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REVIEW

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Protective role of melatonin in early-stage and end-stage liver cirrhosis

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Abstract

The liver is composed of hepatocytes, cholangiocytes, Kupffer cells, sinusoidal endothelial cells, hepatic stellate cells (HSCs) and dendritic cells; all these functional and interstitial cells contribute to the synthesis and secretion functions of liver tissue. However, various hepatotoxic factors including infection, chemicals, high-fat diet consumption, surgical procedures and genetic mutations, as well as biliary tract diseases such as sclerosing cholangitis and bile duct ligation, ultimately progress into liver cirrhosis after activation of fibrogenesis. Melatonin (MT), a special hormone isolated from the pineal gland, participates in regulating multiple physiological functions including sleep promotion, circadian rhythms and neuroendocrine processes. Current evidence shows that MT protects against liver injury by inhibiting oxidation, inflammation, HSC proliferation and hepatocyte apoptosis, thereby inhibiting the progression of liver cirrhosis. In this review, we summarize the circadian rhythm of liver cirrhosis and its potential mechanisms as well as the therapeutic effects of MT on liver cirrhosis and earlier-stage liver diseases including liver steatosis, nonalcoholic fatty liver disease and liver fibrosis. Given that MT is an antioxidative and anti-inflammatory agent that is effective in eliminating liver injury, it is a potential agent with which to reverse liver cirrhosis in its early stage.

KEYWORDS
hepatic stellate cells, liver cirrhosis, melatonin, oxidative stress, regression

1 | BACKGROUND

The liver is the largest organ with synthesis and secretion functions and is composed of hepatocytes, cholangiocytes, Kupffer cells (KCs), sinusoidal endothelial cells, hepatic stellate cells (HSCs) and dendritic cells, among others.[1] Liver cirrhosis is the end-stage disease of liver disease induced by various hepatotoxic factors including infection, chemicals, high-fat diet (HFD) consumption, surgical procedures and genetic mutation, as well as biliary tract diseases such as sclerosing cholangitis and bile duct ligation (BDL).[2] Chronic liver injury impairs epithelial cells and triggers a fibrogenic response to recruit inflammatory cells (eg, macrophages and T cells), consequently inducing the activation and proliferation of extracellular matrix (ECM)-producing cells including fibroblasts and HSCs in liver tissue. The subsequent

Hu and Zhao contribute equally to this manuscript.

collagen deposition process gives rise to uncontrolled wound-healing pathophysiology and irreversible formation of intrahepatic scar tissue.[3,4] After liver fibrosis progresses into the end stage, multiple complications including acute or chronic liver failure, portal hypertension and hepatocarcinoma will follow.

In liver tissue, superoxide anions, hydrogen peroxide and hydroxyl radicals can be converted into stable reactive oxygen species (ROS) with strong toxicity, such as nitric oxide and peroxynitrites.[5] Thus, therapies targeting ROS inhibition are in great demand for inhibiting injury caused by oxidative stress and improving the prognosis of liver cirrhosis. Melatonin (MT), also known as *N*-acetyl-5-methoxytryptamine, is isolated from the pineal gland and participates in regulating multiple physiological functions including sleep promotion, circadian rhythms and neuroendocrine processes.[6-8] In the pineal gland, tryptophan is hydroxylated by tryptophan-5-hydroxylase to generate 5-hydroxytryptophan, which is then decarboxylated into 5-hydroxytryptamine (serotonin) by L-aromatic amino acid decarboxylase. After that, the serotonin is acetylated to generate *N*-acetylserotonin, which is finally converted to MT.[9] The hydroxylated MT generated by hepatic cytochrome P_{450} mono-oxygenases is conjugated with sulphate to generate active 6-sulfatoxymelatonin.[10] MT not only exerts a strong antioxidant effect to protect cells and tissues from radical damage[11] but also inhibits proinflammatory cytokines including tumour necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 b decreasing NF-κB during the development of hepatic fibrosis.[12,13] MT dramatically inhibits the activation of leucocyte, macrophage, mononuclear cells, mast cell and neutrophil infiltration in animal models of liver fibrosis.[14] Most importantly, MT contributes to a reduction in the amount of ECM deposition and significantly reduces histopathological changes in liver tissue.[15]

This review summarizes the circadian rhythm of liver cirrhosis and its potential mechanisms, as well as the therapeutic effects of MT on liver cirrhosis and earlier-stage liver diseases including liver steatosis, nonalcoholic fatty liver disease (NAFLD) and liver fibrosis (Figure 1). We describe how MT protects against liver injury against liver injury by inhibiting oxidation, inflammation, HSC proliferation and hepatocyte apoptosis, thereby inhibiting progression of liver cirrhosis. When the optimal dose, time-point, route and duration of administration are determined for MT to target oxidative liver damage in animal models and human beings, it may become a standard agent for liver disease treatment.

