Update on new aspects of the renin–angiotensin system in liver disease: clinical implications and new therapeutic options

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ABSTRACT

The RAS (renin–angiotensin system) is now recognized as an important regulator of liver fibrosis and portal pressure. Liver injury stimulates the hepatic expression of components of the RAS, such as ACE (angiotensin-converting enzyme) and the AT1 receptor [AngII (angiotensin II) type 1 receptor], which play an active role in promoting inflammation and deposition of extracellular matrix. In addition, the more recently recognized structural homologue of ACE, ACE2, is also up-regulated. ACE2 catalyses the conversion of AngII into Ang-(1–7) [angiotensin-(1–7)], and there is accumulating evidence that this ‘alternative axis’ of the RAS has anti-fibrotic, vasodilatory and anti-proliferative effects, thus counterbalancing the effects of AngII in the liver. The RAS is also emerging as an important contributor to the pathophysiology of portal hypertension in cirrhosis. Although the intrahepatic circulation in cirrhosis is hypercontractile in response to AngII, resulting in increased hepatic resistance, the splanchnic vasculature is hyporesponsive, promoting the development of the hyperdynamic circulation that characterizes portal hypertension. Both liver fibrosis and portal hypertension represent important therapeutic challenges for the clinician, and there is accumulating evidence that RAS blockade may be beneficial in these circumstances. The present review outlines new aspects of the RAS and explores its role in the pathogenesis and treatment of liver fibrosis and portal hypertension.

INTRODUCTION

Cirrhosis, and its inevitable consequence of portal hypertension, represents the final pathway for liver fibrosis due to all chronic liver diseases. As a result of the hepatitis C epidemic 20–30 years ago and the increase in steatohepatitis related to obesity and alcohol misuse, the incidence of cirrhosis is rising and it is responsible for the death of over 800,000 people worldwide annually [1,2].

The function of the RAS (renin–angiotensin system) in cardiac and renal disease has been the subject of considerable recent interest, as it may regulate fibrosis and tissue repair, and is consequently a target for treatment

Key words: angiotensin II, angiotensin-converting enzyme, cirrhosis, liver fibrosis, portal hypertension, renin–angiotensin system.

Abbreviations: ACE, angiotensin converting-enzyme; ACEi, ACE inhibitor; ADAM17, a disintegrin and metalloproteinase 17; Ang-(1–7), angiotensin-(1–7); AngI, angiotensin I; AngII, angiotensin II; ARB, AT1 receptor blocker; AT1 receptor, AngII type 1 receptor; AT2 receptor, AngII type 2 receptor; BP, blood pressure; EABV, effective arterial blood volume; ECM, extracellular matrix; HSC, hepatic stellate cell; MasR, Mas receptor; NASH, non-alcoholic steatohepatitis; NOS, NO synthase; eNOS, endothelial NOS; RAS, renin–angiotensin system; ROS, reactive oxygen species; TGF-β1, transforming growth factor-β1; TNF-α, tumour necrosis factor-α; VSMC, vascular smooth muscle cell.

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with RAS blockers [3,4]. In addition to the systemic RAS, in many tissues there is local expression of many, if not all, RAS components. Thus there exist ‘local RAS systems’ – functionally autonomous systems in individual organs, regulated by stimuli within the same organ, which play an important role in the local response to disease or injury [5–7]. Recent research suggests that not only is there activation of the systemic RAS in cirrhosis, but there is also activation of the hepatic RAS [8–12]. The present review will outline relevant aspects of RAS physiology, evidence of its role in the development of liver fibrosis and the circulatory abnormalities that characterize portal hypertension, and the use of drugs targeting the RAS in these conditions.

**THE RAS**

In the 1930s, two groups independently discovered that venous blood from ischaemic kidneys had a pressor effect and that the substance causing this effect was temperature-sensitive and non-dialysable [13]. One group named it angiotonin; the other, hypertensin. Following decades of work, during which the structure of this substance was identified as a peptide containing eight amino acids, both centres compromised and agreed to call it angiotensin in 1958 [14]. Since then it has been well documented that AngII (angiotensin II), as we now know it, is central to the regulation of BP (blood pressure) by influencing vascular smooth muscle tone and extracellular fluid homoeostasis.

It has subsequently emerged that the effects of AngII are far more extensive and diverse than the original investigators could have appreciated. The ‘classical’ RAS, comprising AngII, ACE (angiotensin converting-enzyme) and the AT1 receptor (AngII type 1 receptor) is responsible for not only vasoconstriction and sodium homoeostasis by acting on VSMCs (vascular smooth muscle cells), but also has a role in inflammation, cytokine production and cell proliferation [15–19]. Other components of the RAS that may mediate inflammation and fibrosis include the (pro)renin receptor, which is likely to have intracellular functions beyond its role as a receptor for renin and prorenin [22].

