Neuroendocrine Regulation in Liver Fibrosis

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Liver fibrosis is a complicated process that involves different cell types and pathological factors. The excessive accumulation of extracellular matrix (ECM) and the formation of fibrotic scar disrupt the tissue homeostasis of the liver, eventually leading to cirrhosis and even liver failure. Myofibroblasts derived from hepatic stellate cells (HSCs) contribute to the development of liver fibrosis by producing ECM in the area of injuries. It has been reported that the secretion of the neuroendocrine hormone in chronic liver injury is different from a healthy liver. Activated HSCs and cholangiocytes express specific receptors in response to these neuropeptides released from the neuroendocrine system and other neuroendocrine cells. Neuroendocrine hormones and their receptors form a complicated network that regulates hepatic inflammation, which controls the progression of liver fibrosis.

neuroendocrine hormones | neuroendocrine regulation | hepatic stellate cells | tissue homeostasis | liver fibrosis

1. Introduction

The liver is the largest gland in the body that plays a critical role in maintaining homeostasis of the body and has many important physiologic functions. Hepatocytes occupy 60–80% of the total cell populations in the liver, and the other cells include hepatic stellate cells (HSCs), cholangiocytes, Kupffer cells, liver sinusoidal endothelial cells, hepatic stem/progenitor cells, etc. [1].

Liver fibrosis is the process of abnormal accumulation of extracellular matrix (ECM) and eventual formation of fibrous scar in response to chronic liver injury caused by various diseases, such as excessive alcohol drinking, viral hepatitis, non-alcoholic fatty liver disease as well as cholestasis. Without proper treatment, the fibrous scar formed due to long-term liver fibrosis may destroy the normal structure of the liver and lead to the loss of hepatocytes and dysfunction of the liver, eventually resulting in liver failure or cancer, which seriously threatens human life [2].

Nowadays, the function of neuroendocrine regulation in liver fibrosis is a new territory that provides new clues for the treatment of chronic liver diseases. In previous studies, it has been reported that the liver is innervated by sympathetic, parasympathetic, and peptidergic nerves, including afferent and efferent fibers. Many neuropeptides produced in the liver have been identified [3]; for example, neuropeptide Y (NPY), substance P (SP), and α-calcitonin gene-related peptide (α-CGRP) are abundant in the connective tissue within the lobules [4]. Aberrant expressions of some neuropeptides in the hepatic environment may regulate pathological inflammation and tissue

homeostasis, thus promoting or inhibiting the development of liver fibrosis. The liver is also affected by these neural signals as a receptor and effecter. Therefore, clarification of the role of these signaling pathways in the liver is significant for the therapy of liver fibrosis. However, further studies are needed to be performed to elucidate the underlying mechanisms of those neuropeptides' effect on liver diseases before they can be used as the target to improve hepatic fibrosis.

2. The Role of Neuroendocrine Regulation during Liver Fibrosis

2.1. Renin-Angiotensin System

The renin-angiotensin system (RAS) is considered a hormonal system primarily responsible for blood pressure control and fluid homeostasis in the body [5]. Renin, an enzyme first described in the kidney, is also found in the brain. The sympathetic nervous system is amplified during stressful stimuli and with numerous downstream effects, including on the RAS because sympathetic nerves strictly control renin production. The RAS is important in regulating organ function through autocrine, paracrine, or endocrine actions. The classic RAS axis is the conversion of renin to angiotensin II (Ang II) via an angiotensin-converting enzyme (ACE). Ang II exerts its biological effects by binding to two types of receptors, namely angiotensin type 1 receptor (AT1) and AT2 [6]. Several research studies have improved researchers' understanding of RAS, including the identification of Ang-(1-7), a biologically active member of the RAS [7]. Ang-(1-7) is produced by the degradation of Ang II by ACE2, a homolog of ACE [8]. Ang-(1-7) exerts its biological action by binding to the G-protein-coupled receptor Mas [9]. Many studies have pointed out that RAS plays an important role in the development of liver fibrosis. One study reported that the RAS component was significantly elevated in the circulation of bile duct ligation (BDL) rats, with a three-fold increase in Ang II and a two-fold increase in Ang (1-7) compared to normal rats [10]. The classical ACE-Ang II-AT1 axis plays a pro-fibrotic, pro-inflammatory effect in the process of liver fibrosis; however, the ACE2-Ang-(1-7)-Mas axis has a counter-regulatory effect on Ang II to improve liver fibrosis [11]. Accordingly, the balance between both RAS axes likely influences the progression of liver fibrosis.