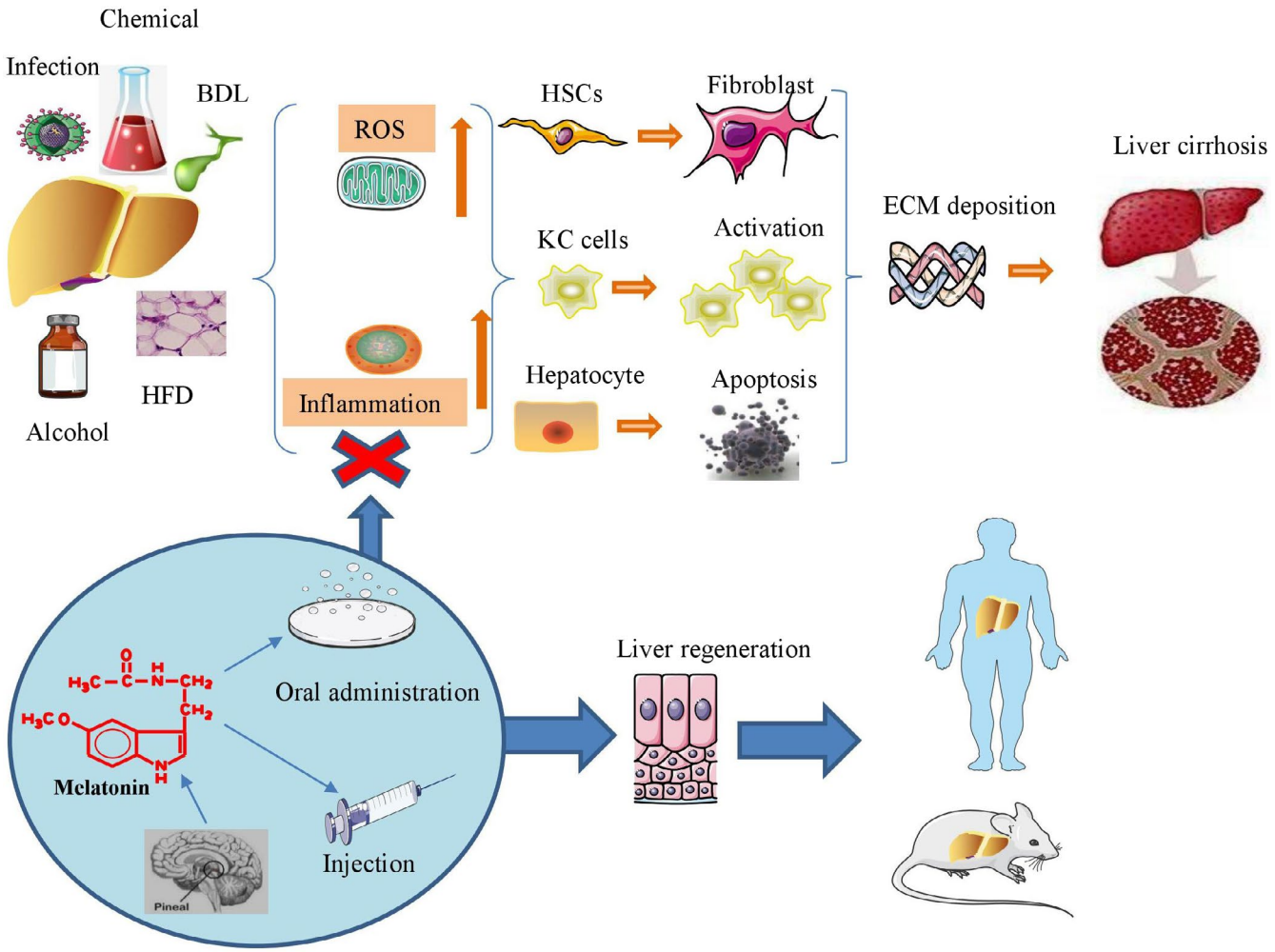

FIGURE 1 Melatonin reduces oxidative stress and inflammation to eliminate collagen deposition and prevent fibrosis progression

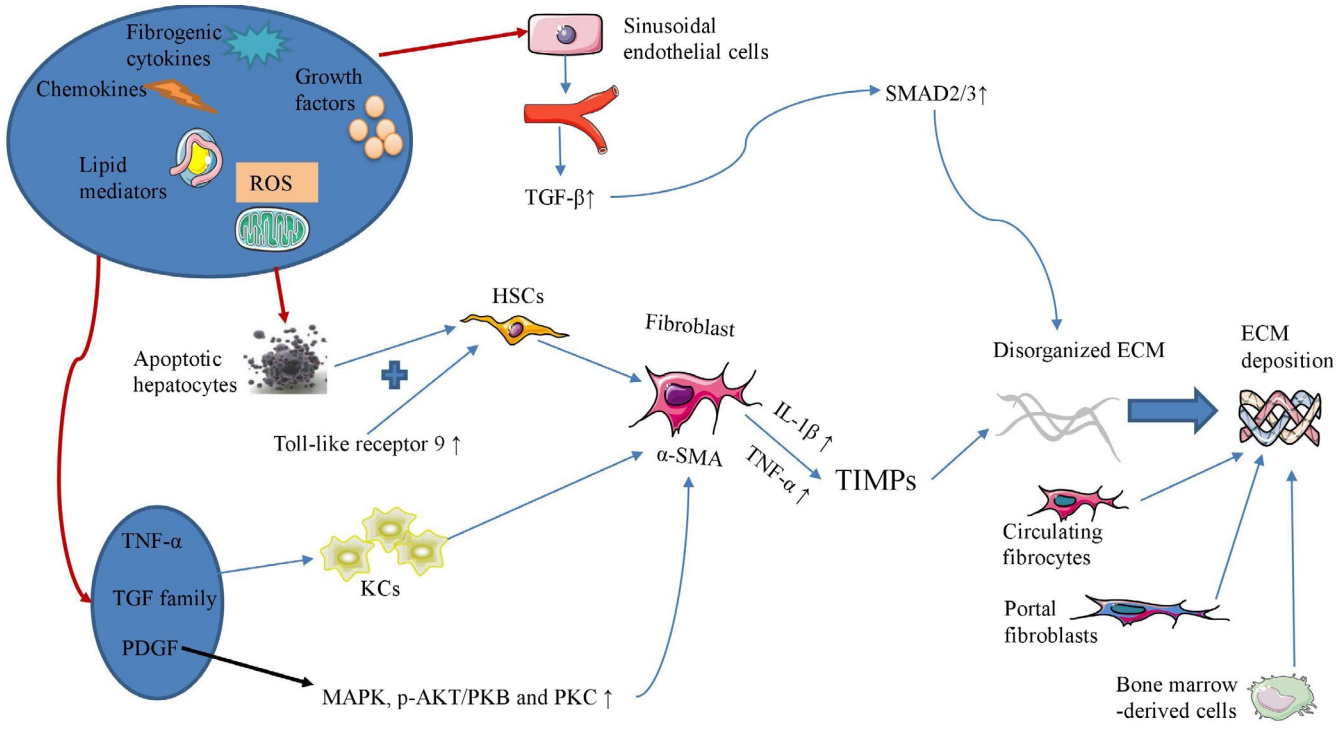

FIGURE 2 Several pathogenetic factors and pathways participate in the initiation and progression of liver cirrhosis

