



Liver

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Introduction

Over the past decades, significant advances in the understanding of the renin–angiotensin system (RAS), especially regarding the local expression of RAS in several organs and tissues, including the kidney, brain, and liver, have hampered the classical view of RAS as a merely circulating hormonal system [1]. Particularly relevant for the reconceptualization of the RAS was the identification of the heptapeptide Ang-(1-7) [2], the ACE homolog enzyme responsible for the conversion of Ang II into Ang-(1-7), ACE2 [3, 4], and the Mas receptor, a G-protein coupled receptor, which mediates the main effects of Ang-(1-7) [5]. In this scenario, RAS is currently viewed as a system composed by two opposing axis: the classical one, including angiotensin converting enzyme (ACE)-Angiotensin (Ang) II-Ang type 1 (AT₁) receptor and the alternative one, comprising ACE2-Ang-(1-7)-Mas receptor [6].

It has also been often postulated that the classical arm mediates pro-inflammatory, pro-thrombotic, and pro-fibrotic processes, mainly through the activation of AT₁ receptors [6], whereas the alternative axis seems to play a protective role by frequently opposing Ang II actions via Mas receptors stimulation [7, 8]. An imbalance in the RAS classical and alternative axis components have

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been implicated in the pathogenesis of a wide range of conditions including liver diseases [7–9]. Accordingly, therapeutic strategies have often been designed in order to inhibit ACE-Ang II-AT₁ receptor and to stimulate ACE2-Ang-(1-7)-Mas receptor activities [7, 9].

The involvement of both axes of the RAS in the pathogenesis of liver diseases has been supported by experimental and clinical studies [9, 10]. Herein, we will discuss current evidence regarding the role of RAS, mainly focusing on ACE2-Ang-(1-7)-Mas receptor arm, in liver physiological and clinical conditions as well as potential therapeutic role of RAS in liver diseases.

ACE2-ANG-(1-7)-MAS Receptor Axis Role in Liver Physiology

The local RAS concept opens the road for the hypothesis that RAS components activity might be tissue/organ specific and function-oriented [1]. Specifically, regarding the liver, the local hepatic RAS is not well defined, although studies about RAS involvement in hepatic diseases have supported a role for this system in liver function [11].

The liver, under physiological conditions, plays a pivotal role in metabolic homeostasis, by regulating glucose and lipid metabolisms [12, 13]. A great body of evidence has pointed out the RAS components as crucial regulators of hepatic-associated metabolic functions [12, 14–17]. For instance, the genetic deletion of the Mas receptor in FVB/N mice leads to a metabolic syndrome-like state characterized by dyslipidemia, increased abdominal fat mass, enhanced muscle triglycerides, glucose intolerance, and reduced insulin sensitivity, as well as a decrease in insulin-stimulated glucose uptake by adipocytes [16, 17]. In line with these findings, the absence of ACE2 expression in mice also increases liver insulin resistance and expression of hepatic lipogenic genes, and decreases the expression of fatty acid oxidation-related genes. These changes in hepatic metabolic activity were associated with enhanced liver oxidative stress and inflammation, all of which supporting the idea that ACE2 ameliorates hepatic insulin resistance, improves insulin signaling, and is involved in protection against oxidative stress in the liver [14, 15].

An *in vitro* approach also reinforced the protection of ACE2-Ang-(1-7)-Mas receptor arm in liver metabolic function. The exposure of HepG2 hepatocytes cells to the Ang-(1-7) increased liver glucose uptake and intracellular glycogen synthesis. The amelioration of insulin resistance in the liver was associated with the activation of the Akt/PI3K/IRS-1/JNK insulin-signaling pathway. Importantly, the protective effect of Ang-(1-7) was partially blocked by the Mas receptor antagonist, A779, indicating that the beneficial effects of the alternative RAS arm are dependent of Mas receptor activation [15]. The same authors further demonstrated, by employing the same *in vitro* approach, that the activation of the ACE2-Ang-(1-7)-Mas receptor axis decreased liver oxidative stress, inflammation, and lipid accumulation partly by regulating lipid-metabolizing genes through ATP/P2 receptor/CaM signaling pathway [14]. A more recent study provided *in vivo* evidence of significant effects of Ang-(1-7) on metabolic pathways involved in lipid homeostasis. A transgenic rat

