Liver disease and the renin–angiotensin system: Recent discoveries and clinical implications

John S Lubel, Chandana B Herath, Louise M Burrell and Peter W Angus

Department of Medicine, The University of Melbourne, Austin and Northern Health, Melbourne, Victoria, Australia

Abstract

The renin–angiotensin system (RAS) is a key regulator of vascular resistance, sodium and water homeostasis and the response to tissue injury. Historically, angiotensin II (Ang II) was thought to be the primary effector peptide of this system. Ang II is produced predominantly by the effect of angiotensin converting enzyme (ACE) on angiotensin I (Ang I). Ang II acts mainly through the angiotensin II type-1 receptor (AT1) and, together with ACE, these components represent the ‘classical’ axis of the RAS. Drug therapies targeting the RAS by inhibiting Ang II formation (ACE inhibitors) or binding to its receptor (angiotensin receptor blockers) are now in widespread clinical use and have been shown to reduce tissue injury and fibrosis in cardiac and renal disease independently of their effects on blood pressure. In 2000, two groups using different methodologies identified a homolog of ACE, called ACE2, which cleaves Ang II to form the biologically active heptapeptide, Ang-(1–7). Conceptually, ACE2, Ang-(1–7), and its putative receptor, the mas receptor represent an ‘alternative’ axis of the RAS capable of opposing the often deleterious actions of Ang II. Interestingly, ACE inhibitors and angiotensin receptor blockers increase Ang-(1–7) production and it has been proposed that some of the beneficial effects of these drugs are mediated through upregulation of Ang-(1–7) rather than inhibition of Ang II production or receptor binding. The present review focuses on the novel components and pathways of the RAS with particular reference to their potential contribution towards the pathophysiology of liver disease.

Key words

angiotensin (Ang)-(1–7), angiotensin converting enzyme 2, hepatic fibrosis, renin–angiotensin system.

Accepted for publication 11 February 2008.

Correspondence

Professor Peter W Angus, Department of Gastroenterology and Hepatology, Austin Health, Heidelberg, Vic. 3084, Australia. Email: peter.angus@austin.org.au

Renin–angiotensin system, past and present

‘Classical’ renin–angiotensin system

Most of us can recall the schema of the renin–angiotensin system (RAS) taught in physiology lectures (Fig. 1). The system is often depicted as a simple enzyme cascade starting with the degradation of angiotensinogen (derived from the liver) by circulating renin (secreted from the juxtaglomerular apparatus of the kidney) to form angiotensin I (Ang I). Subsequent enzymatic action by angiotensin converting enzyme (ACE) in the capillaries of the lung yields the predominant effector peptide of the system, angiotensin II (Ang II). Two receptors for Ang II have been cloned and characterized, the angiotensin II type-1 receptor (AT1) is the abundant receptor in adult life, whereas the angiotensin II type-2 receptor (AT2) is present in the fetus and persists in the central nervous system of adults. Binding of Ang II to the AT1 receptor mediates a number of diverse effects including vasoconstriction and sodium hemostasis. Ang II also participates in inflammation and wound healing through the release of critical cytokines and production of extracellular matrix. The effect of Ang II on vascular tone and systemic blood pressure has been extensively studied and is mediated through direct effects on vascular smooth muscle cells or indirectly by increasing vascular sympathetic tone. Sodium-conserving effects occur via reabsorption of sodium by the renal tubules as well as stimulating the adrenal gland to secrete aldosterone. The effect of Ang II to stimulate thirst is mediated through AT1 receptors in the brain.

Figure 1 illustrates the conventional view of the ‘classical’ RAS. This schema is useful as it clarifies how drugs like ACE inhibitors or AT1 receptor blockers (ARB) produce their beneficial therapeutic effects in cardiovascular and renal disease. However, there have been a number of major advances in our understanding of the RAS which have made it clear that the system is far more complex than this ‘classical’ view would suggest (Fig. 2).

