

REVIEW

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

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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.

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).^{1–3} 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.^{4–6} 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.⁷ 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.⁸

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),

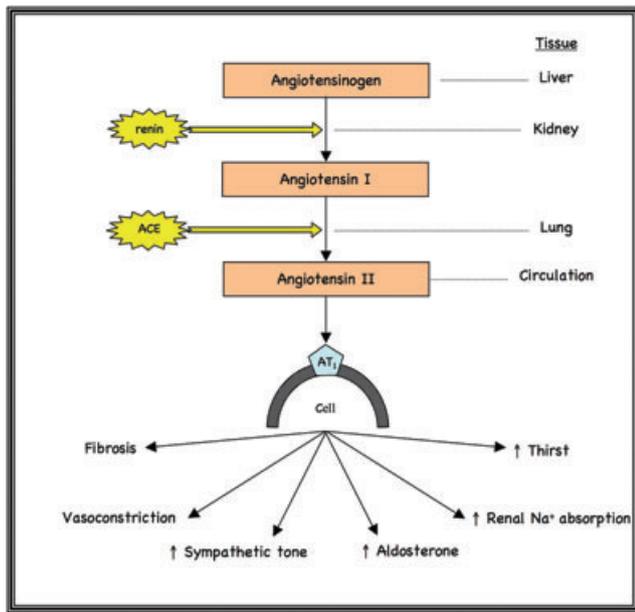


Figure 1 'Classical' renin-angiotensin system (RAS). The RAS is depicted here as a linear cascade leading to the generation of angiotensin II (Ang II) through the enzymatic action of renin on angiotensinogen and angiotensin converting enzyme (ACE) on angiotensin I (Ang I). There are two known receptors for angiotensin II, angiotensin II type-1 receptor (AT₁) and angiotensin II type-2 receptor (AT₂). The AT₁ receptor is thought to play a more important role than the AT₂ receptor in human disease.

where the first amino acid is at the N-terminus and the eighth amino acid is at the C-terminus. Therefore, cleavage of amino acids from either end of the Ang II molecule can generate smaller peptide fragments (Fig. 3). For example, removal of the N-terminus amino acid results in the generation of a peptide consisting of seven amino acids starting from the second amino acid of Ang II, and is denoted by Ang-(2-8) (historically also known as Ang III). Of the fragments that can be generated from Ang II, only three are known to be physiologically relevant.⁹ Two are derived from N-terminus cleavage, Ang-(2-8) (Ang III) and Ang-(3-8) (Ang IV) and one formed by cleavage of a single amino acid from the C-terminus, angiotensin-(1-7) (Ang-(1-7)). Ang III is formed following cleavage of the aspartate-arginine bond of Ang II by aminopeptidase A, and Ang IV can be formed by further cleavage of Ang III by aminopeptidase B or N. Ang III shares many of the properties of Ang II with 40% of the pressor activity and 100% of the aldosterone stimulating activity. Ang IV has its own distinct receptor (AT₄) and has central nervous system effects together with some opposing actions to Ang II.¹⁰ Ang-(1-7) is generated from cleavage of either Ang II or Ang I and has been the focus of much research since the discovery that it has biological functions that oppose those of Ang II.

ACE2 and the 'alternative' renin-angiotensin system

For decades, the 'classical' arm of the RAS was recognized as being the only system of biological relevance. However, this interpretation was challenged in the late 1980s with the discovery of

Ang-(1-7) and description of its diverse biological functions.¹¹ This was followed by studies which clearly demonstrated new components of the RAS, such as ACE2^{12,13} and the Ang-(1-7) receptor, *mas*.¹⁴ These new components, together with the effector molecule Ang-(1-7), form the axis which we now recognize as the 'alternative' arm of the RAS. The discovery of these new RAS components provided some missing connections to the hitherto complex biochemical pathways of the RAS. The new components of the alternative arm of the RAS are reviewed below with a major emphasis on their potential contribution towards the pathophysiology of liver disease.

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-1 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 proteinase, ADAM 17 acts as a 'shedase' 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-

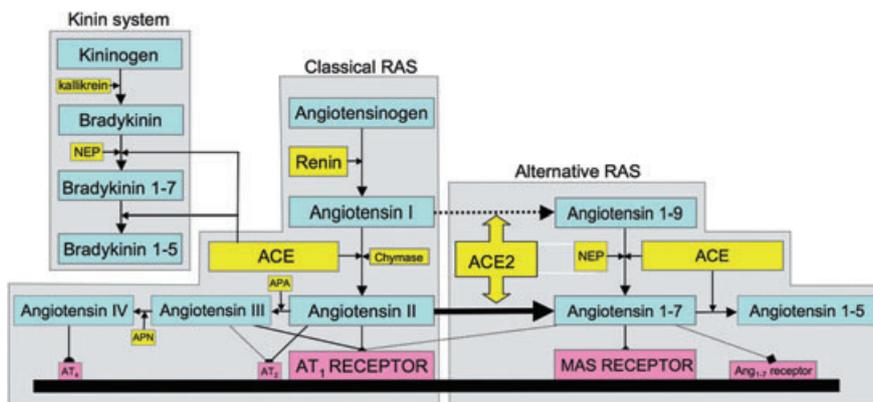


Figure 2 Contemporary renin–angiotensin system (RAS). Angiotensin converting enzyme (ACE) 2 has a central role in the RAS influencing both the ‘classical’ and ‘alternative’ axes, as it degrades angiotensin II (Ang II) while simultaneously generating Ang(1–7). ACE is important in generating Ang II, but 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.

