■ R E V I E W

Liver fibrosis: a balance of ACEs?

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A B S T R A C T

There is an increasing body of evidence to suggest that the RAS (renin–angiotensin system) contributes to tissue injury and fibrosis in chronic liver disease. A number of studies have shown that components of a local hepatic RAS are up-regulated in fibrotic livers of humans and in experimental animal models. Angiotensin II, the main physiological effector molecule of this system, mediates liver fibrosis by stimulating fibroblast proliferation (myofibroblast and hepatic stellate cells), infiltration of inflammatory cells, and the release of inflammatory cytokines and growth factors such as TGF (transforming growth factor)-β1, IL (interleukin)-1β, MCP (monocyte chemoattractant protein)-1 and connective tissue growth factor. Furthermore, blockade of the RAS by ACE (angiotensin-converting enzyme) inhibitors and angiotensin type 1 receptor antagonists significantly attenuate liver fibrosis in experimental models of chronic liver injury. In 2000 ACE2 (angiotensin-converting enzyme 2), a human homologue of ACE, was identified. ACE2 efficiently degrades angiotensin II to angiotensin-(1–7), a peptide which has recently been shown to have both vasodilatory and tissue protective effects. This suggests that ACE2 and its products may be part of an alternate enzymatic pathway in the RAS, which counterbalances the generation and actions of angiotensin II, the ACE2–angiotensin-(1–7)–Mas axis. This review focuses on the potential roles of the RAS, angiotensin II and ACE2 in chronic liver injury and fibrogenesis.

INTRODUCTION

Hepatic fibrosis and its end-stage sequelae of cirrhosis and liver cancer are major causes of morbidity and mortality throughout the world and their prevalence is rising, largely due to the increasing impact of chronic viral hepatitis. While the development of effective antiviral therapies will help reduce this disease burden, there remains a major need to understand the mechanisms involved in hepatic fibrosis in order to design therapies that can prevent or slow its development in other forms of liver disease and in patients with viral hepatitis who are unresponsive to current therapies.

Recent studies have shown that liver fibrosis involves a co-ordinated response to chronic liver injury in which HSCs (hepatic stellate cells) and other cells of myofibroblast lineage play a central role. The pathways that lead to activation of these cells and perpetuation of the fibrogenic response in the liver are incompletely understood; however, it is clear that a range of cytokines, growth factors and vasoactive peptides are involved which may be potential targets for therapeutic intervention. Angiotensin II,

the main effector peptide of the RAS (renin–angiotensin system), has been shown to be a key mediator of tissue fibrosis in a number of diseases, including chronic heart and kidney diseases and diabetes. Although its role in liver disease is less well-established, recent studies indicate that angiotensin II may also play a central role in the pathogenesis of chronic liver disease and that the RAS is a promising potential target for antifibrotic thersapies.

PATHOGENESIS OF LIVER FIBROSIS

The response of repair by fibrosis is common to most chronic inflammatory diseases of major organs, including the heart, kidneys, lungs, pancreas and liver. It has been argued that the 'encapsulation' of a site of injury by fibrosis is designed to restrict further tissue injury [1]. However, if there is ongoing injury, this process can lead to distortion of normal anatomy, impairment of organ function and eventually to organ failure. In cirrhosis of the liver, the end stage of progressive liver fibrosis, the architecture of the liver is disrupted due to the replacement of normal tissue with scar tissue and the growth of regenerating nodules. This results in major changes to hepatic perfusion, increased resistance to portal blood flow and impaired liver function.

There are many primary causes of liver fibrosis with the most common being chronic hepatitis B and C, alcohol and the increasingly important problem of non-alcoholic fatty liver disease. The pathways involved in the fibrogenic response to these and other causes of hepatic fibrosis appear to be broadly similar and share many of the features of chronic fibrotic diseases in other organs. The modern view of hepatic fibrosis is that of a dynamic and a potentially reversible process and the end result reflects a balance between pathways which lead to matrix accumulation and those which result in matrix degradation and fibrosis resolution [1,2].

