

CHAPTER 6

THE ROLE OF THE RENIN-ANGIOTENSIN SYSTEM IN HEPATIC FIBROSIS

J.S. LUBEL¹, F.J. WARNER², AND P.W. ANGUS¹

¹*Department of Gastroenterology, Austin Health, Studley Road, Heidelberg, Melbourne, Victoria, Australia*

²*A.W. Morrow Gastroenterology and Liver Centre, Centenary Institute of Cancer Medicine and Cell Biology, University of Sydney, Sydney, Australia*

1. INTRODUCTION

The liver is the second largest organ of the body and has a multitude of functions including carbohydrate and fatty acid metabolism, lipid transport, protein synthesis, storage of fat-soluble vitamins as well as detoxification and modification of compounds absorbed from the small intestine. It has a dual blood supply, with approximately 75% coming from the portal vein and 25% from the hepatic artery. The primary functional unit of the liver, the hepatic lobule, consists of a hexagonal zone of hepatic parenchyma surrounding a central hepatic vein with a number of portal tracts at the periphery which contain a terminal portal vein, bile ductule and hepatic arteriole. In the normal liver, blood flows from portal venous branches through specialised vascular channels called hepatic sinusoids, into the centrilobular hepatic vein. Hepatic sinusoids lack a distinct basement membrane and their endothelial cells have fenestrations which permit bidirectional free passage of solutes between the sinusoid and a sub-sinusoidal space known as the space of Disse. Hepatocytes, account for 70% of liver mass. These cells, which have microvilli on their basolateral surface to facilitate the interchange of nutrients with the sinusoid, are responsible for most of the metabolic and synthetic functions of the liver.

Chronic liver diseases disturb the normal structure and function of the liver by initiating hepatic fibrosis, a process that can eventually lead to progressive destruction of the normal hepatic architecture, loss of functioning hepatocytes and the development of liver cirrhosis. Angiotensin II, the main effector peptide of the renin-angiotensin system (RAS), is known to play an important role in chronic tissue injury and fibrosis in cardiovascular disease, chronic renal disease and diabetes. Its

role in liver disease is less well established, however, recent studies indicate that, as in other organs, there is a renin-angiotensin-system within the liver and that locally generated angiotensin II plays an important role in the pathogenesis of liver injury and hepatic fibrosis. There is also evidence that in the fibrotic liver angiotensin II contributes to portal hypertension by stimulating contraction of perisinusoidal myofibroblasts and increasing sinusoidal resistance to portal flow. In addition to these local effects in the liver, the systemic RAS is activated in patients with advanced liver disease in response to mesenteric and systemic vasodilatation and has an important homeostatic role in maintaining adequate perfusion pressure to the kidney and other vital organs. It also contributes to renal sodium and water retention by releasing aldosterone and by stimulating secretion of antidiuretic hormone (ADH) from the posterior pituitary. These multiple roles of the RAS in liver disease have lead to major interest in the potential role of RAS antagonists in the prevention of liver fibrosis and the treatment of chronic liver disease and its complications.

2. PATHOGENESIS AND SIGNIFICANCE OF HEPATIC FIBROSIS

There are a large number of chronic liver diseases which cause hepatic fibrosis, including chronic viral hepatitis, alcoholic liver disease, non-alcoholic fatty liver disease, iron overload, diseases of the biliary tract and immune and metabolic liver diseases. Cirrhosis, the end stage of hepatic fibrosis, is characterised by the presence of extensive fibrotic septa separating and surrounding parenchymal nodules of regenerating hepatocytes. This disturbance of the normal hepatic architecture, in conjunction with vasoconstriction within the liver, impedes portal blood flow causing portal hypertension; this is the cause of many of the serious complications of cirrhosis including variceal bleeding, hepatic encephalopathy and ascites. Hepatitis B infection is the most common cause of cirrhosis worldwide. It is also the single most important cause of hepatocellular carcinoma, a disease responsible for nearly one third of the world's cancer-related deaths (Lodato *et al* 2006).

Currently the only proven treatment for hepatic fibrosis is to remove the responsible injurious agent. Recent studies have shown that if this can be achieved, for example by eliminating viral replication in patients with chronic hepatitis B or C, hepatic fibrosis and even early stage cirrhosis can resolve. In patients with end-stage cirrhosis and liver failure, therapy is limited to symptom control and the prevention of life threatening complications such as variceal bleeding whilst cure can only be achieved with liver transplantation. Unfortunately despite major advances in antiviral therapy in recent years, many patients with chronic hepatitis do not respond to therapy. There are also a number of chronic liver diseases for which we currently do not have effective treatment. There is therefore an ongoing need to develop anti-fibrotic therapies that can be used to prevent fibrosis progression and the development of cirrhosis.