2 | THE POTENTIAL MECHANISMS OF MT TARGETING LIVER CIRRHOSIS

Multiple fibrogenic cytokines, chemokines, growth factors and lipid mediators as well as ROS production promote the transdifferentiation of quiescent HSCs into a myofibroblastic phenotype, and the interaction between apoptotic hepatocytes and Toll-like receptor 9 also induces HSCs to transdifferentiate into myofibroblasts.[16,17] On the other hand, the capillarization of sinusoidal endothelial cells up-regulates transforming growth factor (TGF)-β levels, transdifferentiates HSCs into activated myofibroblasts, and triggers hepatocyte apoptosis.[18,19] TGF-β interacts with the type II and type I receptors to activate small mothers against decapentaplegic (SMAD)2/3 and initiate collagen deposition in liver tissue.[20] In addition, activated HSCs produce tissue inhibitors of metalloproteinases (TIMPs) and induce ECM deposition in liver tissue via up-regulation of IL-1β and TNF-α.[21] Then, the harsh microenvironment up-regulates the expression of inflammatory cytokines including TNF-α, the TGF family and platelet-derived growth factor (PDGF), activating KCs and aggravating the progression of liver fibrosis.[20] The PDGF-β signalling pathway subsequently activates other signalling pathways including the MAPK, p-AKT/PKB and PKC pathways to enhance the proliferation and fibrogenesis of HSCs.[22] In addition to HSCs and KCs, circulating fibrocytes, portal fibroblasts and bone marrow-derived cells also take part in ECM deposition during liver fibrosis.[23,24] Although excessive ROS has been demonstrated to up-regulate the cell death rate of HSCs, nontoxic ROS is able to promote the activation, proliferation and collagen production of HSCs.[25] After the balance of matrix metalloproteinases (MMPs) and TIMPs is damaged, the deposition of scar tissue will result in irreversible liver fibrosis even if the stimulating factors are withdrawn.[26] Therapeutic approaches targeting elimination of liver fibrosis should interrupt each step, such as inflammation, hepatocyte apoptosis, cholangiocyte proliferation, myofibroblast activation and ECM deposition.[27] As MT exhibits strong antioxidant activity and inhibits inflammation, HSC proliferation and hepatocyte apoptosis, interventions targeting the pathogenetic factors or pathways in this manner will help to block the initiation and progression of liver cirrhosis (Figure 2).

3 | CIRCADIAN RHYTHM IN PATIENTS WITH LIVER CIRRHOSIS AND HEPATIC ENCEPHALOPATHY

Melatonin is generally regulated by light and darkness in both diurnal and nocturnal animals, as light eliminates MT synthesis, whereas darkness, peaking in the middle of the night, permits MT synthesis. The central MT synthesis pattern has been shown to be disturbed in patients with hepatic cirrhosis and is correlated with the severity of liver injury. Patients with liver cirrhosis had markedly elevated MT levels during daytime hours, and both the onset time of the MT increase and the time of the peak MT concentration were consistently and significantly delayed in these patients.[28] The elevated daytime MT levels in patients with liver cirrhosis are probably related to a decreased metabolic clearance rate, decreased liver blood flow, lowered activity of 6-β-hydroxylase and competition with bilirubin in the intrahepatic transport system.[29] The disturbed circadian 6-sulfatoxymelatonin rhythm impairs the quality of nighttime sleep

and increases daytime sleepiness in patients with liver cirrhosis, and bright light therapy has been demonstrated to exert no beneficial effects on them, perhaps due to the severity of central circadian disruption at baseline.[30] However, there is a controversy about the relationship between circadian abnormalities and impaired sleep quality. Montagnese et al found that although patients with liver cirrhosis demonstrated delayed peak serum MT, the urinary 6-sulfatoxymelatonin levels of patients and healthy volunteers did not differ. They concluded that there was no association between circadian abnormalities and impaired sleep quality.[31]

Liver cirrhosis induces a type of brain dysfunction, namely, hepatic encephalopathy (HE), which includes multiple symptoms ranging from subclinical alterations to coma with or without neurological or psychiatric abnormalities.[32] Early in 1954, Sherlock found that there existed a so-called sleep-wake inversion, which combined restless nights and excessive daytime sleepiness in patients with HE induced by liver cirrhosis.[33] A considerable proportion of patients with cirrhosis have the complication of HE with seriously impaired hepatic MT metabolism, but patients with subclinical HE showed impairment of life activities without clinical neurologic abnormalities. However, the understanding of their pathophysiology remains limited, and their treatment is problematic. Abnormal pituitary hormone and MT circadian patterns are present in liver cirrhosis before development into HE, and MT is the only hormone associated with the severity of liver insufficiency and serves as an early indicator of impending HE.[34] After fasting treatment, MT was detected in ascitic fluid in multiple patients with HE, and the high levels of MT in these patients may account for some of the clinical manifestations of HE including daytime sleepiness and fatigue.[35] Chojnacki et al enrolled 75 patients with alcohol-induced liver cirrhosis and 25 healthy control individuals; they found that the levels of serotonin, urinary 5-hydroxyindoleacetic acid and 6-sulfatoxymelatonin were lowest while the level of MT was highest in patients with Child-Pugh C grade, and the disturbance of serotonin and MT homeostasis in patients with liver cirrhosis may be associated with advanced HE.[36] However, although ammonia level is highest in patients with grade 3 HE, there is no correlation between MT and ammonia levels in these patients.[37]