Fibrosis of the liver is characterized by both an overall increase in the concentration of matrix proteins in liver tissue, including collagens, elastin, structural (basement) glycoproteins, proteoglycans and pure carbohydrates (hyaluronan), and a change in the matrix composition profile [1]. In normal liver, the space of Disse contains non-fibril-forming collagens (collagen Types IV, VI and XIV), proteoglycans and glycoproteins (fibronectin, laminin and tensacin). With injury, the space of Disse expands as the ECM (extracellular matrix) is remodelled with an initial increase in fibronectin and tensacin, then the subsequent deposition of Type III and Type I collagen, elastin and laminin. Progressive fibrosis results in further matrix deposition both within the space of Disse and throughout the liver parenchyma, and eventually leads to the laying down of extensive and confluent bands of scar tissue that distort the hepatic parenchyma.

A perivascular mesenchymal cell called the HSC is considered the predominant fibrogenic cell type in the liver [1]. In normal liver, HSCs are quiescent and are the main site of retinoid (vitamin A) storage [3]. Following injury, HSCs become activated and transform into interstitial myofibroblasts that are capable of producing the ECM components of fibrotic tissue, as well as a broad array of profibrotic and pro-inflammatory cytokines and chemokines [1,2]. Stellate cell activation is initiated in response to paracrine stimuli from neighbouring cells such as hepatocytes and Kupffer cells as well as changes in the normal ECM. This process is perpetuated further by a range of mediators secreted from surrounding cells and by a number of potent stellate-cell-derived autocrine profibrogenic stimuli, including TGF-β1 (transforming growth factor-β1) and platelet-derived growth factor. There is now considerable evidence that angiotensin II, the main effector peptide of the RAS, is one of the key mediators of this response and is involved in both the recruitment of inflammatory cells [4] and transformation of HSCs into an activated phenotype [5].

RAS

Since the RAS and its pivotal role in regulating cardiovascular function was first described by Skeggs and co-workers [6], the components and physiology of this endocrine system have been the focus of substantial ongoing research. The circulating RAS is best known for its role as a haemodynamic regulator. Angiotensin II, the principal effector of the RAS, causes vasoconstriction both directly and indirectly by stimulating AT1 (angiotensin II type 1) receptors present on the vasculature and by increasing sympathetic tone and stimulating arginine vasopressin release. In addition, angiotensin II regulates blood pressure by modulating sodium and water reabsorption, directly by stimulating AT1 receptors in the kidney, or indirectly by stimulating the production and release of aldosterone from the adrenal glands or thirst via a central action [7].