In the healthy liver, extracellular matrix (ECM) consists of collagens (predominantly type IV), glycoproteins, proteoglycans and glycosaminoglycans which provide a structural and functional framework for cellular migration, adhesion,

differentiation, proliferation and fibrogenic activation (Schuppan *et al* 2001). The space of Disse contains a delicate ECM with basement membrane-like composition, which permits solute diffusion from blood within the sinusoids to the surrounding cells. Hepatic fibrosis results in major changes to both the volume and composition of ECM. There are increases in the interstitial fibrillar collagens types I and III as well as non-fibrillar collagens (types IV and VI). Proteoglycan and glycosaminoglycan content also increases with the overall composition of the ECM changing from a low-density form to a denser more complex interstitial type. The collagenous and non-collagenous ECM content of the liver is increased by at least 3-5 fold in the cirrhotic liver. There is an associated loss of hepatocyte microvilli and structural changes to the sinusoidal endothelium which include loss of fenestrations, and deposition of fibrillar collagen in the space of Disse. These changes impair the transfer of sinusoidal nutrients to hepatocytes facilitating further hepatocyte injury and liver dysfunction.

The pattern of liver fibrosis ultimately depends on the site and intensity of injury. Hence, biliary obstruction results in bile ductular and periductular myofibroblast proliferation and initially produces periportal and then linking portal-portal fibrosis, viral hepatitis infection leads to portal and then portal-central fibrosis whilst disruption to hepatic venous outflow (as occurs in right ventricular failure or Budd Chiari syndrome) causes centrolobular hepatocyte necrosis and leads to centrolobular fibrosis (central to central septa) (Cassiman and Roskams 2002).

The cell type involved in hepatic fibrosis which has been most studied is the hepatic stellate cell (HSC). In part, this is due to the relative ease with which this cell can be isolated, purified and subcultured from both human and animal liver tissue. However, it has become clear from characterisation of cellular markers and electron microscopic studies that there is a diverse population of myofibroblasts within the liver that may also contribute to hepatic fibrosis. These include portal and septal myofibroblasts, cells residing in vessel walls, centrilobular myofibroblasts, and even marrow-derived HSC and myofibroblasts (Cassiman 2002, Kallis, Alison and Forbes 2006, Russo *et al* 2006). It is likely that the expression and importance of these myofibroblast subtypes vary in different human diseases and animal models.

The HSC normally resides in the space of Disse and is responsible for the storage of vitamin A. It is maintained in a quiescent, non-fibrogenic phenotype, in part, by the surrounding ECM composed predominantly of collagen IV (the collagen type present within the lamina densa of the basal lamina) and other non-collagenous components such as laminins. Following repetitive injury, changes in composition of the ECM in conjunction with the release of proinflammatory and profibrotic cytokines released from damaged hepatocytes, Kupffer cells and inflammatory cells, leads to a cascade of events culminating in the transformation of HSCs into activated myofibroblasts. These activated cells deposit extracellular matrix (ECM) but in addition express contractile proteins which enable them to modulate sinusoidal blood flow. HSCs are capable of producing a broad array of profibrotic and proinflammatory cytokines and chemokines including transforming growth factor beta-1 (TGF β 1), platelet derived growth factor (PDGF) and angiotensin II, all of which

can act in both a paracrine and autocrine manner to further perpetuate fibrosis (Friedman, Maher and Bissell 2000). As will be discussed below, recent data have shown that angiotensin II is involved in both the recruitment of inflammatory cells in response to liver injury (Sewnath *et al* 2004) and transformation of hepatic stellate cells to their activated phenotype (Bataller *et al* 2003).

Hepatic fibrosis is a dynamic 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. Matrix metalloproteinases (MMPs) capable of enzymatically degrading ECM are secreted from many liver cells including HSCs, Kupffer cells, hepatocytes and macrophages (Knittel *et al* 1999). Certain MMPs (MMP-2, MMP-9 and MMP-3) may contribute to the pathogenesis of fibrosis by facilitating liver remodelling, altering both the quantity and the composition of the ECM. Other MMP subtypes (MMP-1, MMP-8) degrade fibrillar collagens in the fibrotic liver and therefore drive fibrosis resolution. Counterbalancing the effects of MMPs is a group of tissue inhibitors of metalloproteinases (TIMPs), which promote collagen and matrix deposition by preventing ECM degradation by matrix proteinases (Arthur 2000).