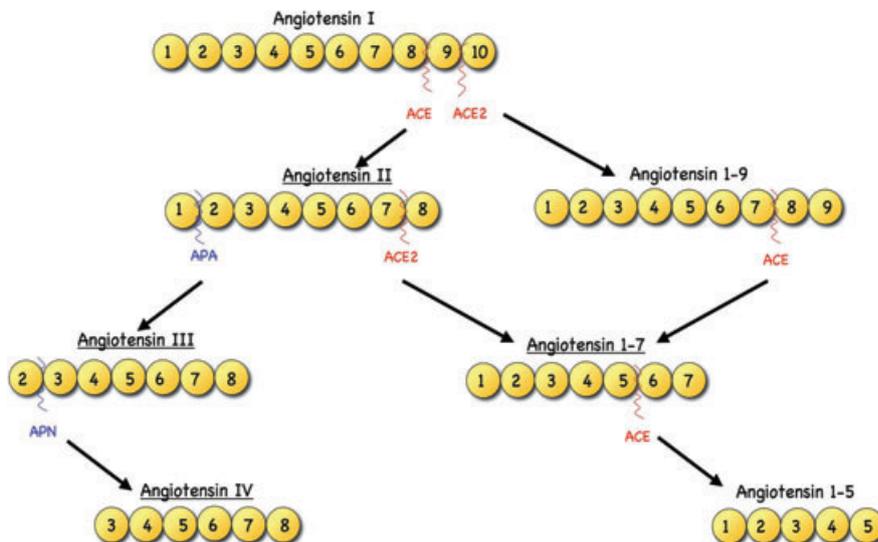


Figure 3 Peptide structure and fragments of angiotensin I. Angiotensin I is a decapeptide (Ang(1–10)) which can be fragmented by various enzymes into four peptides with biological activity; angiotensin II (Ang(1–8)), angiotensin III (Ang(2–8)), angiotensin IV (Ang(3–8)) and angiotensin 1–7 (Ang(1–7)). 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.

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 and generating Ang(1–7) (Fig. 2).

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).³¹ 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.³² 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.³³ A possible explanation for this is that Ang(1–7) has ACE inhibitory properties that prevent ACE-mediated degradation of bradykinin.³⁴

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

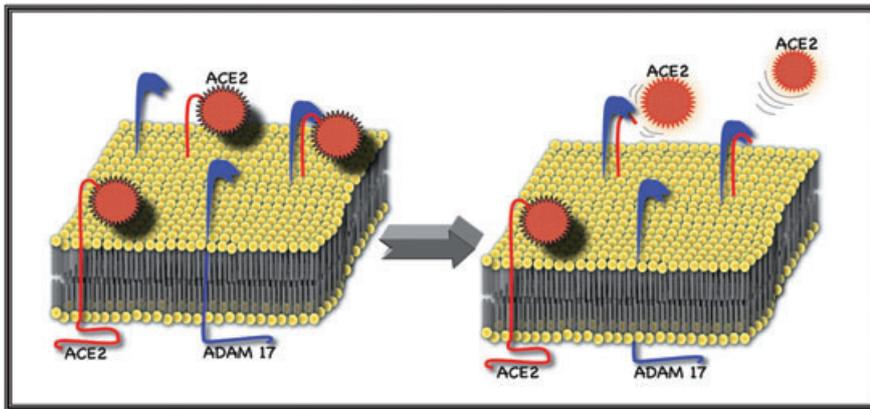


Figure 4 Release of angiotensin converting enzyme (ACE) 2 from the cell membrane by ADAM 17. ACE2 (shown in red) is a transmembrane protein which undergoes shedding by the proteinase ADAM 17 (shown in blue) to release free, circulating ACE2. Modified from Lambert *et al.*¹¹⁹

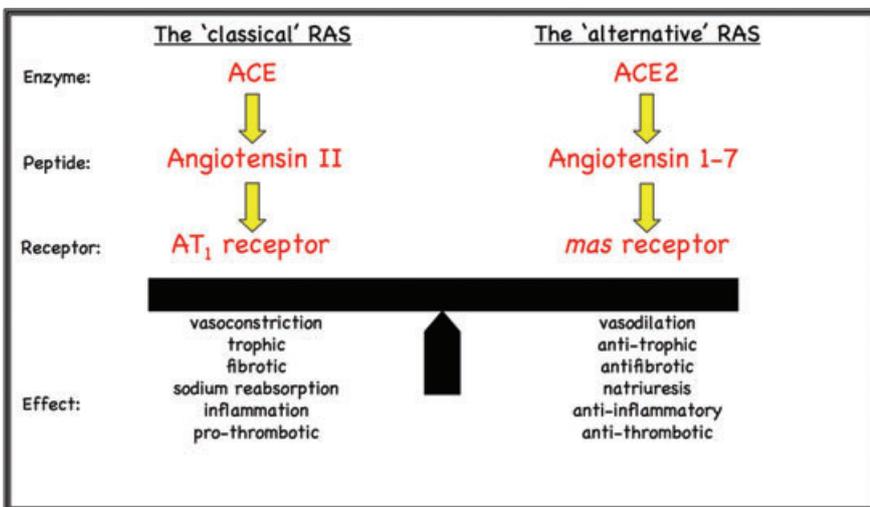


Figure 5 Counterbalancing effects of the two axes of the renin-angiotensin system (RAS). The RAS can be thought of as two counterbalancing axes. The angiotensin converting enzyme (ACE)/angiotensin II/AT₁ receptor axis causes vasoconstriction, salt retention, inflammation, fibrosis and thrombosis, whereas the ACE2/angiotensin 1-7/mas receptor axis has opposing effects.

RAS.^{35,36} 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.^{37,38} 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.³⁹ 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,⁴⁰ 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 promotes 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 AT_{1A} receptor-deficient mice⁴¹ are protected from hepatic fibrosis whereas AT₂ receptor-deficient mice have worse fibrosis.⁴²

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.^{43–53} 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).⁵⁴ [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.⁵⁵

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.⁵⁶ 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.⁵⁷

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 β 1 (TGF- β 1) and type IV collagen in the losartan and ursodeoxycholic acid group, but no significant changes in fibrosis score between the

groups.⁵⁸ Another report described outcomes in patients with hepatitis C treated with low-dose interferon (IFN alpha 3×10^6 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.⁵⁹ 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).⁶⁰

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- β 1, serum ferritin and aminotransferase levels. Five patients showed improvement in the grade of hepatic necroinflammation.⁶¹ 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.⁶² 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.⁶³ 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 (beta-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, beta-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.^{35,65} 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,^{53,69} 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 effect on HVPG, but did cause a drop in MAP of 7.8%.⁷³

Irbesartan, another ARB, produced only modest reduction in portal pressures ($12\% \pm 6.6\%$, $P < 0.05$) 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.⁵⁷ 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.^{87,88} 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 beta-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-