overexpressing Ang-(1-7) presented a decrease in adiposity index along with a reduction in lipogenesis, suggesting a direct effect of Ang-(1-7) on adipose tissue lipid metabolism, independent of the stimulatory effect of insulin. Moreover, specifically in the liver, overexpression of Ang-(1-7) decreased the concentration of triacylglycerol and inhibited fatty acid synthase (FAS) and fatty acid transport protein (FATP) expression in the liver, suggesting a decrease in de novo fatty acid synthesis and fatty acid uptake [12]. Interestingly, exercise training prevented metabolic syndrome and nonalcoholic fatty liver disease (NAFLD) in fructose-fed rats by increasing hepatic ACE2-Ang-(1-7)-Mas receptor axis activity [18]. Of note, NAFLD is one of the most common chronic liver diseases worldwide and an important risk factor for nonalcoholic steatohepatitis, type 2 diabetes, and cardiovascular diseases [19, 20]. There is evidence that the activation of the counter-regulatory arm (ACE2-Ang-(1-7)-Mas) is beneficial in NAFLD and metabolic-associated syndromes [14, 15, 21–23].

Taken together, these studies provided strong evidence of the physiological role of RAS locally expressed in the liver in glycemic and lipid metabolisms, paving the road for the investigation of the ACE2-Ang-(1-7)-Mas receptor arm as a promising therapeutic strategy in liver diseases associated with metabolic dysfunctions.

ACE2-ANG-(1-7)-MAS Receptor Axis Role in the Pathophysiology of Liver Diseases

Liver diseases are major causes of morbidity and mortality worldwide. The leading causes of liver failure are hepatitis B and hepatitis C virus infections, alcohol use, and steatohepatitis related to obesity. Without proper treatment, all types of chronic hepatitis will progress to end-stage liver diseases, including cirrhosis, liver failure, and hepatocellular carcinoma, which ultimately lead to death [24, 25]. It is estimated that liver diseases account for a significant increase in the incidence of cirrhosis and for the death of at least 800,000 people worldwide annually [26].

Although the specific mechanisms underlying hepatic fibrosis pathophysiology remain to be fully revealed, some pathological characteristics of chronic liver diseases might include enhanced fibrosis, oxidative stress, and inflammatory markers [24, 27]. All together, these events lead to significant changes in hepatic perfusion, enhanced portal blood flow resistance as well as liver dysfunction. The end stage of progressive hepatic fibrosis, widely known as cirrhosis, culminates in liver architecture disruption due to fibrous scars and development of regenerating tissue, which, in turn, aggravates liver failure [28, 29].

Emerging evidence has supported the involvement of RAS components in hepatic fibrosis and cirrhosis. Particularly, an upregulation of classical RAS arm components, including angiotensinogen, renin, ACE, Ang II and AT1 receptors has been reported in experimental and clinical liver injury studies [30–34]. Accordingly, inhibition of RAS including the blockade of Ang II activity by lisinopril and captopril (ACE inhibitors) or losartan (AT1 receptor antagonist) prevented RAS pro-fibrogenic effects and restored liver function [30, 35–39].

It is worth mentioning that liver fibrosis and hepatic cirrhosis seem to depend on the balance between the classical (ACE-Ang II-AT1 receptor) and the counter-regulatory (ACE2-Ang-(1-7)-Mas receptor) RAS axes [10, 40, 41]. Indeed, based on the concept that the RAS counter-regulatory axis opposes the classical arm actions, presenting anti-inflammatory, anti-oxidative, and anti-fibrotic effects, it is quite reasonable to expect a protective role for ACE2-Ang-(1-7)-Mas receptor axis in liver diseases [7, 8]. Moreover, considering that cirrhosis might be potentially reversible, particularly in a compensated stage [27], the ACE2-Ang-(1-7)-Mas receptor axis might represent a promise drug target in liver failure.