3. THE RENIN-ANGIOTENSIN SYSTEM

3.1. The Circulating Renin-Angiotensin System

Since the discovery of renin from kidney extracts by Tigerstedt and Bergman in 1898 our understanding of the intricacies of the organisation and function of the renin-angiotensin system (RAS) has expanded considerably (Tigerstedt R 1898, Basso and Terragno 2001). The circulating RAS is best known for its role as a regulator of blood pressure, and fluid and electrolyte homeostasis. Angiotensin II, the principal effector of the RAS, causes vasoconstriction directly by stimulating angiotensin type 1 (AT₁) receptors present on the surface of vascular smooth muscle cells and indirectly by potentiating the release of norepinephrine from postganglionic sympathetic fibres and stimulating antidiuretic hormone (ADH) release from the posterior pituitary. Long-term, angiotensin II regulates blood pressure by modulating sodium and water reabsorption through stimulation of AT₁ receptors in the kidney, and by stimulating the production and release of aldosterone from the adrenal glands. In addition, angiotensin II increases thirst sensation through stimulation of the subfornical organ within the diencephalon (Timmermans *et al* 1992). Other effects that may be important in chronic organ damage include promotion of thrombosis, cardiac hypertrophy and angiogenesis.

The classical enzymatic pathway generating angiotensin II begins with the cleavage of the peptide bond between the leucine and valine residues on angiotensinogen producing the biologically inactive decapeptide, angiotensin I. This process is mediated by renin, an aspartic protease released from juxtaglomerular cells of the kidney into the circulation. The cascade continues with Angiotensin Converting Enzyme (ACE) found predominantly in the capillaries of the lung

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 G protein-coupled receptors. In humans, two angiotensin receptors (AT_1 and AT_2) with differing affinities for angiotensin II have been described (Timmermans *et al* 1992). Angiotensin II can further be cleaved at either its carboxy- or amino-terminus to produce biologically active angiotensin fragments. Thus, from the amino-terminus, Angiotensin III (2-8) can be formed following cleavage of the aspartate-arginine bond of angiotensin II by aminopeptidase A and angiotensin IV (3-8) can be formed following further cleavage of angiotensin III by aminopeptidase B and N (Ardaillou 1997). Angiotensin III shares many of the properties of angiotensin II with 40% of the pressor activity and 100% of the aldosterone stimulating activity of angiotensin II. Angiotensin IV has its own distinct receptor (AT_4) and has central nervous system effects together with some opposing actions to angiotensin II (von Bohlen und Halbach 2003). Enzymatic cleavage of the carboxy-terminus of angiotensin II or angiotensin I can produce the biologically active fragment angiotensin (1-7).

There has been renewed interest in the circulating RAS following the simultaneous discovery by two independent research groups in 2000 of an ACE homologue called ACE2. These two enzymes share 61% protein sequence similarity but have distinct enzymatic actions and tissue distributions (Donoghue *et al* 2000, Tipnis *et al* 2000). Like ACE, ACE2 is a zinc metalloprotease and a type 1 integral membrane protein expressed predominantly on the cell surface and as such acts as an ectoenzyme (Warner *et al* 2005). In its membrane bound form it comprises an extracellular N-terminal domain containing the active site and a short intracellular C-terminal anchor. The highest levels of ACE2 are seen in the kidney, heart, testis and gastrointestinal tract (particularly ileum, duodenum, jejunum, caecum and colon) with lower levels expressed in liver and lung (Harmer *et al* 2002, Hamming *et al* 2004, Donoghue 2000, Tipnis 2000). ACE2 can be released from the cell surface by the action of a secretase-like enzyme and the soluble ACE2 formed can be detected in plasma and urine (Lambert *et al* 2005, Lew *et al* 2006, Ocaranza *et al* 2006, Rice *et al* 2006). In contrast to ACE, 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 different substrate specificity to that of ACE (Warner *et al* 2004) and also is not inhibited in-vitro by ACE inhibitors such as captopril, lisinopril or enalaprilat (Tipnis 2000).

ACE2 has activity on a number of biologically active peptides including angiotensin I and angiotensin II, des Arg⁹ bradykinin, apellin 13 and dynorphin A (1-13) (Vickers *et al* 2002). The enzyme has a preference for hydrophobic or basic residues at the carboxy-terminus as well as for propyl residues at the penultimate position (Table 1) (Turner 2003).