The classical enzymatic pathway by which angiotensin II is formed begins with the cleavage of the precursor angiotensinogen to the decapeptide angiotensin I by renin, an aspartic protease released from juxtaglomerular cells of the kidney into the circulation. The cascade continues with ACE (angiotensin-converting enzyme) cleaving a dipeptide from the C-terminus of angiotensin I to form angiotensin II. The actions of angiotensin II are mediated via specific seven transmembrane GPCRs (G-protein-coupled receptors). In humans, two angiotensin II receptors have been described, AT1 and AT2, that bind angiotensin II with differing affinities [8]. Angiotensin II is cleaved further by a variety of enzymes to produce the bioactive angiotensin fragments angiotensin III (Ang
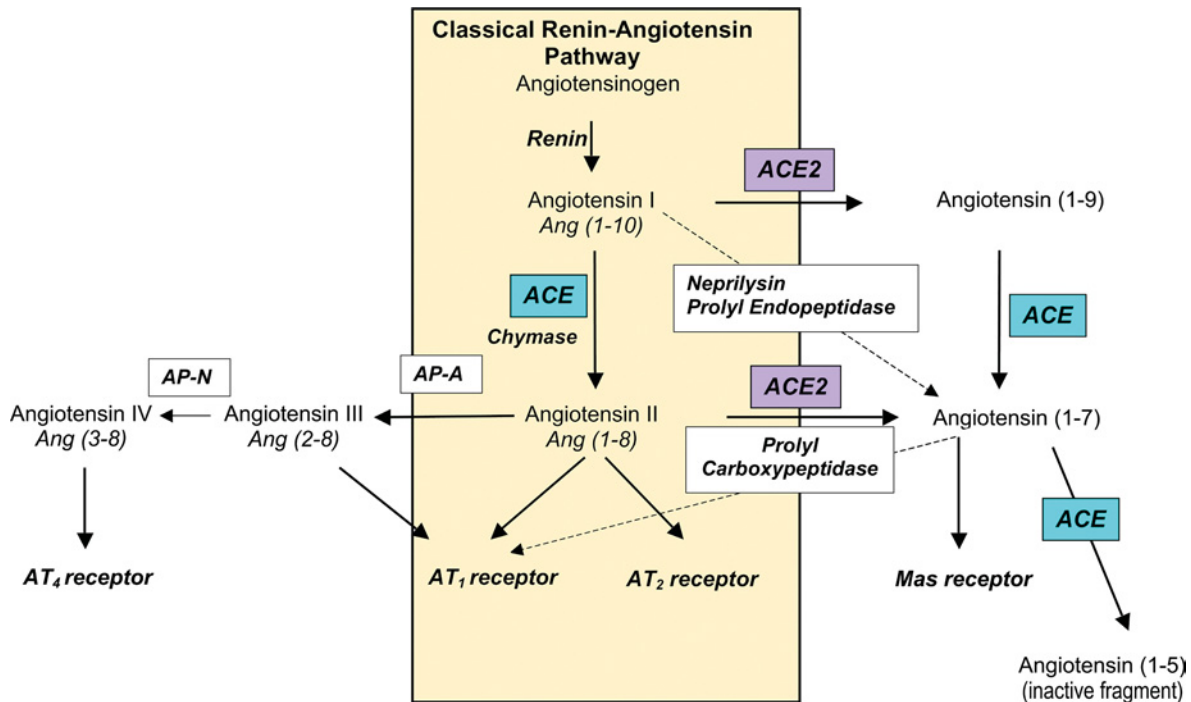
Figure 1   A schematic diagram of the RAS consisting of a classical view of the RAS, a linear sequence of enzymatic reactions yielding the effector peptide angiotensin II that can interact with AT1 and AT2 GPCRs (tinted box) as well the more complex system capable of producing bioactive angiotensin fragments with independent actions to those of angiotensin II
Ang, angiotensin; AP-N, aminopeptidase N, AP-A, aminopeptidase N.

2–8), angiotensin IV (Ang 3–8) and angiotensin-(1–7) [9]. While this depiction (Figure 1) represents the 'classical RAS' described in textbooks, the role of these other angiotensin fragments, the enzymes [ACE2, NEP (neprilysin), aminopeptidase A, prolyl carboxypeptidase and prolyl endopeptidase] implicated in their generation and metabolism, and the identification of new angiotensin receptors (AT4 and Mas) have all been pivotal in re-defining the RAS and its physiological and pathological roles over the last decade.

INTRA-HEPATIC RAS

Apart from the circulating RAS, the existence of local or intra-organ RASs have been described in a number of organs, including the heart, kidney, lung, pancreas and liver [5,10,11]. These local systems have been shown to be responsive to various stimuli of physiological and patho-physiological importance. Moreover, the locally gene-rated angiotensin peptides fragments have a plethora of actions and have been implicated in cell growth, anti-proliferation, apoptosis, ROS (reactive oxygen species) generation, hormonal secretion, pro-inflammatory and pro-fibrogenic actions.

The roles of the hepatic RAS in the function of normal liver and in liver fibrosis are less well-described than that of the heart and kidney. However, it is clear that most of the key components of the enzymatic cascade which lead to the formation of angiotensin II in other organs are the same and are present in the liver. One common theme throughout the literature is that with liver injury an up-regulation and/or redistribution of RAS components, including angiotensinogen, renin, ACE, angiotensin II and AT1 receptors is observed [5,12,13]. The main source of the RAS precursor angiotensinogen is the hepatocyte [12,14], but low levels of protein have also been detected in Kupffer cells and in bile duct epithelium [15]. Studies in humans and rodents show plasma renin concentration and activity, and its substrate angiotensinogen, are in-creased in cirrhotic livers compared with that of controls [14,16–18]. The product of angiotensinogen cleavage by renin, angiotensin I, has not been demonstrated in the liver tissue; however, there is evidence to suggest *de novo* generation of angiotensin I may be produced locally in hepatomesenteric vascular beds as well as in circulating plasma [19]. In contrast, angiotensin II is present in both plasma and liver tissue from normal animals and is increased significantly in rat models of liver disease [20] and in cirrhotic patients [21]. Other RAS components ex-pressed in the normal liver tissue include ACE and AT1 receptor proteins which are both predominantly localized to vascular endothelium, but are also observed in hepa-tocytes and bile duct epithelial cells [12,22–24]. In the

fibrotic liver, ACE and AT1 receptor protein expression is also localized to fibrous septa, mesenchymal cells (HSCs and myofibroblasts) and Kupffer cells [12,22,24].