4 | MT APPLICATION IN CLINICAL TRIALS

The fasting and postprandial plasma MT levels and portal hypertension rose significantly after treatment with MT or tryptophan, particularly in liver cirrhosis patients, which is attributable to portal systemic shunting and decreased liver degradation.[38]

Nonalcoholic steatohepatitis (NASH) patients underwent treatment with Essentiale Forte and tryptophan or MT for 4 weeks demonstrated reduced expression levels of γ-glutamyl transpeptidase (GGTP), triglycerides and proinflammatory cytokines including IL-1, IL-6 and TNF-α, while these patients showed no significant alteration in alanine transaminase (ALT) level.[39] In addition, NAFLD patients underwent treatment with Essentiale Forte and tryptophan or MT for 14 months demonstrated reduced expression levels of GGTP, triglycerides, low-density lipoprotein cholesterol and proinflammatory cytokines including IL-1, IL-6 and TNF-α, while these patients demonstrated no significant difference in ALT level and other biochemical parameters.[40] A 12-week course of MT can not only reduce levels of liver enzymes during the treatment period but also maintain the alterations after discontinuation in patients with NASH.[41] Pakravan et al enrolled 100 patients with NAFLD for 3 months of MT treatment and found that the levels of diastolic blood pressure, aspartate aminotransferase (AST) and high-sensitivity C-reactive protein were significantly lower and the liver grades better in patients with NAFLD who received MT than in those received placebo.[42]

5 | MT EFFECTIVELY REDUCES LIVER INJURY IN ANIMAL MODELS OF LIVER CIRRHOSIS

5.1 | Chemically induced liver fibrosis

Chemicals including carbon tetrachloride (CCl_4), thioacetamide (TAA) and dimethylnitrosamine (DMN) are commonly used to generate animal models of liver cirrhosis to investigate the effects of MT on reversing liver fibrosis (Table 1).

A majority of such studies have focused on the effects of MT on reversing CCl_4-induced liver cirrhosis in animal models and further clarified the underlying mechanisms as follows. MT was demonstrated to significantly attenuate oxidative stress-induced injury and the expression of Bax and other apoptotic proteins in CCl_4-induced liver fibrosis.[43] Furthermore, Hong et al[15] demonstrated that a high dose of MT significantly reduced the serum levels of laminin, hyaluronic acid and hydroxyproline, thus attenuating liver fibrogenesis in a dose-dependent manner. In addition to down-regulating α-smooth muscle actin (α-SMA) and up-regulating peroxisome proliferator-activated receptor alpha (PPARα), MT up-regulated expression of brain and muscle Arnt-like protein 1 (BMAL1), circadian locomotor output cycles kaput (CLOCK), period 2 (PER2), cryptochrome 1 (CRY1) and RAR-related orphan receptor-α (RORα) to maintain the circadian clock machinery in CCl_4-treated mice.[44] MT treatment significantly abolished the activation of HSCs and increased the expression of nuclear factor erythroid-2-related factor 2 (Nrf2) while inhibiting the expression of profibrogenic genes such as MMP-9, collagens I and III, TGF-β, PDGF, connective tissue growth factor, amphiregulin and phospho-SMAD3 in mice with CCl_4-induced liver fibrosis.[45] In addition, the levels of NF-κB in liver tissue and the levels of proinflammatory cytokines such as TNF-α and IL-1β released from KCs were down-regulated in rats with CCl_4-induced fibrosis.[13] An important pathway, the sphingosine kinase 1/sphingosine 1-phosphate (SphK1/S1P) axis, can also be inhibited by MT during the development of liver fibrogenesis.[46] After a 6-week treatment, MT not only significantly reduced the inflammatory process as shown by decreased levels of NF-κB/p65, inducible nitric oxide synthase and inflammatory infiltrate but also significantly decreased angiogenesis as demonstrated by reduced expression of TGF-β1, α-SMA and vascular endothelial growth factor, subsequently slowing the

TABLE 1 The potential mechanisms through which MT may attenuate chemically induced liver cirrhosis

Chemical type	Animal	MT dose	Time	Effect	Mechanism	Ref.
CCl_4	Rat	5, 10 and 20 mg/kg/d	6 wk	Decreases serum transaminase activity; reduces liver fibrosis scores	NF-κB in liver tissue and proinflammatory cytokines such as TNF-α and IL-1β in KC cells	16
CCl_4	Rat	25 mg/kg/d	1 mo	Reduces injury	Oxidative stress↓; Bax↓	46
CCl_4	Mouse	5 or 10 mg/kg/d	2 or 4 wk	Prevents all pathological changes; alleviate the progression of liver fibrosis	HSC activity↓; differentiation of HSCs into myofibroblasts↓	47
CCl_4	Mouse	5 or 10 mg/kg/d	2 or 4 wk	Abrogates the activation of HSCs, maintains normal histopathology; decreases levels of serum transaminases	Profibrogenic genes↓; MMP-9↓; Nrf2↑	48
CCl_4	Mouse	5 or 10 mg/kg/d	2 or 4 wk	Down-regulates the levels of TGF-β and collagen I	SphK1/S1P axis↓	49
CCl_4	Rat	20 mg/kg/d	6 wk	Decreases the deterioration of liver cirrhosis	NF-κB/p65↓; iNOS↓; inflammatory infiltrate↓; angiogenesis↓	50
CCl_4	Rat	2.5, 5, and 10 mg/kg/d	8 wk	Reduces levels of hepatic hydroxyproline; reduces hepatocyte apoptosis; decreases HSC activation	Necroptosis-associated inflammatory signalling↓; damage-associated molecular patterns↓; (Toll-like receptor 4 expression, p38, c-Jun N-terminal kinase phosphorylation, and NF-κB translocation)↓	51
CCl_4	Mouse	5 or 10 mg/kg/d	2 or 4 wk	Abrogates HSC activation	Autophagic response↓; ER↓; phospho-IRE1↓; ATF6↓; phospho-PERK↓	52
CCl_4	Rat	2.5, 5, and 10 mg/kg/d	8 wk		Mitochondrial dysfunction↓; mitochondrial swelling↓; glutamate dehydrogenase release↓	53
CCl_4	Rat	20 mg/kg/d	1 mo	Decreases the levels of lipid deposition, ALT and hydroxyproline; increases the level of albumin	Oxidative stress↓; matrix balance↑	54
TAA	Rat	5 mg/kg/d	Approximately 9 wk	Inhibits excessive oxidative stress	Thioredoxin-1↑; autotaxin↓	55
TAA	Rat	10 mg/kg/d	4 wk	Decreases the levels of liver enzymes and proinflammatory cytokines	PON-1↑; GSH↑; GSSG↓	56
TAA	Rat	1 mg/kg/d	1 or 3 mo	Eliminates HSC activation and extensive tissue damage	Oxidative stress↓; α-SMA↓	57
DMN	Rat	100 mg/kg/d	2 wk	Suppresses hepatic fibrotic changes, but exerts no effects on changes in biochemical parameters when administered alone	Hydroxyproline↑; MDA↑; GSH↓; SOD↓	58