The many diverse functions and interactions of ACE2 are only now being realised (Burrell *et al* 2004, Thomas and Tikellis 2005). This enzyme is not only crucial in cardiovascular and renal injury, but also has been identified as the receptor for the SARS coronavirus (W. Li *et al* 2003). Of particular interest over the past 5 years

Table 1. Peptide substrates for ACE2 showing catalytic efficiency in descending order. Note that ACE2 catalytic efficiency for angiotensin II is 400 fold that of angiotensin I (Vickers 2002)

Substrate	Site of cleavage	Catalytic efficiency (k_{cat}/K_m)
Dynorphin A (1-13)	L-K	$3.1 \times 10^6 \text{ m}^{-1}.\text{s}^{-1}$
Apelin-13	P-F	$2.1 \times 10^6 \text{ m}^{-1}.\text{s}^{-1}$
Angiotensin II	P-F	$1.9 \times 10^6 \text{ m}^{-1}.\text{s}^{-1}$
Des-Arg ⁹ -bradykinin	P-F	$1.3 \times 10^5 \text{ m}^{-1}.\text{s}^{-1}$
Angiotensin I	H-L	$4.9 \times 10^3 \text{ m}^{-1}.\text{s}^{-1}$

has been the role of ACE2 in the formation of the biologically active fragment angiotensin (1-7). ACE2 can generate angiotensin (1-7) directly through enzymatic cleavage of angiotensin II or indirectly by cleaving angiotensin I into the inactive peptide fragment angiotensin (1-9), which is then further enzymatically cleaved by ACE to angiotensin (1-7) (Zisman *et al* 2003, Zisman *et al* 2003). Of these two pathways, the conversion of angiotensin II to angiotensin (1-7) by ACE2 is kinetically favoured *in-vitro* (Vickers 2002, Rice *et al* 2004). Furthermore, *in-vitro* studies show ACE2 to be 10- to 600-fold more potent in hydrolysing Angiotensin II to Angiotensin (1-7) than propyl endopeptidase and propyl carboxypeptidase, peptidases with similar carboxypeptidase actions (Ferrario 2003). These findings suggest that ACE2 is a major angiotensin (1-7) generating enzyme as well as an important enzyme for the degradation of angiotensin II.

The biological effects of angiotensin (1-7) were first described in the rat hypothalamic-hypophysial implant in 1988 in which angiotensin (1-7) stimulated release of vasopressin (Schiavone *et al* 1988). Subsequent animal experiments have shown angiotensin (1-7) to have antihypertensive (Benter *et al* 1995), antiarrhythmic (Ferreira, Santos and Almeida 2001) and cardioprotective properties (Ferreira, Santos and Almeida 2002). The vasodilatory effects of angiotensin (1-7) are mediated through the release of nitric oxide (NO) (Nakamoto *et al* 1995, P. Li *et al* 1997, Brosnihan, Li and Ferrario 1996), prostaglandins (Freeman *et al* 1996, Iyer *et al* 2000) and the release and interaction with bradykinin (P. Li 1997, Gorelik, Carbini and Scicli 1998, Fernandes *et al* 2001, Ueda *et al* 2001). Angiotensin (1-7) has also been shown to have anti-trophic properties in vascular endothelial, smooth muscle cells, cardiac myocytes, and cardiac fibroblasts (Freeman 1996, Strawn, Ferrario and Tallant 1999, Iwata *et al* 2005, Tallant, Ferrario and Gallagher 2005). In addition, anti-inflammatory, anti-fibrotic (Grobe *et al* 2006, Grobe *et al* 2006) and anti-thrombotic properties (Kucharewicz *et al* 2000, Kucharewicz *et al* 2002) have been attributed to angiotensin (1-7).

The putative receptor for angiotensin (1-7) is the G protein-coupled receptor encoded by the *Mas* proto-oncogene (Santos *et al* 2003). This receptor has been shown *in-vitro* to hetero-oligomerize with the AT₁ receptor and act as a physiological antagonist to angiotensin II as well as interact with the AT₂ receptor (Castro *et al* 2005, Kostenis *et al* 2005). Evidence is emerging that AT₂ receptors and other yet unidentified angiotensin (1-7) receptor subtypes may be important

in the biological action of angiotensin (1-7) (Walters, Gaspari and Widdop 2005, Silva *et al* 2006). Some of the actions of angiotensin (1-7) clearly oppose those of angiotensin II and consequently it has been proposed that the RAS can be divided on this basis into two distinct arms that are capable of producing complementary effects (Fig. 1). Thus our conceptual understanding of the RAS has evolved from an endocrine system consisting of a linear sequence of enzymatic reactions yielding the effector peptide angiotensin II to a complex system closely integrated with other systems (such as the kinin-kallikrein system) with the potential of producing effector peptides with counterbalancing effects (angiotensin (1-7) and angiotensin II). Importantly, the RAS is not just a systemic endocrine system but also can function autonomously as a paracrine system within certain organs.