In recent years an ‘alternative’ arm of the RAS has been characterized, comprising the ACE structural homologue ACE2, Ang-(1–7) [angiotensin-(1–7)] and MasR (Mas receptor), which has effects that counterbalance those mediated by the classical arm (Figure 1).

**THE ALTERNATIVE RAS**

Over a decade ago, two groups concurrently discovered a human structural homologue of ACE, named ACE2, which shares more than 40% identity with ACE in its catalytic domain [23–25]. ACE2 is widely expressed in rodents, and in humans is found in high levels in the heart, kidneys, testes and at low levels in colon and lung [25,26]. ACE2 is a type I integral membrane protein that can convert renin substrate AngI to AngII and also convert more than AngII to Ang-(1–7) [angiotensin-(1–7)].
ACE2 is a type 1 transmembrane carboxypeptidase that cleaves an amino acid from the C-terminus of the octapeptide AngII to form Ang-(1–7). ACE2 undergoes surface-shedding by the action of the metalloprotease ADAM17 to release soluble ACE2. The significance of this shedding is not yet clear. This Figure is adapted from ‘Cell membrane detailed diagram’ at http://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_de.svg.

Unlike ACE, ACE2 has only one catalytic domain (located within the HEXXH motif), which cleaves a single C-terminal residue from peptide substrates, whereas ACE normally releases a dipeptide. ACE2 is therefore able to cleave both AngI (angiotensin I) to Ang-(1–9) [angiotensin-(1–9)] and the octapeptide AngII to Ang-(1–7) respectively (Figure 2). In comparison with ACE, the affinity for cleaving Ang I is low, which makes it a poor physiological substrate, whereas, conversely, ACE2 is kinetically favoured to cleave AngII to Ang-(1–7), suggesting that ACE2 opposes the effects of ACE [31–34]. Far from the initial view that it was an inactive metabolite, the truncated protein Ang-(1–7) has recently become the focus of much research. Ang-(1–7) is the endogenous ligand for MasR, a G-protein-coupled receptor encoded by the Mas proto-oncogene [35]. Many MasR-mediated effects counterbalance those of AngII and the AT1 receptor, and include vasodilation, improvement in endothelial function, inhibition of cell proliferation, and anti-arrhythmogenic and antithrombotic effects [36–45]. Increasing evidence is emerging that Ang-(1–7) may also exert effects via other receptors, including the AT2 receptor (AngII type 2 receptor), perhaps by receptor heterodimerization or alterations in post-receptor signalling [46–49].

Expression of the RAS system in chronic liver disease

Activation of the classical arm of the systemic RAS in patients with decompensated cirrhosis, in response to the mesenteric and systemic vasodilation that accompanies the development of portal hypertension, has been recognized for decades [50]. However, we have documented that, following liver injury, hepatic expression of many RAS components, including ACE and the AT1 receptor, is markedly increased and is localized to areas of active fibrogenesis [11]. Furthermore, expression of these components has been documented in activated, but not quiescent, human HSCs (hepatic stellate cells), which play a key role in liver fibrosis [51]. This suggests that, as in other organs, in the liver the RAS may be involved in the pathogenesis of chronic tissue injury and fibrosis [11,12].

It has been demonstrated that the alternative arm of the RAS is also up-regulated in chronic liver disease. Systemic Ang-(1–7) levels are high in cirrhosis and may
also be elevated in the pre-cirrhotic phase [10,52]. Our group has shown that, in patients with chronic hepatitis C, plasma Ang-(1–7) concentrations increase as liver fibrosis progresses (Figure 3). Similar to the classical RAS, hepatic expression of the alternative RAS is augmented in rats with experimental biliary fibrosis [11,12]. In healthy liver, ACE2 protein is minimally expressed in endothelial cells, bile duct cells and perivenular hepatocytes; however, in both rats with biliary cirrhosis and patients with hepatitis C cirrhosis there is widespread ACE2 protein expression in parenchymal tissue of diseased livers [12]. Organ perfusion experiments demonstrate that fibrotic livers produce increased amounts of Ang-(1–7) from AngII, confirming the presence of functional ACE2 in the diseased liver and suggesting an opposing effect to ACE [10].