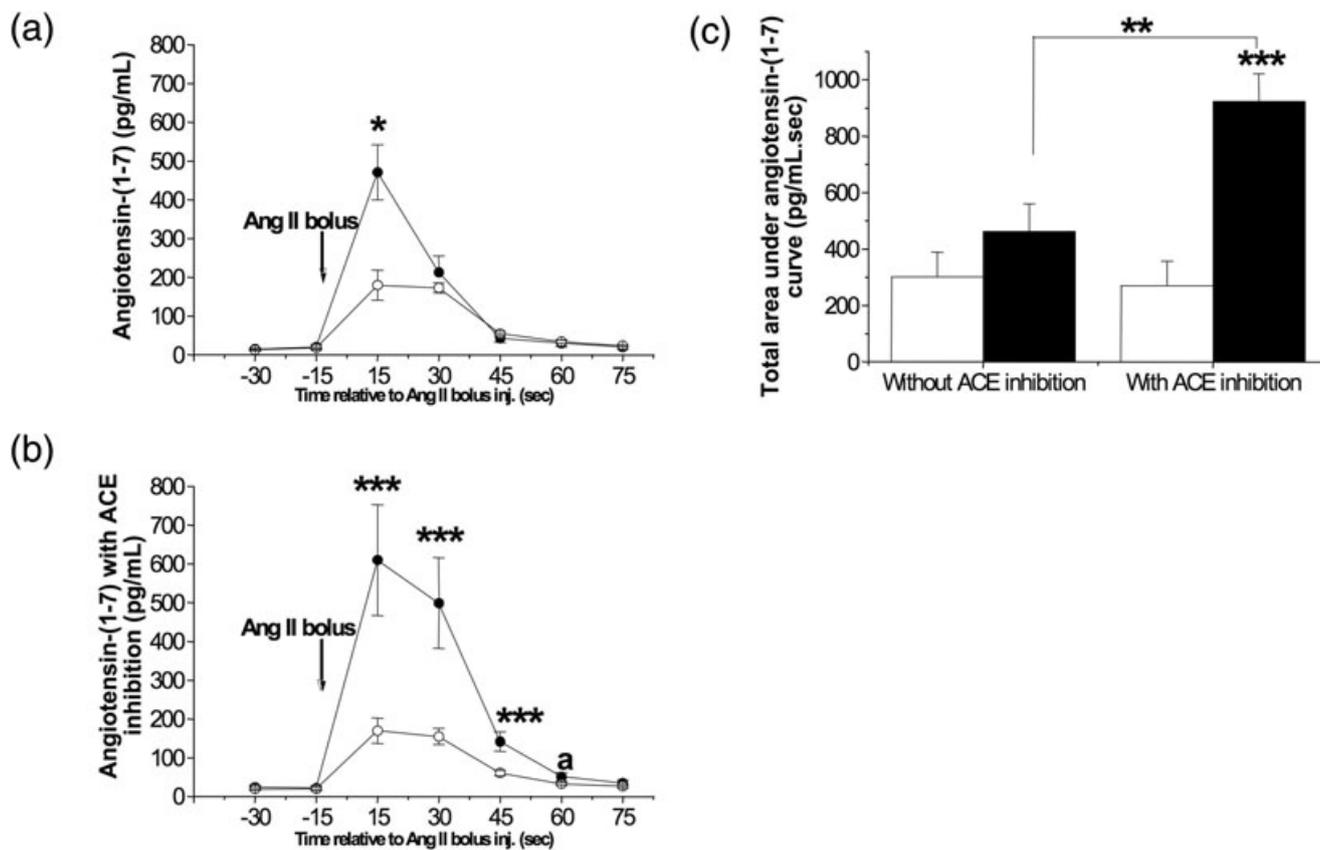


Figure 6 Angiotensin 1-7 synthesis from angiotensin II (Ang II) in perfused rat liver. Ang II injected into fibrotic rat livers (●) generates more Ang(1-7) than healthy livers (○). The production of Ang(1-7) is amplified when the liver is pre-incubated with the ACE inhibitor lisinopril (b). Baseline corrected total area under the Ang(1-7) curves are shown in panel (c). Each circle/bar represents mean ± SEM from 4-5 rats per treatment group. ****P* < 0.001, ***P* < 0.01, **P* < 0.05 baseline-corrected fibrotic vs healthy livers. ^a*P* = 0.05. Reproduced from Herath *et al* with publisher's permission.³⁷

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.⁹² 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 METAVIR fibrosis score, hydroxyproline content, and type 1 collagen mRNA expression.