Although the AT1 receptor is abundant in the liver, the AT2 receptor gene is very low [5,25] or not detectable [12] in normal or diseased liver. The only report so far to attribute AT2 receptor gene expression to a particular liver cell type is that of Bataller et al. [5], who detected the receptor message in isolated human hepatocytes and stellate cells (quiescent, culture-activated and *in vivo*-activated). Despite the existence of AT2 receptors in the liver and a recent study showing that ablation of AT2 receptors augments liver injury and fibrosis [25], the vast majority of reports support the concept that AT1 receptors mediate the inflammatory, proliferative and vascular effects of angiotensin II in the liver [5,26,27]. Moreover, the gene expression of AT1 receptors on septal myofibroblasts appear to correlate with the extent of fibrosis and the degree of portal hypertension [22].

RAS AND HEPATIC FIBROSIS

Experimental evidence

ACEi (ACE inhibitors) and ARBs (AT1 receptor blockers), which prevent either the generation of angiotensin II or angiotensin peptide receptor binding and activation, have been fundamental pharmacological tools for studying the RAS. ACEi such as captopril, lisinopril and perindopril have been found to have multiple benefits in both cardiovascular and renal disease (hypertension, prevention of myocardial infarction and stroke, preventing heart failure, arrhythmias, renal failure, proteinuria and diabetic nephropathy) [28]. Losartan was the first drug of an alternative class of RAS blockers. Subsequently, a large number of ACEi and ARBs have been developed. Collectively, these two classes of drug have been shown to reduce chronic end-organ damage in chronic cardiovascular and renal disease and diabetes. The benefits of these drugs appear to be independent of their antihypertensive effects suggesting that they have direct antifibrotic or tissue-protective effects in these diseases.

Interventional animal studies using RAS inhibitors have provided the strongest evidence that the ACE–angiotensin II–AT1 receptor axis plays a major role in the pathogenesis of hepatic fibrosis. Most of these studies have been performed in rodents and several established models of hepatic fibrosis have been used [23,27,29–41]. Although methodologies have differed widely, there is a surprising degree of uniformity in the results. In almost all of the published studies, both ACEi and ARBs have been shown to have beneficial effects. These include both the attenuation of fibrosis and down-regulation of key inflammatory and profibrotic cytokines known to be involved in the pathogenesis of hepatic fibrosis. The importance of the ACE–angiotensin II–AT1 receptor axis in

hepatic fibrosis is supported further by studies which have shown that inflammation and fibrosis in response to both CCl$_4$ treatment and BDL (bile duct ligation) are attenuated in AT1-knockout mice [26,42]. Furthermore, systemic infusion of angiotensin II stimulates proliferation of bile duct epithelial cells, exacerbates liver fibrosis and increases serum transaminases and endotoxin levels in BDL rat livers, suggesting both local and circulating RAS can contribute to the progression of liver fibrosis [43,44]. One of the most common and serious complications of cirrhosis of the liver is the development of hepatocellular carcinoma. In keeping with the known proliferative and angiogenic effects of angiotensin II, there is increasing evidence that the RAS is involved in the development and growth of this neoplasm. Experiments in mice have shown that the potent angiogenic factor VEGF (vascular endothelial growth factor) is induced by angiotensin II and that the ACEi perindopril significantly attenuates VEGF-mediated tumour development [40,45].