**ROLE OF THE RAS IN LIVER FIBROSIS**

**Mechanisms of hepatic fibrosis**

Hepatic fibrosis occurs when liver tissue is exposed to chronic damage due to insults such as chronic viral hepatitis, alcohol abuse and NASH (non-alcoholic steatohepatitis). It is characterized by the excessive accumulation of ECM (extracellular matrix) proteins, and eventually leads to distortion of the hepatic architecture, the formation of fibrous scars and the development of the characteristic nodules that define cirrhosis [53].

A number of liver cells have been implicated in the pathophysiology of hepatic fibrogenesis, of which the most important and most comprehensively studied is the HSC [53,54]. HSCs are resident perisinusoidal cells that lie in the subendothelial space between hepatocytes and sinusoidal endothelial cells, a location which enables them to easily interact with surrounding cell types. HSCs are quiescent in healthy liver, but undergo a dramatic phenotypic activation into ECM-secreting myofibroblasts in response to chronic liver injury [54]. These activated HSCs are continuously stimulated to produce ECM proteins with collagen type I and III as major components. Major stimuli for activation include growth factors, inflammatory chemokines and cytokines, and ROS (reactive oxygen species) released from hepatocytes, Kupffer cells, biliary cells and inflammatory cells following hepatic injury. The initial changes in gene expression and phenotypic activation in response to these stimuli render HSCs highly responsive to continuous stimulation by other pro-fibrotic cytokines, growth factors and vasoactive peptides [54].

**The classical RAS and liver fibrosis**

A large number of mechanisms have been shown to drive activation of HSCs and other cells involved in hepatic fibrogenesis. However, there has been major interest recently in the role of the RAS, given its importance in other chronic fibrotic diseases and from evidence of the existence of a functioning and up-regulated RAS in experimental liver disease [10,11,55].

In the healthy liver, quiescent HSCs have very low levels of expression of components of the RAS and are unable to secrete AngII [51]. Following liver injury, activated HSCs and other hepatic myofibroblasts express many of the components of the RAS, including ACE and the AT1 receptor, and acquire the ability to synthesize AngII, which induces contraction and proliferation of HSCs via the AT1 receptor [51,56]. In addition, there is a large body of work to support the hypothesis that AngII promotes the activation and differentiation of quiescent HSCs into myofibroblasts [53]. AngII stimulates HSC activation via JNK (c-Jun N-terminal kinase)/ERK (extracellular-signal-regulated kinase) transduction pathways with increased secretion of pro-inflammatory cytokines and oxidative stress [57]. Oxidative stress due to ROS generation by activated NADPH oxidase is likely to be a key driver of AngII-induced hepatic fibrosis, since mice lacking p47phox, a
regulatory subunit of NADPH oxidase, have attenuated experimental liver injury and fibrosis compared with wild-type counterparts [58]. In keeping with this finding, incubation with AngII increases collagen expression and the secretion of TGF-β1 (transforming growth factor-β1) and inflammatory cytokines by human HSCs, and these effects are attenuated by NADPH oxidase inhibition. Moreover, HSCs isolated from p47phox-gene-deleted mice display a blunted response to AngII compared with wild-type animals [58].

Systemic infusion of AngII induces liver fibrosis, whereas blockade of the AT1 receptor ameliorates liver fibrosis in several rat models of liver injury [56,59–64]. Furthermore, studies using gene-deletion mice have demonstrated that AT1-receptor-deficient mice are protected from fibrosis, whereas AT2-receptor-deficient mice have worse liver fibrosis [65–67].

These findings collectively suggest that, in the liver, AngII stimulates the activation of quiescent HSCs and proliferation of activated myofibroblastic HSCs and promotes the release of inflammatory cytokines, as well as the deposition of excessive amounts of ECM proteins, and that these effects are mediated through the AT1 receptor.

The alternative RAS and liver fibrosis

There is increasing evidence that the alternative axis of the RAS plays a protective role that inhibits the development of liver fibrosis. We have demonstrated previously that Ang-(1–7) infusion reduces tissue type I collagen, hydroxyproline content and α-smooth muscle actin expression in the BDL (bile-duct-ligated) model of liver fibrosis [68]. Furthermore, pharmacological blockade of MasR aggravates experimental liver fibrosis [69]. Additional evidence for the potential beneficial effects of the alternate RAS in liver disease comes from a study demonstrating the exacerbation of experimental chronic liver injury in ACE2-deleted mice compared with wild-type littermates as shown by increased collagen staining and expression of α-smooth muscle actin protein [70]. These findings collectively suggest that, as in cardiac fibrosis, the Ang-(1–7)/ACE2/MasR axis is an important inhibitor of liver fibrosis [23].