3.2. The Intra-hepatic RAS

Local or intra-organ renin-angiotensin systems have been described in a number of organs including the heart, kidney, liver and pancreas (Bataller 2003, Leung and Chappell 2003). These local systems have been shown to be responsive to various stimuli of physiological and pathophysiological importance. Moreover, the locally generated angiotensin peptides fragments have a plethora of actions and have been

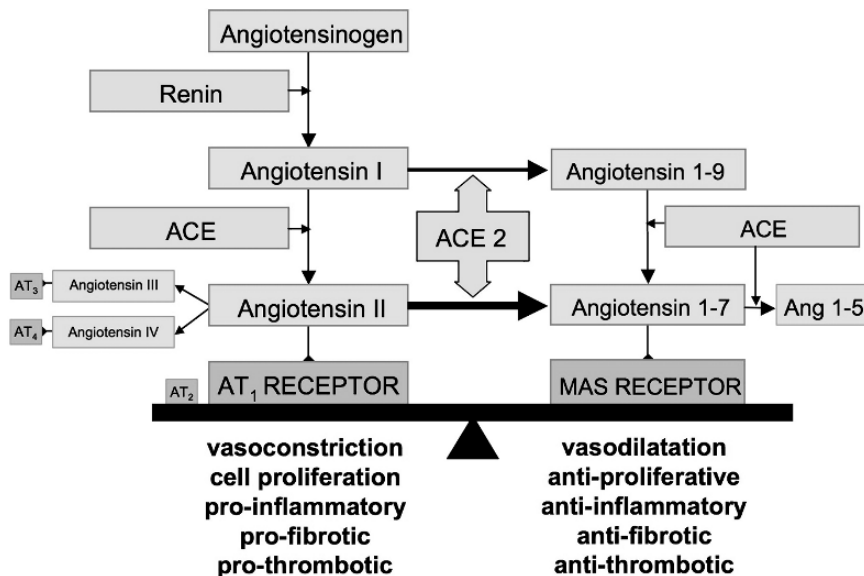


Figure 1. The Renin-Angiotensin System. Peptides are shown in blue boxes, enzymes in yellow boxes and target receptors in pink boxes. The system has four known biologically active peptides, angiotensin II, III, IV and angiotensin 1-7, which act through distinct cellular receptors (AT₁₋₄ and Mas). The two principal arms of this system act via the AT₁ and Mas receptors and have opposing actions

implicated in cell growth, anti-proliferation, apoptosis, reactive oxygen species generation, hormonal secretion, pro-inflammatory, and pro-fibrogenic actions.

The role of the hepatic RAS in normal and diseased liver is less well described than that of the heart and kidney. However, it is clear that most of the key components of the enzymatic cascade that lead to the formation of angiotensin II in other organs are present in the liver. One common theme throughout the literature is the observation that liver injury is associated with an up-regulation and/or redistribution of RAS components including angiotensinogen, renin, ACE, angiotensin II and AT₁ receptors (Sakata *et al* 1991, Paizis *et al* 2002, Bataller 2003). The main source of the RAS precursor, angiotensinogen, is the hepatocyte (Morris, Iwamoto and Reid 1979, Paizis 2002), but low levels of protein have also been detected in Kupffer cells, and in the bile duct epithelium (Sawa 1990). Studies in humans and rodents show plasma renin concentration and activity and its substrate angiotensinogen are increased in cirrhotic livers compared to controls (Morris 1979, Richoux *et al* 1983, Kojima *et al* 1998, Rincon-Sanchez *et al* 2005, Rivera-Huizar *et al* 2006). The product of angiotensinogen cleavage by renin, angiotensin I, has not been demonstrated in liver tissue, however, there is evidence to suggest de novo generation of angiotensin I may occur locally in hepatomesenteric vascular beds as well as in circulating plasma (Admiraal *et al* 1990). In contrast, angiotensin II is present in both plasma and liver tissue from normal animals and increases significantly in rat models of liver disease and in cirrhotic patients (Asbert *et al* 1992, Wang *et al* 2003, Herath *et al* 2006). Other RAS components expressed in the normal liver tissue include ACE and the AT₁ receptor which are both predominantly localised to vascular endothelia, but are also observed in hepatocytes and bile duct epithelial cells (H. S. Wei *et al* 2000, Ikura *et al* 2005). In the fibrotic liver, ACE and AT₁ protein expression is also found in fibrous septa, mesenchymal cells (hepatic stellate cells and myofibroblasts) and Kupffer cells (H. S. Wei 2000, Paizis 2002, Leung *et al* 2003, Ikura 2005).