Abbreviations: α-SMA, α-smooth muscle actin; ALT, alanine transaminase; CCl_4, carbon tetrachloride; DMN, dimethylnitrosamine; ER, endoplasmic reticulum; GSH, glutathione; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; KC, Kupffer cell; IL, interleukin; MMP, matrix metalloproteinase; MDA, malondialdehyde; MT, melatonin; SOD, superoxide dismutase; TAA, thioacetamide; TGF, transforming growth factor; TNF, tumour necrosis factor.

TABLE 2 The potential mechanisms through which MT may attenuate BDL-induced liver cirrhosis

Animal	Dose	Time	Route	Effect	Mechanism	Ref.
Rat	2 mg/g/d	1 wk	Per os	Decreases serum bilirubin and transaminase levels; decreases the percentage of PCNA-positive cholangiocytes; inhibits biliary hyperplasia	cAMP↓; clock genes↓; PKA phosphorylation↓; basal bile and bicarbonate secretion↓	67
Rat	20 mg/kg/d	2 wk	Intraperitoneal	Reduces the hepatosomatic and splenosomatic indices; decreases cholangiocyte proliferation; decreases liver injury and liver fibrosis	Lipid peroxidation↑; antioxidant enzymes↑; inflammation↓; GnRH↓; iNOS↓; TNF-α↓	68
Rat	1 mg/kg/d	7 d	Intracerebroventricular (ICV) cannulas	Alleviates liver injury in cholestatic rats; reduces cholangiocyte proliferation; alleviates fibrosis	Hypothalamic and cholangiocyte GnRH↓	69
Rat	100 mg/kg/d	1 mo	Intraperitoneal	Reverses HSC activation	MDA↓; luminal signal↓; lucigenin signal↓; GSH↑	70
Rat	1 mg/kg/d	14 d	Intraperitoneal	Decreases mortality; prevents kidney injury	Hepatic DDAH2 expression↑, DDAH activity↑; ADMA contents in both the liver and the kidney↓	71
Rat	5 mg/d	4 wk	A slow-release melatonin pellet implanted in the peritoneum	Prevents spatial deficits; decreases ADMA levels in the plasma, prefrontal cortex and dorsal hippocampus	Maintains brain-derived neurotrophic factor in the dorsal hippocampus	72
Rat	500/1000 μg/kg/d	2 wk	Intraperitoneal	Improve spatial performance of rats with BDL-induced liver fibrosis	Plasma MDA↑; liver MDA↑; liver GSH/GSSG ratios↑	73

Abbreviations: BDL, bile duct ligation; GnRH, gonadotropin-releasing hormone; GSH, glutathione; HSC, hepatic stellate cell; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; MT, melatonin; TNF, tumour necrosis factor.

deterioration of liver cirrhosis.[47] MT also attenuated CCl_4-induced liver fibrosis not only by inhibiting necroptosis-associated inflammatory signalling including the necrosome complex, RIP1, RIP3 and the downstream effector (MLKL) but also by decreasing the levels of damage-associated molecular patterns including high-mobility group box 1, IL-1α and IL-33. Other signalling events including Toll-like receptor 4 binding, p38 activity, c-Jun N-terminal kinase phosphorylation and NF-κB translocation were also down-regulated to reduce hepatocyte apoptosis and decreased HSC activation in rats with liver fibrosis after MT administration.[48] Autophagy is a process of programmed cell death and can be activated by CCl_4 administration in mice, while MT treatment significantly inhibited autophagy and reduced the endoplasmic reticulum (ER) stress accompanied with down-regulation of phospho-IRE1, ATF6 and phospho-PERK protein.[49] Chronic CCl_4 exposure led to impaired mitophagy (autophagy in mitochondria) and disturbed mitochondrial biogenesis and mitochondrial dysfunction, while MT attenuated hallmarks of mitochondrial dysfunction as demonstrated by up-regulation of mitochondrial DNA, PTEN-induced putative kinase 1 (PINK1), Parkin, peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α), nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (TFAM).[50] In an intriguing study, Mortezaee et al injected MT after the last dose of CCl_4 in rats for 1 month and set this group as the post-treatment group. They suggested that MT post-treatment was more powerful than cotreatment in reducing liver fibrosis via reduction of oxidative stress and maintenance of matrix balance, including up-regulation of MMP-13 and Bcl2.[51]