ACEis (ACE inhibitors) and ARBs (AT1 receptor blockers) in the treatment of liver fibrosis

ACEis and ARBs block the classical axis of the RAS and therefore might be expected to be beneficial in liver fibrosis. In addition, ACE inhibition prevents the degradation of Ang-(1–7), thus augmenting plasma and local tissue levels. Numerous studies using a variety of experimental models of liver fibrosis have demonstrated anti-fibrotic effects of both ACEis and ARBs, although there are some conflicting findings which may reflect the heterogeneity of disease models, drugs and drug doses used [61,71–80]. There has been particular interest in the therapeutic use of RAS blockade in experimental NASH, given the lack of specific medical treatments for this condition and its association with hypertension and other features of the metabolic syndrome. Although in one study losartan failed to significantly influence either liver injury or progression of fibrosis in an animal model of NASH [81], a similar study using olmesartan demonstrated a 70% reduction in fibrosis, and a study in obese Zucker rats found that both perindopril and irbesartan prevented fatty liver and improved fibrosis with a reduction in the hepatic expression of the potent pro-fibrotic cytokines TNF-α (tumour necrosis factor-α) and TGF-β1 [59,82].

Despite the large number of animal studies, at present there is a relative paucity of clinical data to support the use of these drugs in human liver disease (Table 1). A number of small prospective human studies have demonstrated beneficial effects of ACEis or ARBs in the treatment of liver fibrosis due to chronic hepatitis C [63,83–88]. These studies have administered the drug alone, or in combination with other drugs, for up to 18 months, and have assessed fibrosis using a variety of different ways, including surrogate markers such as serum hyaluronic acid. Only one pilot study, which examined the effects of 6 months of losartan therapy, reported a significant decrease in fibrosis stage as assessed by liver biopsy [85]; however, all showed improvements in fibrosis histological area, fibrosis markers or pro-fibrotic gene expression. In contrast, a recently published analysis of the HALT-C (Hepatitis C Antiviral Long-term Treatment against Cirrhosis) cohort, a prospective, multi-centre trial of antiviral treatment for chronic hepatitis C, did not find any reduction in the liver fibrosis score in the ARB/ACEi users [89]. This result, which conflicts with the majority of the literature, may reflect the increased proportion of diabetics in the ARB/ACEi group, as diabetes is a well-recognized predictor of hepatitis C progression. RAS blockade has also demonstrated beneficial effects on hepatic inflammation and/or fibrosis due to hepatitis C in the post-liver transplant setting [90,91].

Initial human studies examining the anti-fibrotic effects of RAS blockade in NASH were limited by very small patient numbers and the lack of a proper randomized control group, which represents a particular problem in studies of these patients since the disease fluctuates in response to changes in lifestyle. These initial studies suggested a possible beneficial effect of losartan on liver inflammation and fibrosis due to NASH [92,93]. However, a recently published larger trial was unable to demonstrate any additive anti-fibrotic effect of the combination of losartan and rosiglitazone over rosiglitazone alone [94]. Although this result is somewhat discouraging, the trial was underpowered due
Table 1  Clinical trials of ARBs or ACEis for the treatment of liver fibrosis