Angiotensin family of peptides

One key point of understanding is that Ang II is just one member of a family of angiotensin peptides produced by the RAS. Ang II consists of eight amino acids, which, like other peptides, has a free amino group at one end (N-terminus) and a free carboxyl group (C-terminus) at the other. Ang II can also be denoted as Ang-(1–8),
Angiotensin converting enzyme 2

Interest in alternative components of the RAS was re-ignited in the year 2000 when two groups independently discovered an enzyme similar to ACE in human tissue.12,13 This homolog of ACE was initially called hACE but has subsequently been named angiotensin converting enzyme 2 (ACE2). Although structurally similar to ACE, ACE2 has different substrate affinities and resists inhibition by ACE inhibitors. ACE2 is a zinc-metalloproteinase and, like ACE, is a type-I transmembrane protein. It consists of 805 amino acids with a single transmembrane alpha-helical portion, an external N-terminus portion containing the catalytically active enzyme and an internal inactive C-terminus section. A transmembrane protease, ADAM 17 acts as a ‘sheddase’ releasing the active enzyme into the extracellular environment (Fig. 4).15 The released ACE2 (soluble ACE2) is a carboxypeptidase, capable of cleaving a single amino acid from the C-termini of its various substrates, including, Ang II, Ang I, des-Arg9-bradykinin, neurotensin 1–13 and kinetensin (see review by Burrell and colleagues).16 Importantly, ACE2 can generate Ang-(1–7) directly from Ang II or indirectly by cleaving Ang I into an inactive intermediate fragment, Ang-(1–9), which is then cleaved by ACE to produce Ang-(1–7) (Fig. 2). Of these two ACE2 pathways, the conversion of Ang II into Ang-(1–7) is kinetically favoured 400-fold compared to the conversion of Ang I to Ang-(1–9).17,18

In addition to its role in the ‘alternative’ RAS, the ACE2 transmembrane protein has, interestingly, been identified as a receptor site for spike proteins of the severe acute respiratory syndrome (SARS) coronavirus, thereby facilitating infection of target cells.19

Angiotensin 1–7

Much of the work on Ang-(1–7) has been carried out in animals and, to date, this peptide has been shown to have antihypertensive, anti-arrhythmic, and cardioprotective properties20–22 as well as anti-trophic properties in vascular endothelial cells, smooth muscle cells, cardiac myocytes and cardiac fibroblasts.23–26 In contrast to Ang II, Ang-(1–7) also has anti-inflammatory, anti-fibrotic27 and anti-thrombotic properties.28,29 As a result of these studies, Ang-(1–7) has been proposed to represent the effector peptide of a counterbalancing arm of the RAS, capable of opposing the deleterious actions of Ang II. The putative receptor for Ang-(1–7) is the G protein-coupled receptor encoded by the mas proto-oncogene,14 although other receptors may well exist.30 Thus, ACE2 together with Ang-(1–7) and the mas receptor represent an ‘alternative’ arm or axis of the RAS which may present a counter-
balancing system to the deleterious ACE/Ang II/AT1 axis (Fig. 5). Clearly, ACE2 holds a central role in the RAS influencing both axes, as it is capable of simultaneously degrading Ang II while simultaneously generating Ang-(1–7). ACE is important in generating Ang II, but is also responsible for the degradation of Ang-(1–7) into the inactive peptide fragment Ang-(1–5). The RAS interacts with the kinin system through ACE degradation of bradykinin. The two axes of the RAS and the kinin system are shaded grey. Enzymes are shown in yellow boxes and peptides in blue boxes. Aminopeptidase A (APA) and aminopeptidase N (APN) sequentially cleave Ang II to form angiotensin III and angiotensin IV, respectively. Neprilysin (NEP) is involved in both the RAS and the kinin system. Possible peptide–receptor interactions are shown by dashed lines.