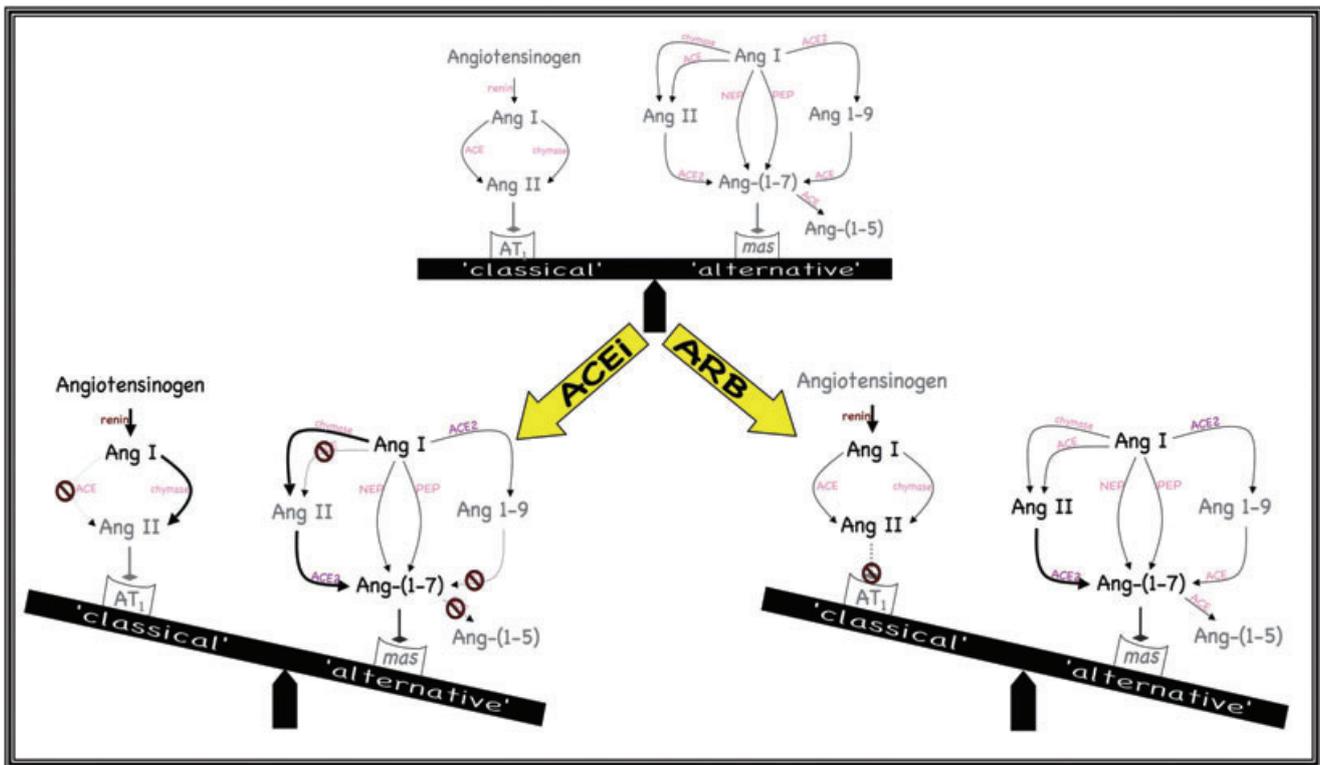


Figure 7 Effects of angiotensin converting enzyme (ACE) inhibitors and angiotensin type-1 receptor (AT₁) receptor blockers (ARB) on the two axes of the renin-angiotensin system (RAS). The RAS is shown as a balance with 'classical' and 'alternative' axes counterbalancing each other. ACE inhibitors (ACEi) 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.

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.⁹⁷ Evidence from studies in ACE2 deletion mice further supports a central role of ACE2 in regulating fibrosis in liver disease.⁹⁸

Despite a number of reports that Ang-(1-7) is a vasodilator,⁹⁹ experiments on rat isolated perfused livers have failed to demonstrate any vasodilatory effect in normal or cirrhotic livers.^{66,100,101} Likewise, experiments in isolated vessels from normal and cirrhotic rats also failed to show any direct vasodilatory effect of this peptide.³⁸ Conversely, Ang-(1-7) has been shown to enhance acetylcholine-mediated vasodilatation in aortic rings from cirrhotic rats.³⁸ The vasodilatory effects of Ang-(1-7) are thought to be mediated through increased production of nitric oxide

(NO).^{34,102} Hence, the absence of a vasodilatory effect by Ang-(1-7) in the cirrhotic rat liver could be explained by the known general impairment of NO-dependent vasodilatation in the cirrhotic liver due to endothelial dysfunction.^{103,104}

In summary, there is considerable evidence supporting the concept that opposing axes of the RAS are involved in the pathogenesis of chronic liver injury. On one side, the ACE/Ang II/AT₁ receptor axis promotes liver injury and deposition of extracellular matrix, on the other, ACE2/Ang-(1-7)/*mas* receptor promotes collagen degradation and resolution of inflammation. Both axes are upregulated in liver disease, but presumably the balance between the two systems is critical in determining the net effect.⁹² For many years researchers in the field of RAS have concentrated on blocking components of the 'classical' system in an attempt to reduce fibrosis. However, both ACE inhibitors and ARB have an impact on other components of the 'classical' RAS apart from Ang II, as plasma renin activity and Ang I levels increase following chronic therapy.¹⁰⁵ This, in part, explains the phenomenon of 'angiotensin II reactivation' and 'aldosterone escape' whereby chronic administration of an ACE inhibitor fails to completely suppress either plasma Ang II or aldosterone production.¹⁰⁶⁻¹⁰⁸ The actual mechanism underlying this phenomenon remains elusive,

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 such tissues would result in both diminished levels of Ang II and simultaneous elevations in tissue Ang-(1-7), thus tipping the RAS balance in favor of the 'alternative' axis. Interestingly, our own studies and those of others have shown that Ang-(1-7) can inhibit ACE activity and gene expression; this would further tend to alter the balance of the two axes towards the 'alternative' axis (Fig. 7).^{34,97}

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 represents a novel set of agents that could be used for the treatment of hepatic fibrosis or portal hypertension. One such drug, AVE0991 is a non-peptide analog of Ang-(1-7). 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.