<table>
<thead>
<tr>
<th>First author</th>
<th>Prospective</th>
<th>Randomized</th>
<th>Controlled</th>
<th>n</th>
<th>Aetiology</th>
<th>Drug</th>
<th>Duration</th>
<th>Fibrosis markers</th>
<th>Liver fibrosis</th>
</tr>
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<tr>
<td>Terui (2002) [86]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>30</td>
<td>HepC</td>
<td>Losartan + UDCA compared with UDCA</td>
<td>Not stated</td>
<td>Improved</td>
<td>Reduced</td>
</tr>
<tr>
<td>Sookoian (2005) [85]</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>23</td>
<td>HepC</td>
<td>Losartan</td>
<td>6 months</td>
<td>—</td>
<td>Reduced</td>
</tr>
<tr>
<td>Yoshiji (2005) [87]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>20</td>
<td>HepC non-responders</td>
<td>Perindopril + IFN compared with nil</td>
<td>1 year</td>
<td>Improved</td>
<td>—</td>
</tr>
<tr>
<td>Yoshiji (2006) [88]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>40</td>
<td>HepC non-responders with HT</td>
<td>Perindopril + IFN compared with nil</td>
<td>1 year</td>
<td>Improved</td>
<td>—</td>
</tr>
<tr>
<td>Debernardi-Vernon (2007) [84]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>67</td>
<td>Cirrhosis, mainly HepC</td>
<td>Candesartan cilexetil</td>
<td>1 year</td>
<td>Improved</td>
<td>—</td>
</tr>
<tr>
<td>Corey (2009) [103]</td>
<td>No</td>
<td>—</td>
<td>Yes</td>
<td>234</td>
<td>HepC</td>
<td>Any ARB or ACEi</td>
<td>Mean 3.4 years</td>
<td>—</td>
<td>Reduced</td>
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<tr>
<td>Colmenero (2009) [83]</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>14</td>
<td>HepC</td>
<td>Losartan</td>
<td>18 months</td>
<td>—</td>
<td>Unchanged</td>
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<tr>
<td>Abu Dayyeh (2010) [89]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>535</td>
<td>HepC (HALT-C cohort)</td>
<td>Any ARB or ACEi</td>
<td>3.5 years</td>
<td>—</td>
<td>Unchanged</td>
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<td>Rimola (2004) [90]</td>
<td>No</td>
<td>—</td>
<td>Yes</td>
<td>128</td>
<td>Post-transplantation HepC</td>
<td>Any ARB or ACEi</td>
<td>Median 41 months</td>
<td>—</td>
<td>Reduced</td>
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<td>Cholongitas (2010) [91]</td>
<td>No</td>
<td>—</td>
<td>No</td>
<td>102</td>
<td>Post-transplantation HepC</td>
<td>Any ARB</td>
<td>Mean 13 months</td>
<td>—</td>
<td>Unchanged</td>
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<td>Yokohama (2004) [92]</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>7</td>
<td>NASH with HT</td>
<td>Losartan</td>
<td>48 weeks</td>
<td>Improved</td>
<td>Reduced‡</td>
</tr>
<tr>
<td>Yokohama (2006) [92]</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>15</td>
<td>NASH with HT</td>
<td>Losartan</td>
<td>48 weeks</td>
<td>—</td>
<td>Reduced</td>
</tr>
<tr>
<td>Georgescu (2009) [94]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>54</td>
<td>NASH</td>
<td>Valsartan compared with Telmisartan</td>
<td>20 months</td>
<td>—</td>
<td>Reduced‡</td>
</tr>
<tr>
<td>Torres (2011) [95]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>137</td>
<td>NASH</td>
<td>Losartan + RSG compared with metformin + RSG compared with RSG</td>
<td>18 months</td>
<td>—</td>
<td>Unchanged</td>
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<td>Kim (2009) [104]</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
<td>47</td>
<td>ALD</td>
<td>Candesartan + UDCA compared with UDCA</td>
<td>6 months</td>
<td>—</td>
<td>Reduced‡</td>
</tr>
</tbody>
</table>

* No change in fibrosis score, but fibrosis area improved.
† Fibrosis score improved in four out of seven patients.
‡ Fibrosis improved in the telmisartan group only.
to restrictions placed upon rosiglitazone during the trial period by the FDA (Food and Drug Administration) in the U.S. In contrast, telmisartan, which is theoretically a more attractive ARB in this setting due to its PPAR-α (peroxisome-proliferator-activated receptor-α) agonist properties, was found to be superior to valsartan for the treatment of liver fibrosis in another randomized trial of patients with NASH [95].

Thus, although there remains insufficient evidence to empirically recommend the use of ARBs or ACEis in the treatment of liver fibrosis, the use of these agents in selected patients, for example, those with hypertension, should be considered. Well-designed placebo-controlled clinical trials that include the histological assessment of liver fibrosis are needed, especially in the treatment of NASH fibrosis, where some of the newer ARBs have shown promise. Further clinical trials of ARBs in liver fibrosis are currently underway and it is hoped that these will provide further guidance to the clinician.

Manipulation of the alternative RAS in the treatment of liver fibrosis
Given that the ACE2/Ang-(1–7)/MasR axis has effects that counterbalance those of AngII and the AT1 receptor, it is tempting to speculate that pharmaceutical attenuation of this arm might also be a useful therapeutic strategy. One option is to enhance ACE2 expression and activity through pharmacological or genetic intervention. Human recombinant ACE2 has been shown to be effective in treating cardiovascular and renal disease in animals, and had no adverse effects in healthy volunteers in a Phase 1 clinical trial [96–98]. A single study using recombinant ACE2 to treat liver injury due to a toxic or cholestatic experimental liver insult in animals demonstrated its ability to attenuate fibrosis [70]. To our knowledge, no other methods for increasing ACE2 activity, for example using a synthetic ACE2 activator, have been trialled in liver disease. Another method of targeting the alternative RAS is via MasR. The oral MasR agonist AVE-0991 was found to be effective in restoring cardiac function in several animal models of cardiomyopathy [99–101]. In addition, although intravenous Ang-(1–7) is unstable and has a short half-life in the circulation, an oral formulation has recently been developed and was cardioprotective in infarcted rats [102]. Studies of these agents in the treatment of experimental liver fibrosis are warranted.