In the liver, angiotensin II regulates cell growth and fibrosis and is involved in key events of inflammation and wound healing. The cell type that is pivotal in these processes is the activated HSC. Following injury, expression of AT1 receptors is increased on activated HSCs, and these cells demonstrate increased responsiveness to angiotensin II compared with quiescent HSCs [46]. Incubation of the activated HSC with angiotensin II results in a dose-dependent increase in the intracellular calcium concentration, cell contraction and cellular proliferation through a MAPK (mitogen-activated protein kinase)-dependent pathway, and these effects are blocked by the ARB losartan [43,46]. ARBs block other dose-dependent profibrotic and pro-inflammatory effects of angiotensin II on HSCs, including the expression of inflammatory cytokines and growth factors such as TGF-β1, IL (interleukin)-1β and connective tissue growth factor, the expression of the transcription factor NF-κB (nuclear factor κB), and the production of ECM and fibrotic markers such as smooth muscle α-actin and collagen [9,27,31,32,47–49]. Angiotensin II is also a powerful chemoattractant for activated HSCs concentrating these cells at the site of hepatic injury [47]. These effects may be amplified by up-regulation of key components of a local RAS by liver injury [12,46], creating an autocrine loop in which liver injury increases angiotensin II production and this in turn perpetuates liver damage and fibrosis.

A recent study has shown that these profibrogenic effects of angiotensin II in human HSCs are at least in part mediated via the generation of ROS by NADPH oxidase [47]. This proposed mechanism is supported by the finding that hepatic fibrosis following BDL is markedly attenuated in NADPH-oxidase-deficient mice [47]. NADPH oxidase is expressed in other hepatic cell types, including Kupffer cells, and sinusoidal endothelial cells, and these cells may also contribute to fibrogenesis through the formation of ROS [50,51].

The hepatic RAS also contributes to fibrosis by regulating the balance between ECM deposition and degradation, which depends on the relative activity of MMPs (matrix metalloproteinases) and their inhibitors TIMP (tissue inhibitors of metalloproteinases). TIMP-1 is a broad specificity inhibitor of MMPs which acts by forming 1:1 complexes with MMPs. Angiotensin II up-regulates TIMP-1 mRNA expression in activated HSCs through AT1 receptor interaction and subsequent PKC (protein kinase C) intracellular signalling pathways. This has been verified in two animal models of fibrosis (pig serum and CCl_4), where down-regulation of TIMP-1 gene expression followed administration of ACEi or ARBs [52].

In addition to its direct profibrotic effects, angiotensin II is an amplifier of the general inflammatory response to chronic liver injury and induces acute-phase reactants, oxidative stress, the release of inflammatory and fibrogenic cytokines [IL-6, IL-1, TGF-β1 and TNF-α (tumour necrosis factor-α)] and ECM deposition [43,44,47,53]. Aside from its complex interactions with other cell types, angiotensin II induces the secretion of MCP (monocyte chemoattractant protein)-1 and IL-8 from activated HSCs [54,55]. MCP-1 is a low-molecular-mass secretory protein that potently stimulates leucocyte recruitment and activation. Up-regulation of MCP-1 gene expression is thought to be mediated via Rho intracellular signalling pathways following angiotensin II binding to the AT1 receptor [54]. Other events that occur as a result of AT1 receptor activation include the release of a number of transcription factors, e.g. AP-1 (activator protein-1), STAT (signal transducer and activator of transcription) and NF-κB [56,57], which are crucial for many of the downstream pro-inflammatory effects of angiotensin II such as the production of the cytokine IL-6. Furthermore, activation of the transcription factor NF-κB is a fundamental positive feedback mechanism by which angiotensin II, acting at AT1 receptors found on hepatocytes, stimulates the transcription of angiotensinogen, the precursor of angiotensin II [58,59]. Kupffer cells also express AT1 receptors and may contribute to these pro-inflammatory effects of angiotensin II [24,60]. For example, Kupffer cells, the resident hepatic macrophage, are activated in alcoholic liver disease and are stimulated by angiotensin II to produce TNF-α and TGF-β1 [61]. The production of these cytokines by Kupffer cells is significantly reduced by the ARB losartan, but not the ACEi captopril, confirming the role of the AT1 receptor in this cell type [39].