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References

- Dorer FE, Kahn JR, Lentz KE, Levine M, Skeggs LT. Purification and properties of angiotensin-converting enzyme from hog lung. *Circ. Res.* 1972; **31**: 356-66.
- Skeggs LT, Lentz KE, Gould AB, Hochstrasser H, Kahn JR. Biochemistry and kinetics of the renin-angiotensin system. *Fed. Proc.* 1967; **26**: 42-7.
- Johnston CI. Franz Volhard Lecture. Renin-angiotensin system: a dual tissue and hormonal system for cardiovascular control. *J. Hypertens. Suppl.* 1992; **10**: S13-26.
- Murphy TJ, Alexander RW, Griendling KK, Runge MS, Bernstein KE. Isolation of a cDNA encoding the vascular type-1 angiotensin II receptor. *Nature* 1991; **351**: 233-6.
- Sasaki K, Yamano Y, Bardhan S *et al.* Cloning and expression of a complementary DNA encoding a bovine adrenal angiotensin II type-1 receptor. *Nature* 1991; **351**: 230-3.
- Kambayashi Y, Bardhan S, Takahashi K *et al.* Molecular cloning of a novel angiotensin II receptor isoform involved in phosphotyrosine phosphatase inhibition. *J. Biol. Chem.* 1993; **268**: 24 543-6.
- Touyz RM, Schiffrin EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. *Pharmacol. Rev.* 2000; **52**: 639-72.
- Timmermans PB, Benfield P, Chiu AT, Herblin WF, Wong PC, Smith RD. Angiotensin II receptors and functional correlates. *Am. J. Hypertens.* 1992; **5** (Pt 2): S221-35.
- Ardaillou R. Active fragments of angiotensin II: enzymatic pathways of synthesis and biological effects. *Curr. Opin. Nephrol. Hypertens.* 1997; **6**: 28-34.
- von Bohlen und Halbach O. Angiotensin IV in the central nervous system. *Cell. Tissue Res.* 2003; **311**: 1-9.
- Schiavone MT, Santos RA, Brosnihan KB, Khosla MC, Ferrario CM. Release of vasopressin from the rat hypothalamo-neurohypophysial system by angiotensin-(1-7) heptapeptide. *Proc. Natl Acad. Sci. USA* 1988; **85**: 4095-8.
- Donoghue M, Hsieh F, Baronas E *et al.* A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1-9. *Circ. Res.* 2000; **87**: E1-9.
- Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. *J. Biol. Chem.* 2000; **275**: 33 238-43.
- Santos RA, Simoes e Silva AC, Maric C *et al.* Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. *Proc. Natl Acad. Sci. USA* 2003; **100**: 8258-63.
- Lambert DW, Yarski M, Warner FJ *et al.* Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). *J. Biol. Chem.* 2005; **280**: 30113-19.
- Burrell LM, Johnston CI, Tikellis C, Cooper ME. ACE2, a new regulator of the renin-angiotensin system. *Trends Endocrinol. Metab.* 2004; **15**: 166-9.
- Rice GI, Thomas DA, Grant PJ, Turner AJ, Hooper NM. Evaluation of angiotensin-converting enzyme (ACE), its homologue ACE2 and neprilysin in angiotensin peptide metabolism. *Biochem. J.* 2004; **383** (Pt 1): 45-51.
- Vickers C, Hales P, Kaushik V *et al.* Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. *J. Biol. Chem.* 2002; **277**: 14 838-43.
- Li W, Moore MJ, Vasilieva N *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* 2003; **426**: 450-4.
- Benter IF, Ferrario CM, Morris M, Diz DI. Antihypertensive actions of angiotensin-(1-7) in spontaneously hypertensive rats. *Am. J. Physiol.* 1995; **269** (Pt 2): H313-19.
- Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1-7): cardioprotective effect in myocardial ischemia/reperfusion. *Hypertension* 2001; **38** (Pt 2): 665-8.
- Ferreira AJ, Santos RA, Almeida AP. Angiotensin-(1-7) improves the post-ischemic function in isolated perfused rat hearts. *Braz. J. Med. Biol. Res.* 2002; **35**: 1083-90.

