ECM gene expression	[71]
Silymarin, *Silybum marianum*	Inhibit HF by suppressing the activation of HSCs	[71]
Matrine and Oxymatrine, *Sophorae flavescentis* (roots)	Inhibit PDGF and TGF-β1 actions	[118]
Taurine, *Calculus bovis*	Inhibit TGF-1 action, collagen formation, reduce oxidative stress	[119]
Rehin, emodin, *Rheum palmatum* (roots and rhizome)	Inhibit TGF-β1 expression, anti-HSC proliferation	[120]
Tetramethylpyrazine, *Ligusticum chuanxiong* (rhizome)	Anti-oxidation, synergic anti-hepatic fibrosis effect with rehin,	[121]
Ginkgo biloba (leaves)	Suppress NF-κB activation, inhibit TGF-β1 and collagen gene expression	[122]
Gypenoside, *Gynostemma pentaphyllum*	Inhibits HSCs proliferation by arresting HSCs at G1 phase	[123]
Salvia miltiorrhiza	Reduces the synthesis of TGFβ-1, procollagens I and III and TIMPs	[124]

Table 7: Modern approaches of gene therapy used in the treatment of liver fibrosis

Target achieved	Gene/Oligonucleotides inserted	Method of gene delivery
Reduction of Collagen deposition	Rat interferon-α,[145] human pro-matrix metalloproteinase-1,[146] antisense complementary to the 3′-portion of rat TGF- β1 mRNA,[147] human interleukin-10,[148] recombinant α-melanocyte stimulating hormone,[149] mouse smad-7[150]	Adenovirus, Recombinant Adeno-associated virus, Electroporation
Decreased expression of Collagen-I mRNA	Mitochondrial superoxide dismutase (SOD),[151] rat hemeoxygenase-1,[152] human interleukin-10[148]	Adenovirus, Recombinant Adeno-associated virus, Electroporation
Down regulation of TGF-β and TNF-α	Mitochondrial superoxide dismutase (SOD),[151] matrix metalloproteinase-8,[153] rat hemeoxygenase-1,[152] human interleukin-10,[148] recombinant α-melanocyte stimulating hormone[149]	Adenovirus, Recombinant Adeno-associated virus, Electroporation
Reduction in oxygen free radical formation	Mitochondrial superoxide dismutase (SOD)[151]	Adenovirus
Reduced expression of α-SMA	Rat interferon-α,[145] human pro-matrix metalloproteinase-1,[146] antisense complementary to the 3′-portion of rat TGF- β1 mRNA,[147] mouse smad-7[148]	Adenovirus
Down regulation of TIMP-1 mRNA	Rat interferon-α,[145] human interleukin-10,[148] recombinant α-melanocyte stimulating hormone[149]	Adenovirus, Electroporation
Reduction of PDGF protein expression	Antisense mRNA complementary to the 5′-coding sequence of PDGF B-chain[154]	Adenovirus
Upregulation of MMP-2 and MMP-9	Antisense mRNA complementary to the 5′-coding sequence of PDGF B-chain[154]	Adenovirus
Reduction of fibrous regions and pseudo module formation	Antisense mRNA complementary to the 5′-coding sequence of PDGF B-chain,[154] human hepatocytes growth factor[155]	Adenovirus, Electroporation

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HF suggest that the angiotensin II type 1 receptor antagonist suppresses proliferation, collagen synthesis, and expression of profibrogenic cytokines (TGF-β1 and CTGF) in activated HSCs.[115-117] Two mechanisms of action have been suggested: first, the angiotensin II type 1 receptor antagonist inhibits activated HSCs by blocking angiotensin II type 1 receptors expressed on the surface of HSCs; second, it suppresses the activation of HSCs as a result of the decrease in TGF-β1. Studies on the use of botanicals or their active compounds for treating HF are summarized in Table 6.[70,71,118-124]