Figure 2  Contemporary renin–angiotensin system (RAS). Angiotensin converting enzyme (ACE) 2 has a central role in the RAS influencing both the ‘classical’ and ‘alternative’ axes, as it degrades angiotensin II (Ang II) while simultaneously generating Ang-(1–7). ACE is important in generating Ang II, but is also responsible for the degradation of Ang-(1–7) into the inactive peptide fragment Ang-(1–5). The RAS interacts with the kinin system through ACE degradation of bradykinin. The two axes of the RAS and the kinin system are shaded grey. Enzymes are shown in yellow boxes and peptides in blue boxes. Aminopeptidase A (APA) and aminopeptidase N (APN) sequentially cleave Ang II to form angiotensin III and angiotensin IV, respectively. Neprilysin (NEP) is involved in both the RAS and the kinin system. Possible peptide–receptor interactions are shown by dashed lines.

Figure 3  Peptide structure and fragments of angiotensin I. Angiotensin I is a decapetide (Ang-(1–10)) which can be fragmented by various enzymes into four peptides with biological activity; angiotensin II (Ang-(1–8)), angiotensin III (Ang-(2–8)), angiotensin IV (Ang-(3–8)) and angiotensin 1–7 (Ang-(1–7)). Further enzymatic degradation of Ang 1–7 yields the inactive fragment angiotensin 1–5 (Ang-(1–5)). Aminopeptidases are shown in blue and cleave amino acids from the N-terminus, whereas carboxypeptidases are shown in red and cleave amino acids from the C-terminus. Amino acids are given numerical values, where 1, aspartic acid; 2, arginine; 3, valine; 4, tyrosine; 5, isoleucine; 6, histidine; 7, proline; 8, phenylalanine; 9, histidine; 10, leucine.

Interactions between the RAS and the kallikrein–kinin system

Angiotensin converting enzyme is known to participate actively in the kallikrein–kinin system by degrading bradykinin (Fig. 2). Inhibitors of ACE can therefore lead to the accumulation of bradykinin, which may contribute to the antihypertensive properties of these drugs, as well as to some of the observed side-effects, such as chronic cough and angioedema. In the liver, bradykinin binds to the B2 receptor and causes increases in hepatic resistance and elevation of portal pressure. In other vascular beds, bradykinin induces vasodilatation on binding to the B2 receptor, and Ang-(1–7) has been shown to induce bradykinin-mediated relaxation in porcine coronary arteries. A possible explanation for this is that Ang-(1–7) has ACE inhibitory properties that prevent ACE-mediated degradation of bradykinin.

Concept of local renin–angiotensin systems

In recent years, scientists have departed from the traditionally held view of the RAS being exclusively a circulating endocrine system and have realized that many organs, such as the heart, kidney, liver and pancreas, constitutionally express all the ‘classical’ RAS components required for a functioning, autonomous intra-organ
RAS. These locally generated angiotensin peptide fragments have been demonstrated to have a multitude of actions, being implicated in cell growth, cell proliferation, apoptosis, reactive oxygen species generation, inflammation, and fibrogenesis. Although conceptually separate, the local intra-organ RAS and the systemic RAS must interact and the final peptide products will depend on the interplay between the two.

Renin–angiotensin system in liver disease

Despite the discovery of Ang-(1–7) and the recognition that many of its actions oppose Ang II, the importance of this heptapeptide fragment of Ang II remained elusive until recently. It is now clear that in the diseased liver, not only are the ‘classical’ RAS components such as renin, ACE, Ang II and the AT1 receptor overexpressed, but, importantly, components of the ‘alternative’ RAS, such as ACE2, Ang-(1–7) and the mas receptor are also upregulated. The implication from these studies is that the ‘classical’ components contribute to the fibrotic process whereas the ‘alternative’ components may be upregulated in an attempt to restore the status quo.