Human studies

As a result of experimental studies *in vivo* and *in vitro*, there has been considerable interest in the potential role of ACEi and ARBs in the prevention and treatment of liver fibrosis. The efficacy, ease of use and excellent safety profile of RAS blockers in the treatment of patients with cardiovascular and renal disease makes them an attractive potential therapy for the treatment of human liver disease. There have been only a small number of studies examining the effects of RAS inhibition on fibrosis in human liver disease and there are no large randomized trials. This may at first seem surprising considering the wealth of supportive evidence that has come from animal and *in vitro* studies. However, studies of antifibrogenic therapies are difficult to perform in man because of the need to perform multiple biopsies. In addition, fibrosis progresses very slowly in most common diseases such as hepatitis C and non-alcoholic fatty liver disease, making it difficult to detect possible beneficial effects of antifibrotic therapy unless studies are conducted over a number of years.

One small study ($n = 7$) found that administration of the ARB losartan at 50 mg/day for 48 weeks in patients with NASH (non-alcoholic steatohepatitis) reduced serum TGF-β, ferritin and aminotransferases [62]. Five patients had improvement in the grade of hepatic necro-inflammation. Importantly, this small study had no control group and was not analysed on an intention-to-treat basis. In a subsequent study [63], the pre- and post-treatment biopsies of seven patients with NASH treated with losartan (at 50 mg/day for 48 weeks) were compared with eight patients with non-alcoholic fatty liver disease who acted as a control group. At the end of 48 weeks, the treatment group had a significant improvement in necroinflammatory grade and stage of fibrosis, had significantly fewer activated HSCs and had a mild increase in quiescent HSCs [63]. However, the lack of a proper randomized control group is a particular problem in studies of patients with NASH since the disease can improve in response to changes in lifestyle.

A number of studies have reported possible antifibrotic effects of RAS blockers in patients with hepatitis C [41,63a,64]. In one study [63a], 30 HCV (hepatitis C virus)-infected patients with mild fibrosis were treated with losartan at 50 mg/day and ursodeoxycholic acid at 600 mg/day while controls received ursodeoxycholic acid alone. There were significant differences in serum markers of hepatic fibrosis (TGF-β1 and Type IV collagen) in the losartan and ursodeoxycholic acid group, but no significant changes in fibrosis score (METAVIR scoring system) were observed. The full details of this study have not been published [64]. Another report published in letter form [41] only described outcomes in patients with hepatitis C treated with low-dose IFNα (interferon-α; 3×10^6 units, three times a week for 12 months) in combination with the ACEi 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); however, histological analysis was not performed. Unfortunately, it is impossible to determine from this study whether any of the observed effects were due to perindopril itself as

a perindopril monotherapy group was not included [41]. Finally, a retrospective review [64], which studied liver histology in liver transplant patients with recurrent hepatitis C who were taking RAS-blocking drugs ($n = 27$) compared with those who were not ($n = 101$), showed that the group taking RAS blockers were less likely to develop severe hepatic fibrosis (bridging fibrosis or cirrhosis) at 1 and 10 years post-transplantation compared with the control group [15 % compared with 35 % at 1 year ($P < 0.05$), and 35 % compared with 70 % at 10 years ($P < 0.005$) respectively] [64].

ACE2, AN ANGIOTENSIN II-DEGRADING ENZYME

ACE2 is the first human homologue of ACE to be described [65,66]. Northern blot analysis reveals the highest levels of ACE2 expression in kidney, heart, testis and gastrointestinal tract [65,66]. However, more extensive surveys have shown detectable levels of the *ACE2* gene and protein expression in other tissues, such as the liver and lung [67,68]. Like ACE, ACE2 is a type I integral membrane protein and is found to be expressed predominantly at the cell surface as an ectoenzyme [69]. Its cell-surface location is also consistent with the observation that the ectodomain of ACE2 is shed from the plasma membrane through the action of the metalloprotease ADAM17, which produces a soluble active form of the enzyme [65,70]. Soluble ACE2 has been detected in plasma and urine [69,71,72].

ACE2 has been classified as a member of the M2 zinc metalloproteinase family along with somatic and testicular forms of ACE. The 805 amino acid sequence of ACE2 displays approx. 41 % identity with human somatic ACE [66]. Unlike ACE, however, ACE2 contains only a single catalytic domain compared with the two active sites (N- and C-domains) of somatic ACE. Furthermore, ACE2 is a carboxypeptidase rather than a peptidyl dipeptidase. As a consequence of its mechanism of action, ACE2 has a different substrate specificity to that of ACE [73] and also is not inhibited *in vitro* by ACEi such as captopril, lisinopril or enalaprilat [66]. ACE2 cleaves a single hydrophobic or basic residue from the C-terminus of a limited range of biologically active peptides including angiotensin I and angiotensin II, des Arg^9 bradykinin, apellin 13 and dynorphin A-(1–13) [73].