ROLE OF THE RAS IN PORTAL HYPERTENSION
Portal hypertension contributes to many of the life-threatening complications of cirrhosis, including variceal bleeding, ascites, spontaneous bacterial peritonitis and hepatorenal syndrome. The development of portal hypertension results from both an increase in the intrahepatic resistance to portal flow and an associated vasodilation of the mesenteric vascular bed, which leads to an increase in mesenteric blood flow, much of which bypasses the liver through portosystemic collaterals (Figure 4).

There is considerable experimental evidence that the RAS is involved in the pathogenesis of increased hepatic resistance in cirrhosis. This resistance has a fixed component that results from architectural distortion and obliteration of the normal sinusoidal vascular bed. However approximately 30% of the increase is mediated by contraction of the sinusoidal vascular bed by activated HSCs and VSMCs [105–107]. The phenotypic transformation of HSCs into myofibroblasts following liver injury is promoted by AngII and is associated with the expression of RAS components and the acquisition of a contractile response to AngII via the AT1 receptor [56]. In addition, ACE expression and activity is increased in the cirrhotic liver, providing a source of increased local AngII synthesis [11]. Thus not only are there elevated systemic AngII levels in cirrhosis, but there are also likely to be elevated local levels which promote activation and contraction of myofibroblasts. This concept is supported by the observation that, in perfused liver experiments, the hepatic circulation in cirrhosis is hyper-responsive to AngII [108]. These findings have stimulated interest in the therapeutic use of RAS inhibitors in portal hypertension.

It is not yet known whether the alternate arm of the RAS is also involved in regulating hepatic vascular tone in cirrhosis. ACE2 and MasR expression are strongly up-regulated in cirrhotic liver [12]. We have demonstrated that, although Ang-(1–7) has no direct vasodilatory effect in the perfused cirrhotic rat liver, it ameliorates AngII-induced vasoconstriction [109]. This area remains under investigation.

ACEis and ARBs in the treatment of portal hypertension
Pharmaceutical treatment of portal hypertension is primarily aimed at preventing variceal bleeding by reducing splanchnic vasodilation and/or hepatic resistance to portal flow. The mainstay of established pharmacological treatment for portal hypertension is β-blockade (blockade of β-adrenoceptors). Non-selective β-blockers lower portal pressure by reducing cardiac contractility and increasing splanchnic vascular tone and are effective in both primary and secondary prophylaxis of variceal bleeding [110,111]. However, β-blockers are contraindicated or poorly tolerated by up to 20% of patients with cirrhosis, and almost 50% of patients do not achieve a therapeutically useful fall in the hepatic–portal venous gradient [111–113]. Although portal pressure is directly correlated with the presence of varices, lowering pressure with β-blockers does not prevent the development of varices in patients with cirrhosis [113].
Figure 4  Overview of the pathogenesis of portal hypertension in cirrhosis

The primary event in the pathogenesis of portal hypertension is likely to be sinusoidal vasoconstriction as a result of minor architectural changes and contraction of myofibroblasts (activated HSCs). Increased hepatic resistance triggers the up-regulation of eNOS in the splanchnic circulation, which leads to the release of NO. The resulting splanchnic vasodilation consequently exacerbates portal hypertension as a result of increased inflow to the portal circulation. There is also intrinsic non-endothelial vascular hypocontractility, the cause of which is unclear at the present time.

Given the considerable experimental evidence that AngII plays a key role in mediating increased hepatic resistance in the cirrhotic liver, a substantial number of studies have now been conducted investigating the effects of ARBs or ACEis in the treatment of portal hypertension. A recent meta-analysis of 19 trials concluded that data from the relatively small number of controlled studies suggests that ARBs and/or ACEis are effective in reducing portal pressure compared to placebo, but not as effective as β-blockade in all-comers with cirrhosis [114]. However, sub-analysis suggests that ARBs and/or ACEis are effective as β-blockers in patients with early, Childs A, cirrhosis, with a clinically significant mean reduction in portal pressure of 17% (Figure 5). This is at least partly attributable to the fact that more advanced disease is associated with activation of other vasoconstrictive pathways, such as the sympathetic nervous system, and increased endothelin and vasoconstrictive prostanooid production, which in turn contribute to increased intrahepatic vascular tone [53,115–117]. Moreover the increased hepatic vascular tone in these patients appears to be further aggravated by an insufficient release of NO [118]. Importantly, there is a significant incidence of adverse events, such as renal failure and hypotension, in patients with decompensated cirrhosis.