Although the AT₁ receptor is abundant in the liver, the expression of the AT₂ receptor gene is very low or not detectable in normal or diseased liver (Paizis 2002, Bataller 2003, Nabeshima *et al* 2006). The only report so far to attribute AT₂ receptor gene expression to a particular liver cell type is that of Bataller and co-workers who detected low levels of the receptor messenger RNA in isolated human hepatocytes and all HSC phenotypes (quiescent, culture activated and in vivo activated) (Bataller 2003). Despite the possible existence of AT₂ receptors in the liver, and a recent study showing that ablation of AT₂ receptors augments liver injury and fibrosis (Nabeshima 2006), the vast majority of reports support the concept that AT₁ receptors mediate most of the inflammatory, proliferative and vascular effects of angiotensin II in the liver (Bataller 2003, Kanno, Tazuma and Chayama 2003, Yoshiji *et al* 2003, Bataller *et al* 2005). Moreover, the gene expression of AT₁ on human myofibroblasts has been shown to correlate with the extent of fibrosis and degree of portal hypertension (Ikura 2005).

4. HEPATIC FIBROSIS AND THE RAS

There is increasing evidence that in the liver, angiotensin II regulates cell growth and fibrosis and is involved in key events of inflammation and wound healing. One cell type that is pivotal in these processes is the activated hepatic stellate cell. Following injury, expression of AT₁ receptors is increased on activated hepatic stellate cells and these cells demonstrate increased responsiveness to angiotensin II compared to quiescent HSC (Bataller *et al* 2000). Incubation of the activated HSCs with angiotensin II results in a dose dependent increase in intracellular calcium concentration, cell contraction and cellular proliferation through a mitogen-activated protein kinase (MAPK) -dependent pathway and these effects are blocked by losartan, an angiotensin II type 1 receptor antagonist (ARB), (Bataller 2000). ARBs block other dose dependent profibrotic and proinflammatory effects of angiotensin II on HSCs including the expression of inflammatory cytokines and growth factors such as TGF- β 1, IL-1 β , CTGF, and NF- κ β , production of extracellular matrix (ECM) and fibrotic markers, smooth muscle α -actin and collagen (H. S. Wei 2000, Ohishi *et al* 2001, Yoshiji *et al* 2001, Bataller 2003, Kurikawa *et al* 2003, Y. Zhang *et al* 2003, Y. J. Zhang *et al* 2003). Angiotensin II is also a powerful chemo-attractant for activated HSCs concentrating these cells at the site of hepatic injury (Bataller *et al* 2003). These effects may be amplified by upregulation of key components of a local RAS by liver injury (Paizis 2002, Bataller 2003), creating an autocrine loop in which liver injury increases angiotensin II production and this in turn perpetuates liver damage and fibrosis.

A recent study showed that these profibrogenic effects of angiotensin II in human hepatic stellate cells are at least in part mediated via the generation of reactive oxygen species (ROS) by NADPH oxidase. This proposed mechanism is supported by the finding that hepatic fibrosis following bile duct ligation is markedly attenuated in NADPH oxidase-deficient mice (Bataller 2003). 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 (Whalen *et al* 1999, Kono *et al* 2000).

The importance of the RAS in hepatic fibrosis is supported by studies which have shown that inflammation and fibrosis in response to both CCL4 treatment (Kanno 2003) and bile duct ligation (Yang *et al* 2005) are attenuated in AT₁ knockout mice. Supporting evidence has also come from *in-vivo* studies which have shown that angiotensin II infusion stimulates proliferation of bile duct cells, exacerbates liver fibrosis and increases serum transaminases and endotoxin levels in BDL rat livers (Bataller *et al* 2005). Interestingly, angiotensin II infusion increases the number of vascular thromboses of small hepatic vessels within portal tracts in both BDL and sham operated animals, the putative mechanism being an increase in tissue factor procoagulant activity (Bataller 2005). This prothrombotic effect of angiotensin II may contribute to further liver injury and collagen deposition by causing local hypoxia (Corpechot *et al* 2002).