In liver disease, architectural changes to the microscopic structure of the liver occur as a result of inflammation and fibrosis. These changes lead to capillarization of the hepatic sinusoids, increased extracellular matrix (ECM) formation and elevated hepatic resistance; the latter impedes liver blood flow and leads to portal hypertension. Stretching of the portal vein (as with increased hepatic resistance to blood flow) and oxidative stress together cause release of vasodilators, including nitric oxide, which induce a number of compensatory mechanisms important for restoring the functional blood volume. These mechanisms are effected via sodium and water preservation and stimulation of the sympathetic nervous system, which together contribute to the development of ascites, edema, hepatorenal syndrome, and a hyperdynamic circulation, all of which are typically seen in patients with advanced liver disease. The RAS is involved with all these processes. As the result, manipulation of the RAS with either antagonists of the ‘classical’ pathway, or agonists of the ‘alternative’ pathway could have potential therapeutic benefits. Balanced against the possible benefits are the potential side effects of such therapy, as the compensatory mechanisms activated by the systemic RAS are necessary to maintain an adequate circulation.
Renin–angiotensin system and hepatic fibrosis

Hepatic stellate cells (HSC) are thought to play a pivotal role in fibrogenesis within the liver, and there is a large body of evidence to support the hypothesis that Ang II promotes activation, and dedifferentiation of these cells into myofibroblasts. Furthermore, Ang II encourages myofibroblast contraction, proliferation and release of inflammatory cytokines as well as the deposition of extracellular matrix (ECM). Although both of the Ang II receptors (AT₁ and AT₂) are expressed in the liver, the AT₁ receptor is far in abundance and is thought to be responsible for most of the Ang II-mediated effects. Studies using gene-deletion mice have demonstrated that AT₁ receptor-deficient mice are protected from hepatic fibrosis whereas AT₂ receptor-deficient mice have worse fibrosis.

A great deal of evidence supporting the role of the RAS in hepatic fibrosis has come from animal studies using ACE inhibitors and angiotensin receptor blockers (ARB). Numerous studies using a variety of animal models have demonstrated antifibrotic effects of these drugs. However, there appear to be some conflicting observations reported in the literature. For example, losartan treatment failed to influence either liver injury or progression of fibrosis in an animal model of non-alcoholic steatohepatitis (NASH). [Editor’s note: A detailed review of animal models of NASH has been written by Larter and Yeh for a later article in this Basic Science Miniseries.] In contrast, a study with a similar model of NASH but using the ARB olmesartan, demonstrated a 70% reduction in fibrosis in the ARB-treated group.

The avid interest in RAS-blocking drugs is, in part, related to their relative safety in humans and widespread use in cardiovascular and renal medicine. Despite the large number of animal studies, there is a relative paucity of human data to support the use of these drugs in human liver disease. In part, this could be due to the need to perform multiple liver biopsies to histologically confirm resolution of fibrosis, which, outside the setting of post-transplantation recurrent hepatitis C, is rarely indicated in 2008. In addition, the slow progression of fibrosis in most diseases such as hepatitis C and non-alcoholic fatty liver disease (NAFLD) make it difficult to detect possible beneficial effects of antifibrotic therapy, unless studies are conducted over a number of years.

A pilot study examining the effects of 6 months of losartan treatment on liver fibrosis in chronic hepatitis C demonstrated a significant decrease in fibrosis stage in the treated group compared to control patients. In support of this, a study using candesartan for 48 weeks in 24 compensated Child A and B cirrhotic patients demonstrated a significant reduction of plasma hyaluronic acid levels, a surrogate marker for fibrogenesis. However, in this study, two of three serum markers of fibrosis used showed no improvement, and there were no histological data provided; this makes it difficult to evaluate any effects on architectural changes.