Liver fibrosis caused by *S. mansoni* is of great concern in tropical countries of the world, and owing to repeated anti-schistomic chemotherapy, there are reports of emergence of drug-resistant strains, which have lead researchers to search for suitable alternative medicines. Like in non-parasitic HF, botanicals or phytoextracts are given proper attention in treatment against trematode-induced liver fibrosis due to their friendly interaction and safer action. Many botanicals like *Daucus carota*,[125] *Commiphora molmol*,[126] *Artemisia anmia*,[127] *Combretum* sp.,[128] crude oil of *Nigella sativa*,[129-132] *Zingiber officinale*,[133] *Solanum nigrum*,[134] *Allium sativum*,[135,136] *Curcuma longa*,[137] and *Camellia sinesis*[138] have been reported to possess anti-schistosomal properties. Moreover, among synthetic drugs, high levels of Somatostatin and Paeoniflorin have been shown to reduce fibrosis by inhibiting HSC activation in humans and collagen synthesis in IL-13-stimulated HSC in murine *S. japonicum* infection, respectively.[139,140] Moreover, thiazolidinedione drug, rosiglitazone, and glucocortocoid dexamethasone, have been observed to attenuate HF by activating the PPAR-γ ligand in murine schistosomiasis.[141,142]

GENE THERAPY APPROACH FOR HF

HF involves the expression and suppression of a number of genes. Many attempts have been made to induce the cessation or the reversal of the process, but all of them did not produce desirable results, either due to the low uptake by the target cells or major side effects on other cell populations.[143] A recent report demonstrated the reversal of HF and cirrhosis in rodent model by targeting HSCs via liposomal drug delivery. The targeting complex contained siRNA against the collagen chaperon heat shock protein 47 (HSP47) bound to vitamin A.[144] These antifibrotic vitamin A–coupled liposomes decrease collagen production and promote degradation of ECM. However, thriving steps are being taken to attenuate experimental liver fibrosis with gene therapy. A brief review of novel strategies relying on gene therapy is given in Table 7.[145-155]

Out of many, stem-cell transplantation still seems to be a more promising alternative approach.[156] It is also believed that cell types including neurons, cardiac muscle cells, skeletal cells, kidney cells, liver cells, etc., have similar origin and arise from bone marrow (BM) stem cells. BM-derived stem cells have great power of regeneration and may develop into specific cellular phenotypes with different functions. These stem cells play an active role in liver repair and hepatic regeneration and may also support regeneration of cardiac/skeletal muscle and brain tissue. Recent studies suggested that hematopoietic stem cells migrate from the bone marrow to the injured liver due to the hypoxic milieu generated at the injured hepatic site and formation of chemo-attractant gradients.[157] Using markers such as aldehyde dehydrogenase, it has been demonstrated that these migrated stem cells fuse with the host hepatocytes or liver cells and help to generate fresh cell types.[158]

CONCLUSIONS

HF is a severe consequence of needless accumulation of excessive connective tissue in the liver. This amassing occurs because either ECM components are overproduced (fibrogenesis) or poorly degraded, or both contribute to it. It would be of great interest if the fibrosis pathology research proceeds to gain insights into proteomics to understand the normal and activated HSCs' function. Only then would a comprehensive understanding of the underlying molecular mechanisms be possible. New propositions can then be put forward for the viewpoint of impairments within disease processes. The transformation from basic research to practical investigations is expected to provide novel measures for prospective therapeutics against liver diseases. Since human liver fibrosis can be caused by various pathologies (viral, alcoholic, metabolic, etc.) and each one of these might respond better to one or another drug, it is advisable to reproduce animal models to evaluate effectiveness and therapeutic potential of relatively safer and targeted drugs. Trials for safer or well-tolerated drugs with use in multiple diseases, even for schistosome-induced fibrosis,[159] are underway and will carry great importance since the real efficacy of drugs that are likely to block activation or transformation of HSCs already tested *in vitro* can be directly assessed. So-called modern therapeutic procedures like gene therapy, BM-derived stem cells, and approaches utilizing siRNA along with hepatocyte transplant to reconstitute normal liver function may be expected as future areas to explore possibilities of treatment against HF.

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