Insights from Preclinical Studies

Numerous studies have investigated the role of the counter-regulatory RAS arm as well as the mechanisms underlying its protective effects on liver function by employing different models of liver fibrosis, including bile duct ligation, carbon tetrachloride (CCl₄) treatment or continuous Ang-(1-7) infusion [42–49]. For instance, an increase in ACE2 expression in the liver parenchyma of rats submitted to bile duct ligation provided the first evidence of a potential role of the counter-regulatory RAS axis in chronic liver disease [47]. Similar findings were found with the progression of liver fibrosis induced by CCl₄ administration in rats. In this model, inhibition of ACE upregulated the mRNA expression of ACE2 and Mas receptor, contributing to liver protection [44]. It is worth noticing that ACE2 activity seems to be important as an endogenous negative regulator of RAS in chronic, but not acute, liver injury, primarily by promoting the conversion of Ang II into Ang (1-7). This statement is supported by the fact that ACE2 knockout mice only presented increased hepatic fibrosis 21 days after bile duct ligation or following chronic administration of CCl₄. On the other hand, no differences were found between ACE2 knockout mice and wild-type littermates when animals were subjected to acute liver injury. Moreover, genetic ablation of ACE2 in one-year-old mice resulted in spontaneous inflammatory cell infiltration and mild liver fibrosis [46].

The hepatic protection exerted by increased expression of ACE2 might rely on the fact that this enzyme catalyzes the pro-fibrotic peptide Ang II in the anti-fibrotic peptide Ang-(1-7). Thus, its catalytic action makes ACE2 a very interesting therapeutic target for liver fibrosis [44, 46]. In line with this hypothesis, an earlier study demonstrated, by employing a liver-specific adeno-associated viral genome 2 serotype 8 vector (rAAV2/8-ACE2) with a liver-specific promoter in bile duct ligation and CCL4 administration models, that the long-term therapeutic effect of recombinant ACE2 rapidly upregulated hepatic ACE2 and attenuated liver fibrosis. In parallel, the recombinant ACE therapy reduced hepatic Ang II levels concomitantly with an increase of Ang-(1-7) concentrations in liver tissue. This study also showed reductions in NADPH oxidase activity, oxidative stress, ERK1/2, and p38 phosphorylation, without unwanted systemic effects [45].

An even more attractive idea was to investigate the anti-fibrotic therapeutic capability of Ang-(1-7)-Mas receptor signaling in chronic liver disease models.

Accordingly, infusion of Ang-(1-7) markedly attenuated hepatic fibrosis in bile duct-ligated rats, decreased hydroxyproline content, and downregulated key genes involved in liver fibrosis and angiogenesis such as collagen 1A1, α -SMA (smooth muscle actin), VEGF (vascular endothelial growth factor), and CTGF (connective tissue growth factor) [43]. On the other hand, the pharmacological blockage of Mas receptors with the antagonist A-779, following a bile duct ligation in rats, induced an elevation in hepatic hydroxyproline and TGF- β 1 concentrations, aggravating liver fibrosis [48]. There is evidence that Ang-(1-7) might exert its anti-fibrotic effects in liver tissue induced by bile duct ligation by means of the regulation of NLRP3 inflammasome/IL-1 β /Smad pathway activation induced by Ang II-mediated reactive oxygen species (ROS) via redox balance modulation [42, 49]. A more recent study showed that the microRNA-21 (mir-21) mediates Ang-II-induced NLRP3 inflammasome activation via the Spry1/ERK/NF- κ B, Smad7/Smad2/3/NOX4 pathways contributing to liver fibrosis. The administration of Ang-(1-7) downregulated mir-21 expression, and protected against bile duct ligation and Ang-II infusion-induced hepatic fibrosis [50].

The protective role of Ang (1-7) in the liver has been also investigated in a murine model of hepatocellular carcinoma. The administration of Ang-(1-7) to H22 hepatoma-bearing mice prevented tumor growth by arresting tumor proliferation, promoting tumor apoptosis and inhibiting tumor angiogenesis. Interestingly, the treatment with Ang-(1-7) decreased AT1 receptor mRNA expression, upregulated mRNA levels of AT2 and Mas receptors, and suppressed H22 cell-endothelial cell communication. These findings suggest that benefits of Ang-(1-7) in hepatocellular carcinoma depend on the complex interaction between AT1, AT2, and Mas receptors [51]. A more recent study, by employing the same hepatocellular carcinoma model, investigated the long-term effects of adeno-associated virus (AAV) serotype-8-mediated Ang-(1-7) overexpression. The anti-tumoral activity of Ang-(1-7) was indicated by a persistent inhibition of the tumor growth and downregulation of angiogenesis along with a decrease in the levels of the proangiogenic factors phosphatidylinositol-glycan biosynthesis class F protein (PIGF) and VEGF [52]. Taken together, these studies reinforce the role of Ang-(1-7) as a promising drug target for liver diseases.