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 β 1, TNF α) and ECM deposition (Bataller 2003, Miyoshi *et al* 2003, Bataller 2005, Sasaki *et al* 2005). In addition to complex interactions with other cell types, angiotensin II induces the secretion of monocyte chemoattractant protein (MCP-1) and IL-8 from activated HSCs (Marra *et al* 1998, Kanno *et al* 2005). MCP-1 is a low molecular weight secretory protein that potently stimulates leukocyte recruitment and activation. Upregulation of MCP-1 gene expression is thought to be mediated via Rho intracellular signalling pathways following angiotensin II binding to the AT $_1$ receptor (Kanno 2005). Other events that occur as a result of AT $_1$ receptor activation include the release of a number of transcription factors; activator Protein 1 (AP-1), signal transducer and activator of Transcription (STATs) and NF κ B (Jamaluddin *et al* 2000, McAllister-Lucas *et al* 2006), which are crucial for many of the downstream pro-inflammatory effects of angiotensin II such as the production of cytokine, IL-6. Furthermore, activation of the transcription factor NF κ B is a fundamental positive feedback mechanism by which angiotensin II acting at AT $_1$ receptors located on hepatocytes stimulates the transcription of angiotensinogen, the precursor of angiotensin (Ron, Brasier and Habener 1990, Brasier, Li and Copland 1994). A number of cell types present within the liver express AT $_1$ receptors and may contribute to these proinflammatory effects of AngII (Leung 2003, X. Zhang *et al* 2004). 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- β 1 (Enomoto *et al* 2000). The production of these cytokines by Kupffer cells is significantly reduced by the angiotensin receptor antagonist (ARB), losartan but not the ACE inhibitor captopril, confirming the role of the AT $_1$ receptor in this cell type (Y. H. Wei, Jun and Qiang 2004).

The hepatic RAS also appears to affect the balance between ECM deposition and degradation which depends on the relative activity of matrix metalloproteinases (MMPs) and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs). TIMP-1 is a broad specificity inhibitor of MMPs which acts by forming 1:1 complexes with MMPs. Angiotensin II upregulates TIMP-1 mRNA expression in activated HSCs through AT $_1$ receptor binding and subsequent protein kinase C (PKC) 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 ACE inhibitors or ARB (Yoshiji 2003).

Paizis *et al*, recently demonstrated that ACE2 gene expression and protein levels are markedly elevated in the BDL liver and cirrhotic human liver tissue, opening the possibility that this putative counter-regulatory arm of the RAS is actively involved in chronic liver disease (Paizis *et al* 2005). In addition angiotensin (1-7), ACE2 and Mas, have recently been shown to increase progressively following bile duct ligation in rats (Herath 2006, Lubel *et al* 2006, Pereira *et al* 2006). Furthermore in this same model, pharmacological blockade of the Mas receptor may worsen liver fibrosis (Pereira 2006). The potential benefits of angiotensin (1-7) are of particular clinical relevance because both ACE inhibitors and angiotensin receptor blockers (ARBs) can result in elevations in angiotensin (1-7) raising the possibility that some

of the beneficial effects of these drugs are mediated by this peptide (Iyer, Ferrario and Chappell 1998, Collister and Hendel 2003).

4.1. Effects of RAS Inhibition on Hepatic Fibrosis in vivo

ACE inhibitors 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). Losartan was the first drug of an alternative class of RAS antagonists which block the angiotensin II type 1 receptor. Subsequently a large number of ACE inhibitors and angiotensin receptor blockers (ARBs) have been developed. These two classes of drug collectively have been shown to reduce chronic end-organ damage in 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.

4.2. Studies in Animal Models

Interventional animal studies using RAS inhibitors have provided compelling evidence that the RAS 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 (Table 2) (Ramos *et al* 1994, H. Wei *et al* 2000, Jonsson *et al* 2001, Ohishi 2001, Paizis *et al* 2001, Yoshiji *et al* 2001, Croquet *et al* 2002, Ramalho *et al* 2002, Toblli *et al* 2002, Yoshiji *et al* 2002, Kurikawa 2003, X. Li *et al* 2003, Tuncer *et al* 2003, Yoshiji 2003, X. Li *et al* 2004, Y. H. Wei 2004, Yoshiji *et al* 2005). Although methodologies have differed widely, there is a surprising degree of uniformity in the results. In almost all published studies, both ACE inhibitors and AT1 receptor blockers 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. A summary of the major findings of these studies is provided in Table 2.

One of the most common and serious complications of cirrhosis 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 vascular endothelial growth factor (VEGF) is induced by angiotensin II and that the ACE inhibitor, perindopril, significantly attenuates VEGF-mediated tumour development. (Yoshiji, Kuriyama and Fukui 2002, Yoshiji *et al* 2002)

4.3. Human Studies

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

Table 2. In-vivo animal evidence of RAS involvement in hepatic fibrosis using either ACE inhibitors (ACEi) or angiotensin receptor blockers (ARBs)