A number of other studies have reported possible antifibrotic effects of RAS blockers in patients with hepatitis C. In one study, 30 hepatitis C virus (HCV)-infected patients with mild fibrosis were treated with losartan 50 mg/day and ursodeoxycholic acid 600 mg/day whereas controls received ursodeoxycholic acid alone. There were significant reductions in serum markers of hepatic fibrosis such as transforming growth factor β1 (TGF-β1) and type IV collagen in the losartan and ursodeoxycholic acid group, but no significant changes in fibrosis score between the groups. Another report described outcomes in patients with hepatitis C treated with low-dose interferon (IFN alpha 3 × 10⁶ IU 3 times a week for 12 months) in combination with the ACE inhibitor, perindopril (4 mg/day). Treatment was accompanied by significant improvement in serum markers of fibrosis (hyaluronic acid, type IV collagen 7S and procollagen III-N-peptide), but histological analysis was not carried out. Although this study did not have a perindopril monotherapy group, a subsequent study by the same group demonstrated that perindopril alone decreased serum fibrosis markers in patients with chronic hepatitis C. The addition of interferon significantly augmented the effect of perindopril monotherapy. Finally, a retrospective review compared liver histology in liver transplant patients with recurrent hepatitis C who were taking RAS-blocking drugs (n = 27) with those who were not (n = 101). The group taking RAS blockers were less likely to develop severe hepatic fibrosis (bridging fibrosis or cirrhosis) at 1 and 10 years after transplantation than were the control group (15% vs 35% at 1 year [P < 0.05], and 35% vs 70% at 10 years, respectively).

Only small studies have looked at RAS blockers and NASH. One such study (n = 7) found that giving losartan (50 mg/day for 48 weeks) in hypertensive patients with NASH reduced serum TGF-β1, serum ferritin and aminotransferase levels. Five patients showed improvement in the grade of hepatic necroinflammation. The study design could have been improved had the investigators examined pre- and post-treatment histology and biochemical markers in a placebo group. In a subsequent study, the pre- and post-treatment biopsies of seven patients with NASH treated with losartan (50 mg/day for 48 weeks) were compared with eight patients with NAFLD who acted as a control group. The treatment group showed a significant improvement in necroinflammatory grade, stage of fibrosis, significantly fewer activated HSC and a mild increase in quiescent HSC at the end of 48 weeks. However, the lack of a proper randomized control group is a particular problem in studies of patients with NASH, as the disease can improve in response to changes in lifestyle.

Renin–angiotensin system and portal hypertension

Fixed changes in hepatic architecture account for approximately 70% of the total resistance to portal blood flow in the cirrhotic liver. The remaining 30% results from a reversible or ‘dynamic’ resistance caused by the contraction of activated myofibroblasts positioned around the sinusoidal endothelial cells within the space of Disse. As portal resistance increases, a number of factors, including distension of the portal venous system, endotoxemia and oxidative stress result in the release of mediators, including nitric oxide, which dilate the mesenteric and systemic vasculature. Activation of compensatory mechanisms designed to restore functional blood volume results in sodium and water retention, stimulation of the sympathetic nervous system and the development of a hyperdynamic circulation. This cascade of events contributes to many of the key features and complications of advanced liver disease including development of ascites, edema and the hepatorenal syndrome. The RAS is involved with all these processes. Manipulation of the RAS with either antagonists of the ‘classical’ pathway, or agonists of the ‘alternative’ pathway therefore has potential for therapeutic benefit.
Variceal bleeding is one of the most important causes of morbidity and mortality in patients with portal hypertension. A number of pharmacological approaches have been developed for the prevention and treatment of this problem. Non-selective β-adrenergic antagonists (β-blockers) lower portal pressure by decreasing cardiac output and constricting the mesenteric vascular bed but have no direct effect on intrahepatic resistance to portal flow. These drugs have become the mainstay of treatment for the prevention of variceal bleeding. However, only 36% of patients achieve the target reduction in portal pressure of 20%, as measured by hepatic venous pressure gradient (HVPG), and they are poorly tolerated in patients with severe liver disease.64 As a result, there is a major interest in the development of other pharmacological therapies which can lower portal pressure. Interestingly, β-blockers interact with the RAS by inhibiting renin release, but have not been shown to impact on the development or progression of hepatic fibrosis. In contrast, the use of either ACE inhibitors or ARB to reduce portal pressure is an attractive proposition, as these drugs have the additional potential benefit of slowing the progression of hepatic fibrosis.