In the liver, the RAS components such as renin and ACE are present in the Kupffer cells [12]. Ang II induces contraction of a-HSCs through AT1 receptors and promotes the proliferation of a-HSCs by activating the mitogen-activated protein kinase (MAPK) pathway, and these effects can be blocked by losartan, a specific AT1 antagonist [13]. Activation of AT1 by Ang II also increases the expression of type I collagen genes, which require activation of both the MAPK and transforming growth factor-β (TGF-β) signaling pathways [14]. Studies have reported that the RAS affects the development of liver fibrosis by regulating the balance of the oxidative stress [15]. Ang II increased NADPH oxidase (NOX) protein and reactive oxygen species (ROS) expression in HSCs, leading to the activation of NOD-like receptor protein 3 (NLRP3) inflammasome in HSCs. However, Ang-(1-7) improves BDL-induced liver fibrosis by decreasing the NOX protein and NLRP3 inflammasome levels and increasing the expression of antioxidants, such as glutathione [15]. Ang(1-7) not only counteracts the oxidative stress effects of Ang II but also counter-regulates Ang II-mediated cell contraction, proliferation, and pro-fibrotic effects [11]. In addition, treatment with the Mas receptor antagonist (A-779) aggravated hepatic fibrosis, further confirming the protective effect of

Ang-(1-7) [10]. In general, two axes of the RAS are involved in the development of hepatic fibrosis and play completely opposite roles, and the balance between these two axes determines the direction of liver fibrosis development.

2.2. Cannabinoid System

The cannabinoid system consists of specific cannabinoid receptors (CB1 and CB2) and exogenous and endogenous (endocannabinoids, ECs) ligands. Studies have reported that CB1 and CB2 receptors are abundantly expressed in neurons as well as central and peripheral immune cells, which are involved in the modulation of inflammatory neurodegenerative diseases [16], and both CB1 and CB2 are weakly expressed in normal liver tissue [17]. The most profoundly studied ECs are anandamide and 2-arachidonoylglycerol, both belonging to the family of endogenous arachidonic acid-derived ligands [16]. When intracellular calcium levels are elevated in postsynaptic neurons, the enzymes that synthesize ECs are activated, leading to the formation and release of ECs. ECs cross the synaptic cleft and stimulate CB1 receptors on the presynaptic membrane to exert their functions [18]. Subsequent studies showed that the expression of ECs was significantly upregulated, probably produced by hepatocytes and nonparenchymal cells in the chronic liver disease [19]. The expression of CB1 and CB2 was higher in transdifferentiated HSCs than in normal livers [20]. These results suggest that the cannabinoid system is involved in the development of liver fibrosis. The latter study on cannabinoid receptors in liver fibrosis found that CB1 and CB2 play differential effects in the process of liver fibrosis [21].

It has been reported that cannabinoid receptors may be activated by ECs. In three mouse models of liver fibrosis (chronic CCl4 intoxication, chronic thioacetamide intoxication, and BDL), CB1 antagonist (SR141716A) and knockout of CB1 gene alleviated liver fibrosis by reducing the expression of fibrosis markers TGF-β and α-smooth muscle actin (α-SMA). However, the determining factor for the improvement of liver fibrosis by CB1 ablation is the decrease in accumulation and increase in apoptosis of myofibroblasts [20]. Another study reported that CB2 ablation aggravated liver fibrosis in chronic CCl4 intoxication mice, suggesting an anti-fibrotic function of CB2. CB2 ameliorates liver fibrosis by inhibiting the growth of hepatic myofibroblasts and promoting apoptosis of hepatic myofibroblasts as well as HSCs. Growth inhibition involves cyclooxygenase-2(COX-2), and oxidative stress leads to apoptosis [21].

In addition, ECs can act independently without dependence on cannabinoid receptors. Anandamide mediates cell necrosis by promoting the formation of ROS, and the increase of intracellular Ca^{+} and pretreatment with antioxidants and Ca^{+}-chelators attenuated HSCs death. Furthermore, cannabinoid receptor antagonists did not resist anandamide-mediated necrosis of HSCs, suggesting that anandamide can exert antifibrotic effects alone [22].