Two angiotensin fragments produced through C-terminal cleavage of either angiotensin II or angiotensin I by ACE2 are angiotensin-(1–7) and angiotensin-(1–9) respectively (Figure 1). Of these two pathways, the conversion of angiotensin II to angiotensin-(1–7) by ACE2 is kinetically more favourable *in vitro* [73–75] and has been shown to exist *in vivo* [76]. Furthermore, studies *in vitro* show ACE2 to be 10- to 600-fold more potent in hydrolysing angiotensin II to angiotensin-(1–7) than prolyl endopeptidase and prolyl carboxypeptidase, peptidases with similar carboxypeptidase actions [77]. It is these findings that have gained ACE2 the reputation as a major angiotensin-(1–7)-generating enzyme.

ACE2, A NOVEL TARGET FOR LIVER FIBROSIS?

In the classical view of the RAS, the ACE–angiotensin II–AT1 receptor axis is considered the primary pathway. However, the role of angiotensin-(1–7) has been of great interest to several groups, since this fragment mediates effects such as vasodilatation, anti-proliferation [78–80], increased baroreflex sensitivity [81,82], potentiation of bradykinin activity at bradykinin (BK2) receptors [83–85], inhibition of C-domain ACE activity [86] and AT1 receptor antagonism [87], some of which oppose the actions of angiotensin II. Future investigations are now required to decipher which of the known angiotensin-(1–7); effects can be attributed to interaction with the Mas receptor or if these purported actions of angiotensin-(1–7) are mediated via interaction with AT1 receptors or cross-talk between the AT2, BK2 receptor and Mas [11].

Two events have been important in supporting the concept of a new counter-regulatory arm in the RAS for which angiotensin-(1–7) is the effector peptide. First, the discovery of ACE2, which degrades angiotensin II and in turn generates angiotensin-(1–7) [65,66] and, secondly, the identification of the *Mas* protooncogene, an endogenous GPCR of angiotensin-(1–7) [88]. Together, these three components form the putative ACE2–angiotensin-(1–7)–Mas axis, a pathway that is postulated to intrinsically regulate the RAS system by modulating angiotensin II levels and its actions at it receptors (Figure 2).

Our studies have shown that, as rats develop advanced biliary fibrosis, increased expression of components of the classic RAS such as ACE, AT1 receptor and angiotensin II is observed [12,89], and this is accompanied by increased hepatic and plasma ACE2 activity, increased Mas expression in the liver and a marked increases in plasma angiotensin-(1–7) [90,91]. By 28 days post-BDL, plasma concentrations of this vasodilatory and anti-proliferative peptide approach those of the vasoconstrictor angiotensin II [90]. Furthermore, in this same model, pharmacological blockade of the Mas receptor worsens liver fibrosis [92]. Together, these findings are consistent with the presence of a local RAS within the liver and show that both the classical and ACE2-dependent pathways are up-regulated in response to chronic injury. Furthermore, the transcription of the *Mas* gene in the liver is closely linked to *ACE2* gene expression [91]. This provides support for the development of an hepatic ACE2–angiotensin-(1–7)–Mas axis as liver fibrosis progresses, in
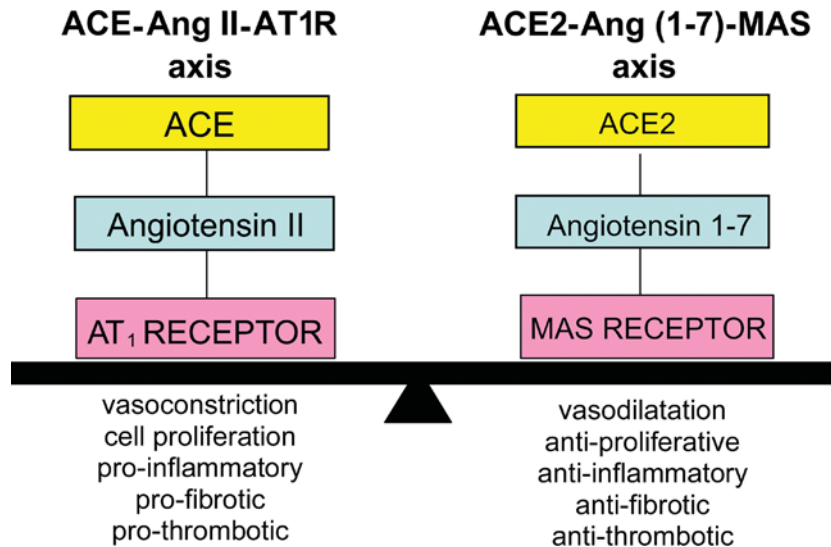
Figure 2   A schematic diagram of the putative ACE2–angiotensin-(1–7)–Mas axis, a counter-regulatory arm of the RAS that produce effects that oppose those of the ACE–angiotensin II–AT1 receptor axis