Ang II is a potent vasoconstrictor, and myofibroblasts derived from HSC express the AT1 receptor and contract in response to Ang II.55,65 Additionally, cirrhotic rat livers are hyperresponsive to Ang II with an increased portal pressure compared to those from healthy rats as a result of increased expression of AT1 receptors.66 This finding is of interest given that the relative importance of Ang II as a mediator of increased portal resistance has been questioned,67 based on a study of hepatic hemodynamics in isolated perfused cirrhotic rat livers which suggested that Ang II-mediated vasoconstriction is attenuated in the cirrhotic liver.68

Following some persuasive animal studies,69,70 the effects of AT1 blockade on portal hypertension have been examined in a number of human studies.70 Despite some encouraging initial studies showing a significant reduction of portal pressure by ARB, subsequent well-designed studies have failed to confirm these findings. Schneider and colleagues reported a dramatic reduction in HVPG with losartan in both moderate and severe portal hypertensive patients, but with only a 3 mmHg drop in mean arterial pressure (MAP).71 These findings were markedly different to a subsequent randomized controlled trial comparing the hemodynamic effects of losartan with propranolol72 given for 6 weeks following an index variceal bleed. Losartan failed to reduce HVPG, yet resulted in a significant reduction of MAP by 8%. Treatment tolerance was equivalent. The hemodynamic effect of losartan was further corroborated by a recent small study of 12 pre-ascitic patients which also found that losartan had no affect on HVPG, but did cause a drop in MAP of 7.8%.73

Irbesartan, another ARB, produced only modest reduction in portal pressures (12% ± 6.6%, P < 0.05) in a randomized, placebo-controlled, double-blind study. Importantly, however, this was associated with significant arterial hypotension and significant renal impairment in 22% of patients. In this study, plasma renin activity before treatment was a predictor of patients that would not tolerate treatment.74 The explanation for this adverse effect is that the RAS is known to play a central role in the homeostatic response to vasodilatation in patients with portal hypertension. The RAS, together with other compensatory systems, the posterior pituitary (through vasopressin secretion) and the sympathetic nervous system, endeavors to restore circulatory volume and organ perfusion by inducing vasoconstriction and sodium and water retention. In patients with advanced cirrhosis, plasma renin, Ang II, ACE and aldosterone levels are all increased75 and, within the kidney, Ang II is critical for maintenance of renal perfusion pressure and an adequate glomerular filtration rate (GFR). As liver disease progresses, the decrease in effective circulatory volume results in vasoconstriction of the glomerular afferent circulation, renal hypoperfusion and a fall in GFR. In response to renal hypoperfusion, Ang II selectively constricts the efferent glomerular arterioles; this restores glomerular perfusion pressure and GFR. The maintenance of adequate renal perfusion is therefore ACE dependent. Furthermore, ACE inhibition results in a rapid fall of GFR.76,77 This adverse effect of RAS inhibition on renal function in patients with advanced cirrhosis represents a major disadvantage for the use of this class of drug for the treatment of portal hypertension.