Chronic administration of TAA significantly decreased superoxide dismutase (SOD) and glutathione (GSH) activity but increased the hepatic content of malondialdehyde (MDA), which is the end product of lipid peroxidation, thus causing excessive oxidative stress in liver tissue. However, MT exhibits anti-inflammatory, antioxidant and fibrosuppressive activity against hepatic fibrogenesis via induction of thioredoxin-1 and elimination of autotaxin.[52] MT also serves as a mediator of oxidative stress and protects the liver from TAA-induced injury by increasing the levels of paraoxonase 1 (PON-1) and GSH and inhibiting the activity of oxidized glutathione (GSSG), consequently decreasing the levels of liver enzymes and proinflammatory cytokines.[53] MT eliminated HSC activation and abrogated TAA-induced oxidative stress, along with the extensive tissue damage that accompanies it, in rats with hepatic fibrosis.[54] On the other hand, although MT exerts no additional effects on normal rats, it effectively attenuated the hepatic fibrotic changes in a DMN-induced liver fibrosis model by down-regulating hydroxyproline and MDA and up-regulating GSH and SOD.[55]

5.2 | Liver fibrosis induced by biliary tract disease

The pathogenesis of primary biliary cirrhosis, primary sclerosing cholangitis (PSC) and autoimmune hepatitis is different from that of chronic liver fibrosis induced by toxic factors, as portal fibroblasts are found around bile ducts, but not as a result of HSC activation in response to profibrogenic or mitogenic stimuli.[56]

Manifestations of primary biliary cirrhosis include increased skin melanin pigmentation, elevated cholesterol and alkaline phosphatase levels, defective T lymphocytes and hyperactive B lymphocytes and hepatic fibrosis.[57-60] After exposure to darkness or administration of MT, multidrug resistance gene 2-knockout ($Mdr2^{-/-}$) mice with primary sclerosing cholangitis showed elevated serum MT level and decreased biliary mass, along with reduction of liver fibrosis and angiogenesis.[61] In addition, MT decreased the mean severity scores of liver parenchymal necrosis, portal fibrosis, biliary duct proliferation and cholangitis in rats with formalin-induced sclerosing cholangitis.[62]

Cholangiocyte proliferation is initiated after BDL at the edge of the portal tract and caused the generation of proliferating bile ductules with portal inflammation and fibrosis; the pathological changes in the liver after BDL are more severe at 4 weeks than at 2 weeks.[63] Fortunately, MT exerts protective effects against BDL-induced injury and inhibits the progression of liver fibrosis (Table 2). MT significantly decreased the expression of clock genes (PER1, BMAL1, CRY1 and CLOCK) as well as cAMP levels and PKA phosphorylation, consequently reducing serum bilirubin and transaminase levels, the percentage of PCNA-positive cholangiocytes and biliary hyperplasia in the cholangiocytes of BDL rats.[64] After administration in vivo, MT was effective in reducing the hepatosomatic and splenosomatic indices, restoring normal levels of lipid peroxidation and antioxidant enzyme expression and inhibiting inflammation, thus decreasing liver injury and liver fibrosis in BDL-induced secondary biliary cirrhosis.[65] On the other hand, MT is able to suppress the release of hypothalamic gonadotropin-releasing hormone (GnRH), a hormone that promotes cholangiocyte proliferation.[65] McMillin et al also concluded that MT alleviated liver injury in cholestatic rats via GnRH receptor 1-dependent paracrine signalling and demonstrated that supernatants from cholangiocytes isolated from BDL rats infused with MT suppressed the activation of HSCs induced by BDL cholangiocyte supernatant.[66] BDL not only increased collagen deposition but also improved MDA expression and luminol and lucigenin signal while decreasing GSH levels, whereas MT serves as a powerful physiological scavenger of hydroxyl radicals to reverse the activation of HSCs.[67] BDL increased the prevalence of kidney and brain injury in animal models. BDL increased the activity of hepatic dimethylarginine dimethylaminohydrolase (DDAH), which serves as an asymmetric dimethylarginine (ADMA)-metabolizing enzyme in both the liver and kidney of rats, while MT therapy effectively decreased mortality and prevented kidney injury characterized as measured by decreased tubulointerstitial injury scores and plasma creatinine and symmetric dimethylarginine levels in BDL rats.[68] Intriguingly, MT maintained brain-derived neurotrophic factor in the dorsal hippocampus of young BDL rats at a level comparable to that of sham controls, consequently preventing spatial deficits and decreasing ADMA levels in the plasma, prefrontal cortex and dorsal hippocampus of these cholestatic rats.[69] However, the dose of MT may influence the outcome of clinical symptoms because the antioxidative stress capacity varies according to the MT dose. Although low-dose and high-dose MT treatment both significantly improved the plasma MDA levels, liver MDA levels and liver GSH/GSSG ratios in BDL rats, only a high dose of MT (1000 μg/kg/d) was able to improve the spatial performance of rats with BDL-induced liver fibrosis.[70]

5.3 | NAFLD and NASH

Nonalcoholic fatty liver disease, a state ranging from simple steatosis to steatohepatitis, advanced fibrosis and cirrhosis, is attributed to specific dietary habits and lifestyles and results in liver dysfunction and end-stage liver cirrhosis. In particular, NASH, which is currently recognized as a serious condition, may also progress to liver cirrhosis because of pathophysiological mechanisms including oxidative stress, lipid peroxidation and excessive hepatocyte apoptosis.[12] Lipid infiltration results in mitochondrial fission and mitochondrial fragments in hepatocytes by disrupting the interaction of SIRT1 and Mitofusin 2, consequently up-regulating glycolytic flux, mitochondrial permeability transition pore (mPTP) opening, ROS production, cell cycle arrest and apoptosis of hepatocytes.[71] Primary hepatocytes isolated from mice with HFD-induced NAFLD showed up-regulated NR4A1 levels and activation of DNA-PKcs and p53, which up-regulated Drp1-mediated mitochondrial fission and mitophagy arrest. Thus, the cultured hepatocytes demonstrated mitochondrial dysfunction including extensive mPTP opening, decreased mitochondrial potential, oxidative stress, calcium overload, mitochondrial respiratory collapse and ATP shortage.[72]