Figure 5  Effects of ARBs or ACEis compared with β-blockade in the treatment of portal hypertension

The reduction in hepatic vein pressure gradient (HVPG) due to ARBs or ACEis in patients with early cirrhosis (Child Pugh class A) is similar to the reduction achieved with β-blockade. Results are calculated from individual data from a meta-analysis of ARBs/ACEis compared with β-blocker treatment trials. The mean percentage change and the 95% confidence limits are represented. This Figure is reprinted from the Journal of Hepatology, 53(2), Puneeta Tandon, Juan G. Abraldes, Annalisa Berzigotti, Juan Carlos Garcia-Pagan and Jaime Bosch, Renin–angiotensin–aldosterone inhibitors in the reduction of portal pressure: a systematic review and meta-analysis, pp. 273–282, Copyright (2010), with permission from Elsevier (http://www.sciencedirect.com/science/journal/01688278).
cirrhosis who receive ARBs [119]. Thus, although ARBs and ACEis may be useful drugs for the treatment of portal hypertension in early stage cirrhosis, further well-conducted trials are needed, particularly for those patients who are intolerant or non-responsive to β-blockade. In addition, the role of these drugs in the prevention of varices should be investigated.

**Splanchnic vasodilation and the RAS**

As outlined above, splanchnic vasodilation is a key event in the formation of the hyperdynamic circulation and the development of portal hypertension (Figure 4). Enlargement of the vascular compartment due to dilation of the mesenteric circulation leads to a reduction in the EABV (effective arterial blood volume) [50]. Activation of the renin–angiotensin–aldosterone and sympathetic systems in an attempt to normalize the EABV causes salt and water retention, leading to expansion of the blood volume and an increase in cardiac output [120]. In advanced cirrhosis, the splanchnic circulation remains vasodilated despite high circulating levels of endogenous vasoconstrictors, and this results in ongoing activation of these systems as they are unable to fully normalize the EABV.

The mechanisms underlying splanchnic vasodilation in cirrhosis, however, remain incompletely understood. It is clear that NO is a key mediator [121–128]. Increased NO production occurs predominantly as a result of the up-regulation of splanchnic eNOS [endothelial NOS (NO synthase)], and this has been shown to occur prior to the development of splanchnic vasodilation [126]. Evidence from animal studies of portal hypertension suggests that, in early cirrhosis, a small increase in portal pressure as a result of increased hepatic vascular resistance may stimulate increased eNOS expression in the splanchnic microcirculation [129,130]. However, denudation of the vascular endothelium or inhibition of NO does not normalize vascular hypocontractility in cirrhosis [121,124,126,131–135]. In addition, mice with targeted gene deletion of eNOS still develop a hyperdynamic circulation in response to portal vein ligation [136]. These findings strongly suggest that, although NO is clearly an important regulator of vascular tone in cirrhosis, there is intrinsic dysfunction of contractile signalling in vascular smooth muscle.

**Role of the classical RAS in splanchnic vasodilation**

Circulating levels of AngII are elevated in cirrhosis, and the administration of ARBs to patients with decompensated cirrhosis may cause circulatory collapse and/or renal failure [119,120]. Thus AT1-receptor-mediated peripheral vasoconstriction appears to be a vital compensatory mechanism that helps maintain systemic BP and renal perfusion in advanced liver disease. However, an important and well-documented finding is that patients with portal hypertension have persistent splanchnic vasodilation, despite marked elevation of systemic AngII levels [137]. Splanchnic hyporeactivity to AngII in cirrhosis has been consistently documented both in animals and humans [135,138–141]. Although some of this is due to overproduction of NO, this is unlikely to be the only mechanism as, for example, in denuded human hepatic arteries from cirrhotic patients undergoing liver transplantation, maximal contraction to AngII was one-third that of control arteries [139].

The mechanism underlying this impaired response to AngII is not yet established. Although one study has documented reduced AT1 receptor number in portal hypertensive rabbits, a more comprehensive human study did not show any change in receptor expression or affinity in affected arteries [142,143]. One mechanism by which G-protein-coupled receptor function can be modified is by binding of receptor-desensitizing proteins, and it has been shown that β-arrestin-2 expression and binding to the AT1 receptor is increased in splanchnic vessels of cirrhotic patients, inhibiting the response to AngII [143].