2.3. Melatonin

Melatonin is a neurohormone synthesized in the pineal gland by *N*-acetyltransferase (AANAT) and is participated in the regulation of many diverse physiological functions, including the promotion of sleep, circadian rhythms as well as neuroendocrine processes. Melatonin has also been detected in a variety of organs, such as the brain, retina,

gastrointestinal tract, and liver [23]. In addition, melatonin has powerful antioxidant and anti-inflammatory functions and modulates mitochondrial homeostasis, which has led to a growing number of studies on melatonin. Several studies suggest that melatonin may play an important role in the treatment of liver fibrosis. Melatonin in the liver may partly come from the gastrointestinal tract via the hepatic portal vein and also from the hepatocyte nuclei [23]. The effects of melatonin are mediated by two signaling mechanisms, including receptor-mediated and non-receptor-mediated pathways.

Cholangiocytes have been reported to express melatonin receptors (MT1 and MT2) [24]. It was reported that melatonin interacted with MT1 in BDL rats to reduce intrahepatic bile duct mass (IBDM) caused by cholangiocytes proliferation via down-regulation of the cyclic adenosine 3′,5′-monophosphate (cAMP) signaling pathway, and also reduced serum transaminase levels, however, these results could only be blocked by MT1 antagonists but not MT2 antagonists, suggesting that the antifibrotic effects of melatonin are mediated by MT1 [24]. Another study reported that intraperitoneal administration of melatonin to CCl_4-treated mice counteracted a range of CCl_4-mediated pro-fibrotic factors, such as upregulation of the TGF-β/Smad signaling pathway and increased expression of collagen I and III genes and activation of HSCs. Moreover, melatonin significantly increased the expression of nuclear factor erythroid2-related factor 2(Nrf2) protein, an important regulator of cellular antioxidant response [25]. Next, the mechanism of melatonin suppresses of HSCs has been investigated. It was found that the nuclear melatonin sensor retinoic acid receptor-related orphan receptor-alpha (RORα) was expressed in HSCs, and melatonin directly suppressed HSC activation via RORα-mediated inhibition of 5-lipoxygenase expression, while the RORα antagonist SR1001 blocked the antifibrotic profile of melatonin [26].

In recent years, the effect of mitochondria in liver fibrosis has attracted a great deal of interest. Mitochondrial dysfunction occurs in all types of liver injury. Chronic CCl4 exposure impaired mitophagy and mitochondrial biogenesis and also mediated the reduction of mitochondrial fission and fusion-associated proteins. Melatonin ameliorates CCL4-mediated liver fibrosis by resisting the impairment of mitophagy and mitochondrial biogenesis [27]. In the multidrug resistance gene 2 knockout ($Mdr2^{-/-}$) mice, administration of the AANAT antagonist miR-200b attenuated the expression of AANAT and melatonin, resulting in increased biliary proliferation, angiogenesis, and hepatic fibrosis. In contrast, overexpression of AANAT or inhibition of miR-200b restored the antifibrotic effects of the melatonin [28]. Overall, extensive studies have demonstrated the powerful antifibrotic effects of melatonin, and it is promising for melatonin to be used as an anti-fibrotic drug in clinics.

2.4. Substance P

Substance P (SP) consists of 11 amino acids and belongs to the tachykinin family. The tachykinin family has six members, including SP, neurokinin A, neurokinin B, neurokinin K, neurokinin γ, and heme kinin, and SP is encoded by the TAC1 gene [29]. After synthesis, SP is packed in vesicles and transported to the central and peripheral endings of primary sensory neurons. SP can be detected in the central nervous system, sensory nerves, immune cells, and peripheral organs such as the liver and lungs. SP exerts its regulatory effects by binding to tachykinin receptors, which are G protein-coupled receptors of three types: neurokinin-1 receptor (NK-1R), NK-2R as well as NK-3R. However, SP has a high affinity to NK-1R and preferentially binds to it, exerting a series of physiological

and pathophysiological regulatory functions, including pro-inflammatory and pro-proliferative effects [29]. Several studies have reported that the SP/NK-1R axis is involved in the fibrotic program of various organs. In this entry, researchers mainly elaborate on the role of the SP/NK-1R axis in liver fibrosis.