In vitro approaches also provided valuable evidence regarding the role of the counter-regulatory RAS axis in liver dysfunction. Culture hepatic stellate cells treated with Ang-(1-7) or the Mas receptor agonist, AVE 0991, expressed less α -SMA and hydroxyproline, while treatment with the Mas receptor antagonist, A779, induced opposite effects [53]. Accordingly, Ang-(1-7) through Mas receptor activation, in cultured hepatic stellate cells, inhibited Ang II-induced phosphorylation of extracellular signal-regulated kinase (ERK)1/2, a classical pathway of tissue fibrosis [46]. Ang-(1-7) also decreases Ang II-induced NLRP3 inflammasome/IL-1 β /Smad pathway activation in hepatic stellate cells, thus preventing α -collagen I (Col1A1) accumulation. This finding suggests a novel potential mechanism by which Ang-(1-7) exerts its anti-fibrotic activity in liver tissue [42, 49]. Moreover, Ang-(1-7) seems to inhibit Ang II-induced NLRP3 inflammasome/IL-1 β /Smad pathway activation in primary hepatic stellate cells also by suppressing mir-21 expression [50].

Insights from Clinical Studies

Data from experimental studies have broadened our knowledge regarding the involvement of ACE2-Ang-(1-7)-Mas receptor axis in liver physiology and pathophysiology. However, a translational approach to human studies seems to become a real challenge. Up to date, there is little evidence provided by clinical studies. A pivotal study first demonstrated widespread parenchymal expression of ACE2 in the liver of hepatitis C cirrhotic patients. The authors also reported increased levels of ACE2 in cultured human hepatocytes exposed to hypoxia [47]. In line with these findings, patients with cirrhosis induced by hepatitis C and mild-to-moderate liver disease presented enhanced circulating levels of Ang-(1-7), possibly as an attempt to counteract ACE-Ang II-AT1 receptor arm pro-fibrotic effects [40, 53]. A more recent study investigated the role of counter-regulatory RAS arm components in liver failure progression from fibrosis to cirrhosis to hepatocellular carcinoma. The concentrations of Ang II, Ang-(1-7), and VEGF were higher in the serum of patients compared with healthy subjects, and increased with the disease progression. Conversely, the liver mRNA expression of ACE2 gradually decreased with the increasing grade of disease severity. Importantly, higher liver expression of ACE2 was associated with patient's longer survival time, indicating that low expression of ACE2 may be a useful indicator of poor prognosis in hepatocellular carcinoma [54]. The evidence of ACE2-Ang-(1-7)-Mas receptor axis involvement in human liver diseases is scarce as well as its potential role as predictive biomarkers or drug targets. Further studies are necessary in order to better address this issue.

Concluding Remarks

The counter-regulatory RAS axis exerts anti-inflammatory, anti-oxidative, and anti-fibrotic effects in liver tissue. In general, ACE2-Ang-(1-7)-Mas axis opposes ACE-Ang II-AT1 receptor arm actions. The balance between both RAS axes may influence clinical and histopathological expression of liver diseases. Most data regarding ACE2-Ang-(1-7)-Mas axis role in hepatic pathophysiology as well as its therapeutic potential in liver diseases were generated from preclinical studies. To date, clinical research focused on the investigation of circulating and local concentrations of ACE2 and Ang-(1-7). Evidence regarding the interaction of AT1, AT2, and Mas receptor is still missing. Moreover, further studies that address the role of counter-regulatory RAS axis molecules as biomarkers of liver fibrosis and/or of disease prognosis as well as potential therapeutic targets are urgently necessary. The design of molecular or genetic methods to increase the expression of ACE2 and increased tissue levels of Ang-(1-7) and/or activation of the Mas receptor may, in turn, result in the development of new pharmacological approaches.

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