- 23 Freeman EJ, Chisolm GM, Ferrario CM, Tallant EA. Angiotensin-(1-7) inhibits vascular smooth muscle cell growth. *Hypertension* 1996; **28**: 104-8.
- 24 Tallant EA, Ferrario CM, Gallagher PE. Angiotensin-(1-7) inhibits growth of cardiac myocytes through activation of the mas receptor. *Am. J. Physiol. Heart Circ. Physiol.* 2005; **289**: H1560-6.
- 25 Strawn WB, Ferrario CM, Tallant EA. Angiotensin-(1-7) reduces smooth muscle growth after vascular injury. *Hypertension* 1999; **33** (Pt 2): 207-11.
- 26 Iwata M, Cowling RT, Gurantz D *et al.* Angiotensin-(1-7) binds to specific receptors on cardiac fibroblasts to initiate antifibrotic and antiproliferative effects. *Am. J. Physiol. Heart Circ. Physiol.* 2005; **289**: H2356-63.
- 27 Grobe JL, Mecca AP, Mao H, Katovich MJ. Chronic angiotensin-(1-7) prevents cardiac fibrosis in DOCA-salt model of hypertension. *Am. J. Physiol. Heart Circ. Physiol.* 2006; **290**: H2417-23.
- 28 Kucharewicz I, Pawlak R, Matys T, Pawlak D, Buczko W. Antithrombotic effect of captopril and losartan is mediated by angiotensin-(1-7). *Hypertension* 2002; **40**: 774-9.
- 29 Kucharewicz I, Chabielska E, Pawlak D, Matys T, Rolkowski R, Buczko W. The antithrombotic effect of angiotensin-(1-7) closely resembles that of losartan. *J. Renin Angiotensin Aldosterone Syst.* 2000; **1**: 268-72.
- 30 Silva DM, Vianna HR, Cortes SF, Campagnole-Santos MJ, Santos RA, Lemos VS. Evidence for a new angiotensin-(1-7) receptor subtype in the aorta of Sprague-Dawley rats. *Peptides* 2006; **28**: 702-707.
- 31 Tom B, Dendorfer A, Danser AH. Bradykinin, angiotensin-(1-7), and ACE inhibitors: how do they interact?. *Int. J. Biochem. Cell Biol.* 2003; **35**: 792-801.
- 32 Gioli-Pereira L, Nascimento EA, Santos EL *et al.* Fate of bradykinin on the rat liver when administered by the venous or arterial route. *J. Gastroenterol. Hepatol.* 2005; **20**: 463-73.
- 33 Gorelik G, Carbini LA, Scicli AG. Angiotensin 1-7 induces bradykinin-mediated relaxation in porcine coronary artery. *J. Pharmacol. Exp. Ther.* 1998; **286**: 403-10.
- 34 Li P, Chappell MC, Ferrario CM, Brosnihan KB. Angiotensin-(1-7) augments bradykinin-induced vasodilation by competing with ACE and releasing nitric oxide. *Hypertension* 1997; **29** (Pt 2): 394-400.
- 35 Bataller R, Sancho-Bru P, Gines P *et al.* Activated human hepatic stellate cells express the renin-angiotensin system and synthesize angiotensin II. *Gastroenterology* 2003; **125**: 117-25.
- 36 Leung PS, Chappell MC. A local pancreatic renin-angiotensin system: endocrine and exocrine roles. *Int. J. Biochem. Cell Biol.* 2003; **35**: 838-46.
- 37 Herath CB, Warner FJ, Lubel JS *et al.* Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1-7) levels in experimental biliary fibrosis. *J. Hepatol.* 2007; **47**: 387-95.
- 38 Paizis G, Tikellis C, Cooper ME *et al.* Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. *Gut* 2005; **54**: 1790-6.
- 39 Lubel JS, Angus PW. Modern management of portal hypertension. *Intern. Med. J.* 2005; **35**: 45-9.
- 40 Bataller R, Brenner DA. Hepatic stellate cells as a target for the treatment of liver fibrosis. *Semin. Liver Dis.* 2001; **21**: 437-51.
- 41 Kanno K, Tazuma S, Chayama K. AT1A-deficient mice show less severe progression of liver fibrosis induced by CCl(4). *Biochem. Biophys. Res. Commun.* 2003; **308**: 177-83.
- 42 Nabeshima Y, Tazuma S, Kanno K *et al.* Anti-fibrogenic function of angiotensin II type 2 receptor in CCl4-induced liver fibrosis. *Biochem. Biophys. Res. Commun.* 2006; **346**: 658-64.
- 43 Jonsson JR, Clouston AD, Ando Y *et al.* Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. *Gastroenterology* 2001; **121**: 148-55.
- 44 Kurikawa N, Suga M, Kuroda S, Yamada K, Ishikawa H. An angiotensin II type 1 receptor antagonist, olmesartan medoxomil, improves experimental liver fibrosis by suppression of proliferation and collagen synthesis in activated hepatic stellate cells. *Br. J. Pharmacol.* 2003; **139**: 1085-94.
- 45 Li X, Meng Y, Yang XS, Mi LF, Cai SX. ACEI attenuates the progression of CCl4-induced rat hepatic fibrogenesis by inhibiting TGF-beta1, PDGF-BB, NF-kappaB and MMP-2,9. *World J. Gastroenterol.* 2005; **11**: 4807-11.
- 46 Ohishi T, Saito H, Tsusaka K *et al.* Anti-fibrogenic effect of an angiotensin converting enzyme inhibitor on chronic carbon tetrachloride-induced hepatic fibrosis in rats. *Hepatol. Res.* 2001; **21**: 147-58.
- 47 Paizis G, Gilbert RE, Cooper ME *et al.* Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. *J. Hepatol.* 2001; **35**: 376-85.
- 48 Ramos SG, Montenegro AP, Goissis G, Rossi MA. Captopril reduces collagen and mast cell and eosinophil accumulation in pig serum-induced rat liver fibrosis. *Pathol. Int.* 1994; **44**: 655-61.
- 49 Tuncer I, Ozbek H, Ugras S, Bayram I. Anti-fibrogenic effects of captopril and candesartan cilexetil on the hepatic fibrosis development in rat. The effect of AT1-R blocker on the hepatic fibrosis. *Exp. Toxicol. Pathol.* 2003; **55**: 159-66.
- 50 Wei HS, Li DG, Lu HM *et al.* Effects of AT1 receptor antagonist, losartan, on rat hepatic fibrosis induced by CCl(4). *World J. Gastroenterol.* 2000; **6**: 540-5.
- 51 Wei HS, Lu HM, Li DG *et al.* The regulatory role of AT 1 receptor on activated HSCs in hepatic fibrogenesis: effects of RAS inhibitors on hepatic fibrosis induced by CCl(4). *World J. Gastroenterol.* 2000; **6**: 824-8.
- 52 Yoshiji H, Yoshii J, Ikenaka Y *et al.* Inhibition of renin-angiotensin system attenuates liver enzyme-altered preneoplastic lesions and fibrosis development in rats. *J. Hepatol.* 2002; **37**: 22-30.
- 53 Ramalho LN, Ramalho FS, Zucoloto S *et al.* Effect of losartan, an angiotensin II antagonist, on secondary biliary cirrhosis. *Hepatogastroenterology* 2002; **49**: 1499-502.
- 54 Ibanez P, Solis N, Pizarro M *et al.* Effect of losartan on early liver fibrosis development in a rat model of nonalcoholic steatohepatitis. *J. Gastroenterol. Hepatol.* 2007; **22**: 846-51.
- 55 Hirose A, Ono M, Saibara T *et al.* Angiotensin II type 1 receptor blocker inhibits fibrosis in rat nonalcoholic steatohepatitis. *Hepatology* 2007; **45**: 1375-81.
- 56 Sookoian S, Fernandez MA, Castano G. Effects of six months losartan administration on liver fibrosis in chronic hepatitis C patients: a pilot study. *World J. Gastroenterol.* 2005; **11**: 7560-3.
- 57 Debernardi-Venon W, Martini S, Biasi F *et al.* AT1 receptor antagonist Candesartan in selected cirrhotic patients: effect on portal pressure and liver fibrosis markers. *J. Hepatol.* 2007; **46**: 1026-33.
- 58 Terui Y, Saito T, Watanabe H *et al.* Effect of angiotensin receptor antagonist on liver fibrosis in early stages of chronic hepatitis C. *Hepatology* 2002; **36** (Pt 1): 1022.
- 59 Yoshiji H, Noguchi R, Fukui H. Combined effect of an ACE inhibitor, perindopril, and interferon on liver fibrosis markers in patients with chronic hepatitis C. *J. Gastroenterol.* 2005; **40**: 215-16.
- 60 Rimola A, Londono MC, Guevara G *et al.* Beneficial effect of angiotensin-blocking agents on graft fibrosis in hepatitis C recurrence after liver transplantation. *Transplantation* 2004; **78**: 686-91.