In addition, this novel pathway intrinsically regulates the RAS system by modulating angiotensin II levels and interaction with its receptors. Ang, angiotensin; AT1R, AT1 receptor.

which increased Mas receptors are available to mediate the angiotensin-(1–7) signal, and may represent an endogenous mechanism by which the liver responds to the potentially harmful effects of an augmented ACE–angiotensin II–AT1 axis.

Blockade of the RAS with ACEi or ARBs has undoubtedly established its key role in managing a number of diseases, including hypertension, heart failure, ventricular remodelling and diabetes. These beneficial outcomes mainly result from blocking the vasoconstrictor, hypertrophic and pro-inflammatory actions of angiotensin II. Several recent studies have shown that ARBs and ACEi increase ACE2 [71,93–95] and angiotensin-(1–7) levels [93–95]. Therefore it may also be that RAS blockade may also protect tissues by not only blocking angiotensin II synthesis or receptor binding, but also by increasing ACE2 expression and/or angiotensin-(1–7) levels. ACE inhibitors lead to an accumulation of angiotensin-(1–7) by preventing its degradation to angiotensin (1-5) [96]. Thus novel approaches to genetically or pharmacologically target ACE2 and its downstream pathways may be therapeutically useful. Recent studies have shown that systemic lentiviral delivery of ACE2 protects rats from angiotensin II-induced cardiac hypertrophy and fibrosis [97] and perivascular fibrosis in spontaneously hypertensive rats [98]. These findings are also in keeping with the recent study by Grobe et al. [99], who showed that chronic infusion of angiotensin-(1–7) attenuates myocyte hypertrophy and interstitial fibrosis caused by long-term infusion of angiotensin II. Interestingly, in all cases, increased ACE2 in normal control animals had no effect on blood pressure or in matrix remodelling, suggesting that ACE2 has a minor role in normal physiology. Thus therapeutic approaches that amplify the ACE2–angiotensin-(1–7)–Mas axis may provide protection against the development of liver injury and progression to cirrhosis.

CONCLUDING REMARKS

Recent studies have provided clear evidence that there is a hepatic RAS that may be of major importance in the pathogenesis of chronic liver disease [23,27,30–41,54]. This system is up-regulated by chronic liver injury and contributes to oxidative stress, recruitment of inflammatory cells and the development of fibrosis. There is ample evidence from *in vitro* studies and work in a number of animal models of liver disease to suggest that blockade of the RAS can ameliorate liver injury, inhibit hepatic fibrosis and lower portal pressure. Whilst ACEi and ARBs have proven to be invaluable pharmacological tools, most of these studies have employed higher doses of drugs than are used clinically and the efficacy of these agents in treating human liver disease remains uncertain. This has led investigators to seek novel approaches for the attenuation of fibrosis, targeting newly identified pathways. ACE2 and its products may be part of an alternative enzymatic pathway in the RAS, which counterbalances the generation and actions of angiotensin II. The ACE2–angiotensin-(1–7)–Mas axis represents one such pathway, and studies in the heart and kidney suggest that genetic targeting of ACE2 can ameliorate fibrosis. Future studies in normal and diseased liver will provide

insight into whether targeting of ACE2 and its products is a realistic therapeutic approach.

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