**Role of the alternative RAS in splanchnic vasodilation**

Ang-(1–7) is generally viewed as a vasodilator, although its effects on vascular tone vary depending on the vascular bed under study [144,145]. Unlike AngII, which mediates vasoconstriction throughout the vascular tree, the renal, cerebral and mesenteric vascular beds appear to be more sensitive to the vasodilatory actions of Ang-(1–7), at least in the rat [40]. There are several studies which demonstrate a small vasodilatory response to Ang-(1–7) in healthy mesenteric vasculature [146,147], whereas others have demonstrated no significant effect [148]. These vasoactive properties of Ang-(1–7) are, in general, NO- and MasR-mediated, although several studies have found that the AT2 receptor may also be involved [46,48,49]. Ang-(1–7) also potentiates the vasodilatory response to bradykinin [38,149–151].

There are several lines of evidence to suggest that the alternative axis of the RAS could contribute to splanchnic vasodilation in cirrhosis. Not only do systemic levels of Ang-(1–7) correlate with the degree of portal hypertension, but regional levels may be markedly different from systemic levels, such that in cirrhotic patients at transplantation, the Ang-(1–7)/AngII ratio is elevated in the splanchnic compared with the peripheral circulation, and negatively correlates with systemic vascular resistance [152]. This ratio is dependent on ACE2 expression, and the vascular endothelium is thought to be a key site of ACE2 activity and the production of Ang-(1–7) [24,153]. It is therefore possible that, in cirrhosis, regional changes in ACE2 expression may promote a shift in the balance of angiotensin
peptide production from one favouring vasoconstriction to vasodilation. This is an area of ongoing research which is hoped will open up the investigation of drugs targeting the alternate axis of the RAS within the mesenteric circulation in cirrhosis.

**Hepatorenal syndrome**

In contrast with the vasodilation that affects the splanchnic vascular bed in portal hypertension, the renal circulation is paradoxically vasoconstricted, manifesting in advanced cirrhosis as the hepatorenal syndrome, a renal perfusion defect which leads to progressive renal failure in anatomically normal kidneys [154,155]. Hepatorenal syndrome completely reverses following liver transplantation, emphasizing the functional nature of the renal insult. It occurs in 40% of patients with cirrhosis associated with portal hypertension and ascites at 4 years of follow-up and heralds a poor prognosis in the absence of liver transplantation [156]. In type 1 hepatorenal syndrome, the most aggressive of the two clinical subtypes, median survival is less than 2 weeks [156].

The pathophysiology of hepatorenal syndrome is incompletely understood, but is directly related to splanchnic pooling resulting in a reduction in the effective blood volume. Cirrhotic patients who develop hepatorenal syndrome are more likely to have activation of homoeostatic vasoconstrictor systems, including the RAS, due to splanchnic vasodilation than similar cirrhotic patients who do not develop the syndrome [157]. These circulating vasoconstrictors result in preferential afferent renal arterial vasoconstriction, with consequent renal hypoperfusion, increased sodium resorption and a progressive fall in GFR (glomerular filtration rate) [158]. It is very likely that the classical RAS contributes towards renal vasoconstriction in cirrhosis, as AngII infusion stimulates renal vasoconstriction and there is an inverse correlation between renal hypoperfusion and activation of the classical RAS in cirrhotic patients [159,160].

Although the renal effects of AngII are well-documented, it is not yet clear whether Ang-(1–7) influences normal renal haemodynamics, and the role of Ang-(1–7) in modifying renal vascular responses in hepatorenal syndrome has not been studied. Ang-(1–7) has a vasodilator effect on pre-constricted rabbit afferent arterioles in vitro via MasR and the release of NO [39], and attenuates pressor response to AngII in rat renal vasculature [161]. However, the physiological effect of these observations are unclear as these effects cannot be replicated in vivo [162].

**CONCLUSIONS**

The RAS has multiple roles in the pathogenesis of liver fibrosis and portal hypertension, and this has stimulated major interest in the possible therapeutic use of ARBs or ACEIs in the treatment of chronic liver disease and its complications. There is very strong evidence that RAS blockers ameliorate liver fibrosis in several experimental models of chronic liver injury. Preliminary evidence from small clinical studies suggests possible benefits in human liver disease, and there are several large controlled studies underway in hepatitis C and NASH. There is also a wealth of information suggesting that AngII plays an important part in mediating intrahepatic vasoconstriction in cirrhosis. Although RAS blockers can precipitate renal failure and hypotension in patients with advanced cirrhosis, they may have a role in the treatment of portal hypertension in patients with less advanced disease, in whom they may prove a useful substitute when β-blockers are contraindicated or poorly tolerated, and further studies in this patient population are needed. Finally, the recent findings suggesting anti-inflammatory, anti-fibrotic and vasodilatory functions of the alternate axis of the RAS in liver disease opens up a new field of therapeutic possibilities for the development of drugs selectively targeting the key enzymes and receptors of this newly discovered arm of the RAS.

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