A recent study by Debernardi-Venon and colleagues examined the effects of candesartan treatment for 48 weeks on 24 compensated Child A and B cirrhotic patients. Treatment was well tolerated, with a mild but significant reduction in HVPG in more than 70% of those treated. Furthermore, 25% of patients treated achieved a 20% reduction in their HVPG. Interestingly, the changes in HVPG correlated well with those observed for plasma hyaluronic acid. However, the treatment group was preselected in that patients were excluded from analysis if they had large varices, evidence of significant arterial hypotension or renal impairment.78 Angiotensin receptor blockers have also been studied in portal hypertensive gastropathy; at least one study has reported a positive benefit from their use.78

The effects of ACE inhibitors on portal pressure have also been examined in a few small studies, but the results generally have been disappointing, with poor agreement between studies.79,82 A number of explanations have been proposed to explain the lack of uniformity in results from clinical studies investigating the benefits and adverse effects of RAS inhibitors. There are known genetic polymorphisms for the AT1 receptor gene and genes responsible for cleaving angiotensin I, including ACE,83 these may confer patient-to-patient variations in response to these drugs. This has led to the suggestion that genetic testing may help determine which patients are likely to have a positive response to therapy.84 In addition, chronic ACE inhibition may not lead to sustained Ang II suppression because of increased renin activity and upregulation of alternative enzymes, such as hepatic chymase, which is capable of generating Ang II from Ang I.85,86 Furthermore, chronic use of ARB also results in hyper-reninemia and elevated Ang II levels; the latter increasingly compete with the AT1 receptor antagonist for binding sites on the AT1 receptor molecule.87 Finally, it has also been claimed that there is tissue-dependent responsiveness to ACE inhibitors and ARB and, at current therapeutic dosing, both classes of drug may not completely inhibit their respective targets.88 To date, no studies have examined the effects on portal pressure of combined therapy with an ACE inhibitor and ARB; theoretically, this may overcome some of the possible issues of Ang II reactivation with use of ACE inhibitors alone.

In summary, the use of RAS inhibitors (other than β-blockers) to reduce portal pressure has been disappointing. At the doses used in clinical trials, these drugs appear to have only minor effects on portal pressure but very significant side-effects, includ-
ing systemic hypotension and renal impairment. These complications are a useful reminder of the homeostatic role the RAS plays in maintaining MAP and GFR in the vasodilated patient with severe liver disease.89–91 Based on the current available evidence, the use of either ACE inhibitors or ARB for reducing portal pressure remains controversial and cannot be recommended outside clinical trials.

Role of the ‘alternative’ RAS in liver disease

As outlined above, there is increasing evidence that both the ‘classical’ and the ‘alternative’ RAS are upregulated in chronic liver disease.38,66 It has recently been suggested that the progression of liver fibrosis may be influenced by a balance between ACE and ACE2 activation.62 In both an animal model of secondary biliary fibrosis and in humans with hepatitis C, ACE2 gene and activity are upregulated.38,66 As fibrosis worsens, the progressive rise in ACE and AT1 gene expressions coincide with an increase in ACE2 and mas expression, together with increased plasma levels of both Ang-(1–7) and Ang II.66,93 Cirrhotic livers have a greater capacity than healthy livers to convert Ang II to Ang-(1–7) because of upregulated ACE2 gene and protein expression (Fig. 6). In addition, the hepatic production of Ang-(1–7) from Ang II is augmented by ACE inhibition.38,66 This increased Ang-(1–7) production in the presence of an ACE inhibitor can be explained by the fact that Ang-(1–7) is cleaved by ACE to produce the inactive peptide Ang-(1–5) (Fig. 2). Inhibition of ACE therefore increases Ang-(1–7) half-life, leading to an increase in net production and accumulation of Ang-(1–7).94–96

Evidence for a beneficial role of Ang-(1–7) in hepatic fibrosis has been provided by a study examining the effects of the mas receptor antagonist [7-D-Ala]-Ang-(1–7) (A779). Treatment with A779 worsened experimental liver injury with increases in TGF-β1 and hydroxyproline levels; this infers that mas receptor stimulation plays a protective role in liver fibrosis.93 Further compelling evidence for a beneficial role of Ang-(1–7) has come from a recent rat study presented at AASLD 2007 by our group.97 We demonstrated that Ang-(1–7) infusion in bile duct-ligated rats attenuated fibrosis as quantified using META VIR fibrosis score, hydroxyproline content, and type I collagen mRNA expression. 