An HFD significantly induced oxidative stress and up-regulated the levels of serum ALT, serum AST, total liver cholesterol and liver triglycerides in NAFLD rats.[73] Application of MT significantly reduces the pathogenetic changes in animal models with NAFLD and NASH according to current evidence (Table 3). Although application with MT did not alter the levels of lipids among HFD rats, MT is demonstrated to effectively reduce liver weight, the ratio of liver weight to bodyweight, portal vein pressure, the expression of HFD-induced plasma protein related to liver steatosis, the necrosis rate of liver cells and the extent of liver damage in animal models.[74,75] Others debated whether MT administration significantly reduced lipid accumulation in vivo to reduce the progression of liver fibrosis via different pathways. MT abolishes lipotoxicity-mediated HSC activation and prevents HFD-induced fibrosis through up-regulation of the mitochondrial respiratory chain and the tricarboxylic acid cycle (TCA) cycle.[71] MT promotes the loss of lipid droplets by directly suppressing HSC activation in vitro and enhancing signalling through RORα, which serves as the nuclear MT sensor in quiescent and activated HSCs, in a dose-dependent manner.[76] MT can regulate lipid metabolism, increase insulin sensitivity, regulate glucose metabolism and reduce ALT, low-density cholesterol bodyweight and liver weights in HFD-fed mice. The protection was mediated by down-regulation of TNF-α, IL-1β, and IL-6 and decreased phosphorylation of P38 and JNK1/2[77]; moreover, MT significantly ameliorated lipid deposition by down-regulating ER stress and up-regulating AKT phosphorylation and fetuin-A expression.[78] MT reversed the pathological progress of NAFLD by reducing hyperglycaemia and metabolic dysfunction and restoring mitochondrial function as well as the cellular longevity

TABLE 3 The potential mechanisms through which MT may attenuate NASH- and NAFLD-induced liver injury

Animal	Treatment	HFD time	MT dose	MT time	MT route	Effect	Mechanism	Ref.
Mouse	HFD	15 wk	10 and 20 mg/kg/d	28 d	Intraperitoneal	Reduces hepatic fat deposition and inflammation; abolishes collagen deposition; prevents fibrosis progression	Enzymatic activity associated with the respiratory chain and TCA cycle↑; interaction between steatotic hepatocyte and HSCs↓	74
Rat	HFD	12 wk	2.5 or 5 or 10 mg/kg/d	12 wk	Intraperitoneal	Reduces hepatic steatosis and inflammation	SOD↑; GSH↑; MDA↓	76
Rat	HFD	4 or 8 or 12 wk	5 or 10 mg/kg/d	4 or 8 wk	Intraperitoneal	Decreases liver weight, liver weight/bodyweight ratio, portal vein pressure; reduces the expression of HFD-induced plasma protein related to liver steatosis and the necrosis rate of liver cells; mitigates liver damage	MDA↓; GSH↑	77
Rat	HFD	10 wk	10 mg/kg/d	6 wk	Intraperitoneal	Reduces the HFD-induced expression of plasma proteins related to liver steatosis and the necrosis rate of liver cells; mitigates liver damage	GPx3, serotransferrin, FBG β chain and C-reactive protein ↑; complement factor B and APOE↑	78
Mouse	HFD	36 wk	10 mg/kg/d	12 wk	Per os	Increases insulin sensitivity; maintains glucose metabolism; reduces ALT, low-density cholesterol, bodyweight and liver weight	Inflammatory factors (TNF-α, IL-1β and IL-6)↓; phosphorylation of P38 and JNK1/2↓	80
Mouse	HFD	11 wk	50 or 100 mg/kg/d	10 wk	Per os	Ameliorates insulin resistance and lipid deposition	ER stress↓; AKT phosphorylation and fetuin-A expression↑	81
Mouse	HFD	9 wk	100 mg/kg/d	8 wk	Per os	Reverses the pathological progress of NAFLD; improves hepatic morphological, ultrastructural and metabolic damage	Glycaemia↓; ER stress↓; mitochondria function↑; metabolic dysfunction↓; longevity of hepatocytes↑	82
Rat	HFD	16 wk	10 mg/kg/d	8 wk	Per os	Decreases the elevated activity of AST and ALT; attenuates the elevation of serum triglycerides levels; reduces the elevated HOMA-IR index; decreases MDA; increases GSH; decreases steatosis and portal fibrosis	Serum total cholesterol and triglycerides↓	83
Rat	MCDD	4 wk	50 mg/kg/d	4 wk	Intraperitoneal	Decreases the number of TUNEL-positive cells as well as hepatocyte apoptosis and NASH grade	MDA↑; GSH and SOD↑, inflammatory factors (IL-1β, IL-6 and TNF-α)↓	15
Rat	Dexamethasone injection	Whole pregnancy period	1 mg/kg/d	Pregnancy period	Per os	Attenuates liver steatosis	Caspase 3↓; TNF-α↓; reverses the methylation of leptin	84
Rat	Zucker diabetic fatty	N/A	10 mg/kg/d	6 wk	Per os	Alleviates liver steatosis and vacuolation; mitigates diabetes-induced mitochondrial abnormalities as well as glycogen and lipid accumulation	ATP generation↑	85

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; ER, endoplasmic reticulum; GSH, glutathione; HFD, high-fat diet; HSC, hepatic stellate cell; IL, interleukin; MDA, malondialdehyde; MCDD, methionine- and choline-deficient diet; MT, melatonin; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; SOD, superoxide dismutase; TNF, tumour necrosis factor.

of hepatocytes in vivo.[79] Pioglitazone (an insulin sensitizer), pentoxifylline (a TNF-α inhibitor) and MT (an antioxidant) alone or in combination reduced severe hepatic steatosis, inflammation and fibrosis in rats with NAFLD. Pentoxifylline was demonstrated to decrease serum TNF-α levels, while pioglitazone and MT significantly reduced serum total cholesterol and triglycerides by down-regulating MDA levels and up-regulating GSH in animal models.[80] Pan et al highlighted the effects of various MT doses, showing that a range of MT concentrations (2.5, 5, 10 mg/kg) were effective in reducing hepatic steatosis and inflammation in HFD rats via increasing SOD and GSH activities, while only the high dose (10 mg/kg) of MT reduced MDA levels in liver tissue from NAFLD rats.[73]