It has been identified that SP nerve fibers are abundant in hepatic portal vascular membranes and intralobular connective tissue [4]. It was found that increased expression of TAC1 and NK-1R was measured in the total liver of $Mdr2^{-/-}$ mice and primary sclerosing cholangitis (PSC) patients, and serum levels of SP were also higher than the normal level. Administration of SP to WT mice also increased IBDM and hepatic collagen deposition suggesting the pro-fibrotic effect of SP [30]. However, the SP antagonist (L-733, 060) reduced the serum levels of transaminases and various fibrosis indicators in Mdr2 mice, which ultimately improved the fibrosis [30]. In addition, the knockdown of NK-1R inhibited IBDM, the expression of type I collagen, and α-SMA in BDL-induced cholestasis mice [31]. Wan and her colleagues examined the senescence of cholangiocytes and HSCs and found that the pro-fibrotic function of SP was mediated by increasing the senescence of cholangiocytes and decreasing the senescence of HSCs [30]. Moreover, SP may induce the proliferation and activation of HSCs through the TGF-β1/Smad3 signaling pathway [32].

2.5. Serotonin

Serotonin, also known as 5-hydroxytryptamine (5-HT), is a biogenic amine that exerts its biological activities by binding to seven major receptor families ($5\text{-}HT_1$ to $5\text{-}HT_7$). 5-HT is best known for its functions in the brain, where it functions as a neurotransmitter at neuronal synapses affecting numerous neurophysiological functions, including learning and memory, pain, appetite, and mood. In addition, the enterochromaffine cells in the gastrointestinal tract are the major site of 5-HT synthesis. 5-HT in the blood is taken up through specialized serotonin transporter (SERT) and stored in the platelets [33]. In response to various stimuli, they accumulated in the damaged tissue, and 5-HT was released. Emerging research suggests that serotonin plays a crucial role in liver diseases. The positive effect of serotonin is demonstrated by platelet-derived serotonin initiating liver regeneration after partial hepatectomy and also promoting tissue repair after ischemia/reperfusion injury [34]. However, serotonin mainly plays a pro-fibrotic role in the development of liver fibrosis.

2.6. Calcitonin Gene-Related Peptide

Calcitonin gene-related peptide (CGRP) is a neuropeptide containing 37 amino acids, which are mainly produced in sensory neurons but are also present in various organs, including liver [35] and adipose tissue [36]. Two isoforms of CGRP are named α-CGRP and β-CGRP. The CGRP receptors are located in the brain, heart, lung, spleen, and liver [37], consisting of three subunits: calcitonin receptor-like receptor, receptor activity-modified protein 1, and receptor component protein. In mice and rat liver, α-CGRP-positive innervation forms a dense network in the fibromuscular layer of the biliary tree around the portal vein and in the stromal compartment of portal areas [38]. Furthermore, hepatocytes produce CGRP when there is liver disease such as cirrhosis [39]. α-CGRP levels were found to be higher in the serum of BDL mice than that in control mice, suggesting a close association between CGRP produced by hepatocytes and afferent nerves in the liver and liver fibrosis [40].

2.7. Neuropeptide Y

Neuropeptide Y (NPY) is a neurotransmitter that is normally produced in the brain as well as in neurons throughout the gastrointestinal tract and with a high concentration in the biliary tree [41]. NPY was also found in the cytoplasm of pericentral hepatocytes and cholangiocytes in the normal liver. However, more NPY was expressed in bile ducts of the cirrhotic liver [42]. NPY exerts its various functions via six main receptor subtypes (Y1–Y6). The expression of NPY in the hypothalamus can be regulated by ghrelin, which is an endogenous peptide produced in the gastrointestinal tract. Ghrelin can exert orexigenic action by regulating NPY production in the arcuate nucleus [43]. Overexpression of NPY in the hypothalamus can lead to hyperphagia and obesity in rats [44]. However, there is no report about whether ghrelin can influence liver fibrosis progression through regulating NPY expression, although ghrelin has been reported to exert an anti-fibrotic and anti-inflammatory effect on the liver [45].

2.8. Endogenous Opioid Peptides

Opioid peptides are produced in the hypothalamus and other areas of the brain [46] and are also present in peripheral nervous systems, cardiovascular and gastrointestinal systems, and immune cells [47]. Opioid peptides, a neurohormone, have specific receptor-mediated growth-regulating functions in neurons as well as non-neurons cells and tissues. Currently, three members of the opioid peptide family are confirmed: enkephalins, endorphins, as well as dynorphins, each derived from a different precursor peptide [48]. Endogenous opioid peptides exert their hormonal effects through binding to three subtypes of opioid receptors (OR): μ OR, δ OR, and κ OR, all of which belong to the G protein-coupled receptor superfamily.