![Figure 6](image_url)
Alpha-smooth muscle actin (α-SMA) gene and protein expression were also reduced, indicating that hepatic stellate cell activation was inhibited by Ang-(1–7). Interestingly, Ang-(1–7) infusion also inhibited ACE gene and protein expression, and resulted in down-regulation of mas receptor gene expression. The Ang-(1–7) infusion group also showed decreased mRNA expression levels for connective tissue growth factor (CTGF, also known as CCN2) and vascular endothelial growth factor (VEGF), two critical growth factors implicated in fibrosis and tissue repair. This is the first direct evidence showing that Ang-(1–7) can ameliorate hepatic fibrosis.

Despite a number of reports that Ang-(1–7) is a vasodilator, experiments on rat isolated perfused livers have failed to demonstrate any vasodilatory effect in normal or cirrhotic livers. Likewise, experiments in isolated vessels from normal and cirrhotic rats also failed to show any direct vasodilatory effect of this peptide. Conversely, Ang-(1–7) has been shown to enhance acetylcholine-mediated vasodilation in aortic rings from cirrhotic rats. The vasodilatory effects of Ang-(1–7) are thought to be mediated through increased production of nitric oxide (NO).

Figure 7 Effects of angiotensin converting enzyme (ACE) inhibitors and angiotensin type-1 receptor (AT1) receptor blockers (ARB) on the two axes of the renin–angiotensin system (RAS). The RAS is shown as a balance with ‘classical’ and ‘alternative’ axes counterbalancing each other. ACE inhibitors (ACEi) cause an initial reduction in angiotensin II (Ang II), but after chronic administration increases in plasma renin activity and plasma angiotensin I (Ang I) levels occur. Both Ang II and aldosterone levels can subsequently rise as a consequence of non-ACE-dependent pathways facilitated by enzymes such as chymase. Both ACEi and ARB result in elevated levels of ACE2 and Ang-(1–7) which possibly contribute to the effects of these drugs. Manipulations of the RAS aimed at tipping the balance in favour of ‘alternative’ components represents a potential target for antifibrotic therapies.
although non-ACE-dependent pathways involving enzymes like chymase, which is capable of generating Ang II, may play an important part.85,109 Interestingly, ACE inhibitors and ARB have a profound impact on the ‘alternative’ system by causing significant increases in Ang-(1–7).94,95,110,111 It has been postulated that some of the beneficial effects observed with ARB and ACE inhibitors are mediated through Ang-(1–7).28,29,112–116 In support of this, ACE2 activity and gene expression are both increased in the heart by ARB or ACE inhibitors.112,117 The elevated ACE2 activity in ACE2 activity and gene expression are both increased in the heart.

Conclusions and future directions

Our understanding of the RAS has considerably expanded since the discovery of ACE2. Emerging evidence supports the hypothesis that the RAS consists of two opposing axes. Manipulation of the RAS, by either blocking the ‘classical’ RAS or by stimulating the ‘alternative’ RAS represents a potential target for antifibrotic and portal hypertension therapy. Limitations to treatment may be the side-effects of such drugs, particularly their impact on arterial blood pressure and renal function. Current therapies such as ACE inhibitors and ARB used in cardiovascular and renal fibrosis have been shown to impact on both the ‘classical’ and ‘alternative’ pathways. The elevated Ang-(1–7) plasma levels caused by these drugs may represent a mechanism by which these drugs exert some of their effects. New drugs which mimic the effects of Ang-(1–7) have been developed. This orally active Ang-(1–7) receptor agonist represents an entirely new class of drug118 spawned from the latest insights into the complexities of the contemporary RAS and has possible novel therapeutic applications in liver disease.

Acknowledgments

Dr John Lubel is a recipient of an Australia National Health and Medical Research Council (NHMRC) scholarship, and Peter Angus and Louise Burrell hold an NHMRC project grant (509315).

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