Model	Strain/Species	RAS Blocker	Histological improvement	OH Proline	Fibrosis markers	Collagen expression	Portal pressure/flow	TGFB	MMP	PDGF	α -SMA	Author	
Bile Duct Ligation (BDL)	SD Rat	Losartan	✓	✓	×		✓					(Croquet et al. 2002)	
	Lewis Rats	Captopril	✓	✓	✓			✓	✓		✓	(Jonsson et al. 2001)	
	SD Rat	Olmesartan	✓	✓		✓		✓			✓	(Kurikawa et al. 2003)	
	SD Rat	Irbesartan	×	×		✓		✓				(Paizis et al. 2001)	
	Wistar Rat	Losartan	✓			✓	✓				✓	(Ramalho et al. 2002)	
	SD Rat	Losartan	✓	✓	✓		✓					(Croquet et al. 2002)	
	Wistar Rat	Perindopril	✓	✓	✓			✓	✓			(X. Li et al. 2003)	
	Wistar Rat	Losartan	✓	✓	✓			✓	✓			(X. Li et al. 2003)	
	Wistar Rat	Perindopril	✓	✓	✓			✓	✓			(X. Li et al. 2004)	
	SD Rat	Lisinopril	✓	✓	✓	✓						(Ohishi et al. 2001)	
Carbon Tetrachloride (CCL ₄)	SD Rat	Captopril	×								×	(Tuncer et al. 2003)	
	SD Rat	Candesartan	✓								✓		
	SD Rat	Enalapril	✓										
	SD Rat	Losartan	✓										
	SD Rat	Losartan & Enalapril	✓										
	SD Rat	Losartan	✓	✓	✓	✓							
	SD Rat	Captopril	✓	✓	✓	✓							
	Fisher Rats	Candesartan	✓										
	SD Rat	Perindopril	✓	✓									(Yoshiji et al. 2003)
	BALB/c mice	Perindopril	✓	✓	✓	✓					✓		(Yoshiji et al. 2005)

potential therapy for the treatment of human liver disease. The effects of AT₁ blockade on portal hypertension have been examined in a number of studies. The rationale for these studies is that angiotensin II increases intra hepatic resistance to portal flow in the cirrhotic liver by mediating contraction of perisinusoidal myofibroblasts and thus contributes to the variable component of portal hypertension (Vlachogiannakos *et al* 2001). Although some studies have shown that these drugs can lower portal pressure, their use has been associated with unacceptable drops in systemic blood pressure and renal blood flow, particularly in patients with advanced liver disease. This is because the systemic renin angiotensin system is activated in such patients in response to systemic and mesenteric vasodilation (Arroyo *et al* 1979, Bosch *et al* 1980, Sakata 1991) and plays a central role in the maintenance of renal perfusion pressure and glomerular filtration.

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 randomised 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 angiotensin II receptor antagonist losartan 50mg/day for 48 weeks in patients with non-alcoholic steatohepatitis (NASH) reduced serum TGF- β , ferritin and aminotransferases. Five patients showed 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 (Yokohama *et al* 2004). In a subsequent study the pre and post treatment biopsies of seven patients with non-alcoholic steato-hepatitis treated with losartan (50mg/day for 48 weeks) were compared with eight patients with non-alcoholic fatty liver disease who acted as a control group. The treatment group showed a significant improvement in necro-inflammatory grade, stage of fibrosis, significantly fewer activated HSCs and a mild increase in quiescent HSCs (Yokohama *et al* 2006) at the end of 48-weeks. However, the lack of a proper randomised control group is a particular problem in studies of patients with NASH since the disease can improve in response to changes in life style.

A number of studies have reported possible antifibrotic effects of RAS blockers in patients with hepatitis C. In one study, 30 HCV infected patients with mild fibrosis were treated with losartan 50mg/day and ursodeoxycholic acid 600mg/day whilst controls received ursodeoxycholic acid alone. There were significant differences in serum markers of hepatic fibrosis (TGF- β 1 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 (Rimola *et al* 2004). Another report published in letterform only 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 (4mg/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 (Yoshiji, Noguchi and Fukui 2005). Finally, a retrospective review which 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) 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 to the control group (15% vs. 35% at 1 year (P<0.05), and 35% vs. 70% at 10 years (P<0.005), respectively) (Rimola 2004).

5. CONCLUSIONS

Recent studies have provided clear evidence that there is an hepatic RAS that may be of major importance in the pathogenesis of chronic liver disease. This system is upregulated by chronic liver injury and contributes to oxidative stress, recruitment of inflammatory cells and the development of fibrosis. The RAS also plays a role in the pathogenesis of portal hypertension and many of the systemic complications of cirrhosis. 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 ACE inhibitors and ARB have proven to be invaluable pharmacological tools, most studies have employed higher doses of these drugs than are used clinically. It remains to be determined whether RAS inhibition will prove to be an effective therapeutic approach for the treatment and prevention of hepatic fibrosis and its complications in human liver disease.

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