2.9. Galanin

Galanin is a 29 amino acid neuropeptide found in small intestine and widely distributed in the amygdala, hypothalamus, locus coeruleus as well as sacral spinal cord. It is involved in many different biological functions [49]. Galanin acts through one of three G-protein-coupled receptors: galanin receptor 1 (GalR1), galanin receptor 2 (GalR2), and galanin receptor 3 (GalR3). GalR1 is distributed in the basal forebrain, hypothalamus, and spinal cord. While GalR2 is more widely distributed in the brain, pituitary, and peripheral tissues. GalR3 has been shown to be expressed only in discrete brain regions [50]. In 2017, Matthew McMillin and his colleagues began researching the relationship between galanin and liver disease. They found that the levels of galanin in serum and liver were significantly elevated in BDL rats. Furthermore, large amounts of galanin were produced by cholangiocytes in BDL rats. Galanin promotes IBDM and cholangiocytes proliferation by interacting with GaR1 expressed in cholangiocytes. In addition, the inhibition of galanin counteracted these changes [51]. Subsequently, they detected the expression of GalR2 in HSCs. Galanin treatment increased HSCs proliferation and fibrogenesis in wild-type and $Mdr2^{-/-}$ mice. Inhibition of Gal1R and GalR2 in $Mdr2^{-/-}$ mice reduced IBDM and the expression of fibrosis markers, whereas GalR2 antagonist (M871) only attenuated liver fibrosis without changing IBDM, suggesting that the promotion of HSCs activation and fibrosis gene expression by galanin is mediated by Gal2R [52].

2.10. Secretin (Sct)

Secretin was originally found in the duodenal S cells. In addition, secretin is also considered to be a neuropeptide hormone because it is also expressed in the brain and regulates the function of the central nervous system [53]. Secretin exerts its function by binding to the secretin receptor (SR), and Sct/SR is expressed only in the large cholangiocytes of the liver [54]. Meng and his team found that the Sct/SR levels in liver sections from PSC patients were significantly higher than in healthy samples. Their study reported that Sct promotes hepatic fibrogenesis in normal and BDL mice, and knockdown of SR or SR antagonist (sec 5–27) treatment ameliorated liver fibrosis. The Sct/SR axis increases the secretion of TGF-β from cholangiocytes, which activates HSCs and promotes fibrosis by paracrine means. Knockdown SR and SR antagonist (sec 5–27) treatment attenuated serum transaminase levels, IBDM, TGF-β, and fibrosis gene expression in BDL and Mdr2$^{-/-}$ mice [55]. In addition, they found that secretin-stimulated TGF-β increased cholangiocytes senescence and decreased HSCs senescence via a paracrine pathway, further promoting liver fibrosis. However, the downregulation of SR counteracted these changes in BDL and Mdr2$^{-/-}$ mice [56]. These experimental data fully illustrate the role of secretin in the progression of liver fibrosis, and targeting the Sct/SR axis may provide a new therapeutic approach for liver fibrosis as well as bile duct disease.

2.11. Other Neuroendocrines Involved in Liver Fibrosis

Histamine is a neurotransmitter primarily released from activated mast cells and interacts with histamine receptors (H1–H4) [57]. Histidine is converted to histamine by l-histidine decarboxylase (HDC), an enzyme expressed mainly by cholangiocytes in the liver [57][58]. Once released, histamine is either used or rapidly stored within mast cells. It was found that histamine/histamine receptor levels were elevated in Mdr2$^{-/-}$ mice and PSC patients, and treatment of histamine receptors antagonists (mepyramine or ranitidine) in Mdr2$^{-/-}$ mice reduced biliary tract injury and fibrosis compared to saline treatment [59]. In addition, depletion of HDC improved liver injury, ductular reaction, inflammation, and fibrosis in Mdr2$^{-/-}$ mice [60]. These studies demonstrate that the HA/HDC axis may be a potential target for the treatment of liver fibrosis.

Cortistatin is a cyclic neuropeptide, which is discovered in the brain cortex and hippocampus with anti-inflammatory and anti-fibrotic effect [61]. Cortistatin can alleviate fibrotic responses in various inflammatory disorders by reducing the production of inflammatory factors [62]. Analysis using public databases demonstrated that the expression of cortistatin was obviously lower in the fibrotic liver compared to normal liver tissue [63]. In 2021, a study showed that cortistatin and cortistatin receptors were expressed in HSCs and that cortistatin-deficient mice undergoing hepatotoxic and cholestatic injury treatment showed a higher mortality rate. Cortistatin treatment reversed these exaggerated fibrotic changes and protected against the development of liver fibrosis after liver injury [64]. However, the role of cortistatin in liver fibrosis has not been fully elucidated and the related mechanisms need further investigation.

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