Melatonin was also found to maintain liver function with decreased levels of ALT, AST and alkaline phosphatase; increase antioxidative functions including down-regulation of MDA and up-regulation of GSH and SOD; decrease inflammatory factors including IL-1β, IL-6 and TNF-α; and consequently decrease the number of TUNEL-positive cells as well as hepatocyte apoptosis and NASH grades in rats fed a methionine- and choline-deficient diet.[12] Prenatal glucocorticoids induced liver steatosis and apoptosis in neonatal rats, while MT reversed the methylation of leptin and decreased levels of caspase 3 and TNF-α to attenuate liver steatosis in these neonatal rats.[81] Furthermore, MT treatment alleviates liver steatosis and vacuolation and mitigates diabetes-induced mitochondrial abnormalities as well as glycogen and lipid accumulation by improving ATP generation in Zucker diabetic fatty rats.[82] To this end, MT significantly maintained the homeostasis of the fatty liver not only by regulating oxidative stress but also by decreasing the levels of proinflammatory cytokines. Although MT reduced the mean liver weights and ratios of liver to bodyweight and decreased hepatic steatosis in rats fed an HFD, there was no evidence showing that MT reversed established steatosis.[83]

5.4 | Alcohol-induced liver fibrosis

Alcohol abuse leads to asymptomatic and reversible alcoholic liver disease, and sustained alcohol consumption also directly results in irreversible liver fibrosis. After ingestion, alcohol is metabolized into the intermediate product acetaldehyde, which participates in the development of hepatic fibrogenesis via activation of the TGF-β/SMAD signalling pathway,[84] which then activates HSCs by inhibiting autophagy and ER stress. In addition, cytochrome P450 2E1 completes a crucial step in alcohol-induced fibrogenesis by promoting overexpression of ROS in the liver,[85] ultimately leading to ECM deposition and alcoholic liver cirrhosis.[86] Ethanol can significantly up-regulate NF-κB translocation and MMP-9 expression but down-regulate TIMP-1 expression, while MT prevents all these changes in addition to alcohol-induced liver injury in mice.[87] Given that alcohol-induced liver cirrhosis is highly dependent on an ROS-related mechanism, MT may serve as a potential agent to inhibit the alcohol-induced pathogenetic process, including elevation of aminotransferases, triglyceride and hepatic steatosis, by up-regulating phosphorylation of AMPK and SOD in alcohol-treated rats.[88] Hu et al demonstrated that MT maintained liver function and reduced the severity of hepatic cell damage and steatosis by reducing inflammatory cell infiltration, tissue lipid peroxidation, neutrophil infiltration and hepatocyte apoptosis. Moreover, MT treatment inhibits the release of ROS and TNF-α in KCs isolated from ethanol-fed mice.[89]

6 | COMBINING MT AND MESENCHYMAL STEM CELLS TO REVERSE LIVER CIRRHOSIS

It is well known that mesenchymal stem cells (MSCs) can improve liver function and eliminate liver injury in human and animal models with hepatic fibrosis, but harsh microenvironments in vitro and in vivo reduce the therapeutic effects of MSCs. Current evidence suggests that MT may improve MSC stemness or MSC transplantation efficacy by enhancing antioxidant capacity and anti-inflammation capacity. MT promoted the hepatic differentiation of MSCs and significantly increased bone morphogenetic protein-2 expression and SMAD1/5/8 phosphorylation by activating the p38, extracellular signal-regulated kinase (ERK) and NF-κB signalling pathways. Moreover, the combined transplantation of MT and MSCs suppressed liver fibrosis in mice and restored liver function significantly more than MSC transplantation alone or MT treatment alone.[90] Preconditioning with MT significantly improved the homing ability of MSCs in vivo, up-regulated the expression of MMPs and Bcl2, and decreased the expression of TGF-β1 and Bax, consequently restoring glycogen storage and decreasing collagen and lipid deposition in the fibrotic liver.[91] Mortezaee et al asserted that preconditioning with 5 μmol/L MT for several passages did not alter MSC features in vitro and exerted no additional effects on reversing the pathological progress of CCl_4-induced liver fibrosis, although MT pretreatment significantly increased the homing ability of MSCs after transplantation in vivo.[92] Both MT and MSCs can protect liver tissue from injury by enhancing antioxidant, anti-inflammatory and antiapoptotic processes; thus, the combination of MT and MSCs is more effective than either agent alone in hindering the progression of liver cirrhosis.

7 | CONCLUSIONS

Melatonin exerts protective effects by inhibiting oxidative stress, inflammatory signalling, autophagy flux, hepatocyte apoptosis and epithelial cell injury; thus, it attenuates the activation of HSCs and the proliferation of fibrogenic effector cells, ultimately reducing ECM deposition. Potentially, re-establishment of the light/dark cycle and the circadian rhythm may increase the endogenous MT level and increase the therapeutic effects of MT in reversing the progression of fibrosis. The combination of MSC transplantation and MT administration will have stronger therapeutic effects than either strategy alone in inhibiting the initiation of HSC activation and ECM deposition. However, the majority of studies concerning MT therapy are conducted in animal models rather than human beings, leaving the therapeutic effects of MT on human liver fibrosis currently

undefined. As we have discussed, MT is an effective antioxidative and anti-inflammatory agent for eliminating cirrhotic liver injury and has great potential for application in the pharmaceutical industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Chenxia Hu wrote the manuscript; Lingfei Zhao revised the manuscript; Jingjing Tao collected the data; Lanjuan Li contributed to manuscript conception; and Chenxia Hu and Lanjuan Li provided financial support for the study. All authors have read and approved the final manuscript.

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