- 61 Yokohama S, Yoneda M, Haneda M *et al.* Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology* 2004; **40**: 1222–5.
- 62 Yokohama S, Tokusashi Y, Nakamura K *et al.* Inhibitory effect of angiotensin II receptor antagonist on hepatic stellate cell activation in non-alcoholic steatohepatitis. *World J. Gastroenterol.* 2006; **12**: 322–6.
- 63 Roskams T, Desmet VJ, Verslype C. Development, structure and function of the liver. In: Burt AD, Portmann BC, Ferrell LD, eds. *Macswen's Pathology Of The Liver*, Fifth edn. Edinburgh: Churchill Livingstone, 2007.
- 64 Feu F, Garcia-Pagan JC, Bosch J *et al.* Relation between portal pressure response to pharmacotherapy and risk of recurrent variceal haemorrhage in patients with cirrhosis. *Lancet* 1995; **346**: 1056–9.
- 65 Bataller R, Gines P, Nicolas JM *et al.* Angiotensin II induces contraction and proliferation of human hepatic stellate cells. *Gastroenterology* 2000; **118**: 1149–56.
- 66 Herath CB, Warner FJ, Lubel JS *et al.* Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1–7) levels in experimental biliary fibrosis. *J. Hepatol.* 2007; **47**: 387–95.
- 67 Rockey DC. Vasoactive agents in intrahepatic portal hypertension and fibrogenesis: implications for therapy. *Gastroenterology* 2000; **118**: 1261–5.
- 68 Ballet F, Chretien Y, Rey C, Poupon R. Differential response of normal and cirrhotic liver to vasoactive agents. A study in the isolated perfused rat liver. *J. Pharmacol. Exp. Ther.* 1988; **244**: 283–9.
- 69 Croquet V, Moal F, Veal N *et al.* Hemodynamic and antifibrotic effects of losartan in rats with liver fibrosis and/or portal hypertension. *J. Hepatol.* 2002; **37**: 773–80.
- 70 Vlachogiannakos J, Tang AK, Patch D, Burroughs AK. Angiotensin converting enzyme inhibitors and angiotensin II antagonists as therapy in chronic liver disease. *Gut* 2001; **49**: 303–8.
- 71 Schneider AW, Kalk JF, Klein CP. Effect of losartan, an angiotensin II receptor antagonist, on portal pressure in cirrhosis. *Hepatology* 1999; **29**: 334–9.
- 72 Gonzalez-Abraldes J, Albillos A, Banares R *et al.* Randomized comparison of long-term losartan versus propranolol in lowering portal pressure in cirrhosis. *Gastroenterology* 2001; **121**: 382–8.
- 73 Tripathi D, Therapondos G, Lui HF, Johnston N, Webb DJ, Hayes PC. Chronic administration of losartan, an angiotensin II receptor antagonist, is not effective in reducing portal pressure in patients with preascitic cirrhosis. *Am. J. Gastroenterol.* 2004; **99**: 390–4.
- 74 Schepke M, Werner E, Biecker E *et al.* Hemodynamic effects of the angiotensin II receptor antagonist irbesartan in patients with cirrhosis and portal hypertension. *Gastroenterology* 2001; **121**: 389–95.
- 75 Zhang Z, Feng H, Leng X, Ma F, Wang B, Du R. [The levels of renin activity, angiotensin converting enzyme and angiotensin II in cirrhotic patients with ascites undergoing portacaval shunt]. *Zhonghua Wai Ke Za Zhi* 1999; **37**: 366–8.
- 76 Daskalopoulos G, Pinzani M, Murray N, Hirschberg R, Zipser RD. Effects of captopril on renal function in patients with cirrhosis and ascites. *J. Hepatol.* 1987; **4**: 330–6.
- 77 Pariente EA, Bataille C, Bercoff E, Lebrec D. Acute effects of captopril on systemic and renal hemodynamics and on renal function in cirrhotic patients with ascites. *Gastroenterology* 1985; **88** (Pt 1): 1255–9.
- 78 Wagatsuma Y, Naritaka Y, Shimakawa T *et al.* Clinical usefulness of the angiotensin II receptor antagonist losartan in patients with portal hypertensive gastropathy. *Hepatogastroenterology* 2006; **53**: 171–4.
- 79 Eriksson LS, Kagedal B, Wahren J. Effects of captopril on hepatic venous pressure and blood flow in patients with liver cirrhosis. *Am. J. Med.* 1984; **76**: 66–70.
- 80 Baik SK, Park DH, Kim MY *et al.* Captopril reduces portal pressure effectively in portal hypertensive patients with low portal venous velocity. *J. Gastroenterol.* 2003; **38**: 1150–4.
- 81 Chiang HT, Cheng JS, Lin M, Tseng WS, Chang JM, Lai KH. Haemodynamic effects of enalaprilat on portal hypertension in patients with HBsAg-positive cirrhosis. *J. Gastroenterol. Hepatol.* 1995; **10**: 256–60.
- 82 Svoboda P, Ochmann J, Kantorova I. Effect of enalapril treatment and sclerotherapy of esophageal varices on hepatic hemodynamics in portal hypertension. *Hepatogastroenterology* 1992; **39**: 549–52.
- 83 Rice GI, Jones AL, Grant PJ, Carter AM, Turner AJ, Hooper NM. Circulating activities of angiotensin-converting enzyme, its homolog, angiotensin-converting enzyme 2, and neprilysin in a family study. *Hypertension* 2006; **48**: 914–20.
- 84 Sookoian S, Castano G, Garcia SI, Viudez P, Gonzalez C, Pirola CJ. A1166C angiotensin II type 1 receptor gene polymorphism may predict hemodynamic response to losartan in patients with cirrhosis and portal hypertension. *Am. J. Gastroenterol.* 2005; **100**: 636–42.
- 85 Komeda K, Jin D, Takai S *et al.* Significance of chymase-dependent angiotensin II formation in the progression of human liver fibrosis. *Hepatol. Res.* 2007.
- 86 Shimizu S, Satomura K, Aramaki T, Katsuta Y, Takano T, Omoto Y. Hepatic chymase level in chronic hepatitis: colocalization of chymase with fibrosis. *Hepatol. Res.* 2003; **27**: 62–6.
- 87 Campbell DJ. Renin-angiotensin system inhibition: how much is too much of a good thing?. *Intern. Med. J.* 2002; **32**: 616–20.
- 88 Campbell DJ. Endogenous angiotensin II levels and the mechanism of action of angiotensin-converting enzyme inhibitors and angiotensin receptor type 1 antagonists. *Clin. Exp. Pharmacol. Physiol. Suppl.* 1996; **3**: S125–31.
- 89 Arroyo V, Bosch J, Mauri M *et al.* Renin, aldosterone and renal haemodynamics in cirrhosis with ascites. *Eur. J. Clin. Invest.* 1979; **9**: 69–73.
- 90 Bosch J, Arroyo V, Betriu A *et al.* Hepatic hemodynamics and the renin-angiotensin-aldosterone system in cirrhosis. *Gastroenterology* 1980; **78**: 92–9.
- 91 Sakata T, Takenaga N, Endoh T, Wada O, Matsuki K. Diagnostic significance of serum angiotensin-converting enzyme activity in biochemical tests with special reference of chronic liver diseases. *Jpn. J. Med.* 1991; **30**: 402–7.
- 92 Warner FJ, Lubel JS, McCaughan GW, Angus PW. Liver fibrosis: a balance of ACEs? *Clin. Sci. (Lond.)* 2007; **113**: 109–18.
- 93 Pereira RM, Dos Santos RA *et al.* The renin-angiotensin system in a rat model of hepatic fibrosis: evidence for a protective role of Angiotensin. *J. Hepatol.* 2006; 1–7.
- 94 Chappell MC, Pirro NT, Sykes A, Ferrario CM. Metabolism of angiotensin-(1–7) by angiotensin-converting enzyme. *Hypertension* 1998; **31** (Pt 2): 362–7.
- 95 Yamada K, Iyer SN, Chappell MC, Ganten D, Ferrario CM. Converting enzyme determines plasma clearance of angiotensin-(1–7). *Hypertension* 1998; **32**: 496–502.
- 96 Allred AJ, Diz DI, Ferrario CM, Chappell MC. Pathways for angiotensin-(1–7) metabolism in pulmonary and renal tissues. *Am. J. Physiol. Renal Physiol.* 2000; **279**: F841–50.
- 97 Lubel JS, Herath CB, Jia Z, Burrell LM, Angus PW. Angiotensin 1-7 reduces bile duct proliferation and hepatic fibrosis in the bile duct ligated rat. *Hepatology* 2007; **46** (S1): A706–7.
- 98 Oesterreicher CH, Seki E, Mincis SD *et al.* Angiotensin-converting enzyme 2 is a negative regulator of chronic liver injury. *Hepatology* 2007; **46** (S1): A298–9.

- 99 Ferrario CM, Trask AJ, Jessup JA. Advances in biochemical and functional roles of angiotensin-converting enzyme 2 and angiotensin-(1-7) in regulation of cardiovascular function. *Am. J. Physiol. Heart. Circ. Physiol.* 2005; **289**: H2281-90.
- 100 Lubel JS, Herath CB, Warner FJ, Jia ZY, Burrell LM, Angus PW. Upregulation of the ACE2/Ang(1-7)/Mas receptor axis in the bile duct ligation (BDL) model of hepatic fibrosis does not affect hepatic sinusoidal resistance. *J. Gastroenterol. Hepatol.* 2006; **21** (Suppl 4): A332.
- 101 Carvalho LT, Nascimento EA, Teixeira FO, Nagaoka MR, Borges DR, Kouyoumdjian M. Hepatic conversion of angiotensin I and the portal hypertensive response to angiotensin II in normal and regenerating liver. *J. Gastroenterol. Hepatol.* 2007; **22**: 1543-8.
- 102 Heitsch H, Brovkovych S, Malinski T, Wiemer G. Angiotensin-(1-7)-stimulated nitric oxide and superoxide release from endothelial cells. *Hypertension* 2001; **37**: 72-6.
- 103 Iwakiri Y, Groszmann RJ. Vascular endothelial dysfunction in cirrhosis. *J. Hepatol.* 2007; **46**: 927-34.
- 104 Hendrickson H, Chatterjee S, Cao S, Morales Ruiz M, Sessa WC, Shah V. Influence of caveolin on constitutively activated recombinant eNOS: insights into eNOS dysfunction in BDL rat liver. *Am. J. Physiol. Gastrointest Liver Physiol.* 2003; **285**: G652-60.
- 105 Mooser V, Nussberger J, Juillerat L *et al.* Reactive hyperreninemia is a major determinant of plasma angiotensin II during ACE inhibition. *J. Cardiovasc. Pharmacol.* 1990; **15**: 276-82.
- 106 Sharman DC, Morris AD, Struthers AD. Gradual reactivation of vascular angiotensin I to angiotensin II conversion during chronic ACE inhibitor therapy in patients with diabetes mellitus. *Diabetologia* 2007; **50**: 2061-6.
- 107 van de Wal RM, Plokker HW, Lok DJ *et al.* Determinants of increased angiotensin II levels in severe chronic heart failure patients despite ACE inhibition. *Int. J. Cardiol.* 2006; **106**: 367-72.
- 108 MacFadyen RJ, Lee AF, Morton JJ, Pringle SD, Struthers AD. How often are angiotensin II and aldosterone concentrations raised during chronic ACE inhibitor treatment in cardiac failure? *Heart* 1999; **82**: 57-61.
- 109 Ikura Y, Ohsawa M, Shirai N *et al.* Expression of angiotensin II type 1 receptor in human cirrhotic livers: Its relation to fibrosis and portal hypertension. *Hepatol. Res.* 2005; **32**: 107-16.
- 110 Ferrario CM, Jessup J, Gallagher PE *et al.* Effects of renin-angiotensin system blockade on renal angiotensin-(1-7) forming enzymes and receptors. *Kidney Int.* 2005; **68**: 2189-96.
- 111 Kasper SO, Basso N, Kurnjek ML *et al.* Divergent regulation of circulating and intrarenal renin-angiotensin systems in response to long-term blockade. *Am. J. Nephrol.* 2005; **25**: 335-41.
- 112 Ferrario CM, Jessup J, Chappell MC *et al.* Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 2005; **111**: 2605-10.
- 113 Iyer SN, Ferrario CM, Chappell MC. Angiotensin-(1-7) contributes to the antihypertensive effects of blockade of the renin-angiotensin system. *Hypertension* 1998; **31** (Pt 2): 356-61.
- 114 Collister JP, Hendel MD. The role of Ang (1-7) in mediating the chronic hypotensive effects of losartan in normal rats. *J. Renin Angiotensin Aldosterone Syst.* 2003; **4**: 176-9.
- 115 Iyer SN, Chappell MC, Averill DB, Diz DI, Ferrario CM. Vasodepressor actions of angiotensin-(1-7) unmasked during combined treatment with lisinopril and losartan. *Hypertension* 1998; **31**: 699-705.
- 116 Iyer SN, Yamada K, Diz DI, Ferrario CM, Chappell MC. Evidence that prostaglandins mediate the antihypertensive actions of angiotensin-(1-7) during chronic blockade of the renin-angiotensin system. *J. Cardiovasc. Pharmacol.* 2000; **36**: 109-17.
- 117 Ishiyama Y, Gallagher PE, Averill DB, Tallant EA, Brosnihan KB, Ferrario CM. Upregulation of angiotensin-converting enzyme 2 after myocardial infarction by blockade of angiotensin II receptors. *Hypertension* 2004; **43**: 970-6.
- 118 Santos RA, Ferreira AJ. Pharmacological effects of AVE 0991, a nonpeptide angiotensin-(1-7) receptor agonist. *Cardiovasc. Drug Rev.* 2006; **24**: 239-46.
- 119 Lambert DW, Hooper NM, Turner AJ. Angiotensin-converting enzyme 2 and new insights into the renin-angiotensin system. *Biochem. Pharmacol.* 2008; **75**: 781-6.