Therapeutic potential of targeting the renin angiotensin system in portal hypertension

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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 and non-alcoholic steatohepatitis. Much of the morbidity and mortality that occurs in cirrhosis is due to the development of portal hypertension. However, despite major advances in our understanding of the pathogenesis of portal hypertension, current treatment options are limited.

It is clear that the renin angiotensin system (RAS) contributes to organ dysfunction and chronic tissue injury in a range of conditions including diabetes, cardiovascular and renal disease, primarily through the vasoactive and profibrotic effects of its key effector peptide, angiotensin II\(^1\). More recently, the RAS has also been...
implicated in the pathogenesis of both hepatic fibrosis and portal hypertension\(^2\). This is supported by studies which have shown that RAS blockers are able to reduce fibrosis in experimental models of chronic liver injury and that they can lower portal pressure in both animal models and in man, primarily by inhibiting angiotensin II mediated intrahepatic vasoconstriction\(^3\). This review will briefly outline current therapeutic approaches to the management of portal hypertension, and focus on the evidence supporting the role of the RAS and the use of RAS blockers in this condition, and recent advances in RAS research that could lead to the development of new treatments.

**CURRENT TREATMENT OF PORTAL HYPERTENSION**

The initiating mechanism for the development of portal hypertension is thought to be the development of increased intrahepatic resistance to portal inflow. This is mostly caused by increased deposition of extracellular matrix and disruption of the normal hepatic vascular architecture\(^4\). However, a significant proportion of portal resistance is attributable to intrahepatic vasoconstriction which is caused by the contraction of activated perisinusoidal hepatic stellate cells and of vascular smooth muscle cells in portal venules\(^5\). It is this variable component of intrahepatic resistance, mediated by powerful intrahepatic vasoconstrictors such as angiotensin II and endothelin\(^6\), which is potentially amenable to pharmacological therapies.

The second and equally important contributor in the development of portal hypertension is splanchnic vasodilatation which increases portal blood flow\(^7\). The mechanisms responsible for splanchnic vasodilatation in portal hypertension are incompletely understood, however, there is considerable evidence from both animal and human studies to suggest that nitric oxide (NO) generated by endothelial NO synthase (NOS) plays a central role\(^8\). One of the key consequences of portal hypertension is the development of portosystemic collaterals, of which the most important clinically are oesophageal varices. These vessels divert much of the increased mesenteric inflow away from the liver. However, even when portal blood flow is entirely diverted through collaterals, portal hypertension persists because of concomitant increases in portal venous inflow caused by increasing splanchnic vasodilatation. It should be noted that although the formation of these collaterals has been assumed to be the result of dilatation of pre-existing vascular channels, recent studies have implicated a process of neoangiogenesis which has been shown to contribute to both the formation of portosystemic collaterals and increased splanchnic blood flow\(^7\).

Bleeding from oesophageal varices is responsible for much of the mortality and morbidity that occurs in patients with portal hypertension. Varices are present in about 50% of patients at diagnosis, and this increases to about 90% on long-term follow up. The risk of variceal rupture is 10%-30% per year depending on their size and appearance and the severity of liver disease, and the risk of mortality from a single episode is around 15%-20%\(^9\). Thus prevention or control of variceal bleeding has been the primary aim of the drug treatments that have been used in an attempt to lower portal pressure.

In theory portal pressure should fall in response to drugs that reduce portal inflow or those that lower intrahepatic resistance to portal inflow. The drugs most widely used in the prevention of variceal bleeding are non-selective β-blockers (NSBB). They reduce portal pressure by reducing cardiac output via β-1 receptors and causing splanchnic vasoconstriction by blocking β-2 receptors, resulting in unopposed α-1 activity. Randomized clinical trials showed NSBB reduce portal pressure and the risk of bleeding from oesophageal varices\(^10\-26\).

However around 15% of cirrhotic patients are intolerant of NSBB treatment, and up to 60% fail to achieve the treatment response required to prevent variceal bleeding defined as a fall in hepatic venous pressure gradient (HVPG) to less than 12 mmHg or a decrease of greater than 20% from baseline\(^27\). Although portal pressure is directly correlated with the presence of varices, lowering pressure with NSBB does not prevent the development of varices in patients with cirrhosis\(^28\).

Another approach is to reduce intrahepatic resistance with drugs that increase the delivery of NO to the intrahepatic circulation (e.g., nitrates), or drugs that block α-adrenergic activity (e.g., prazosin, clonidine). Although modest reduction in HVPG can be achieved with these drugs, their use as monotherapy is not recommended as they not only act on the intrahepatic circulation but also exert a vasodilatory effect on the systemic circulation, leading to arterial hypotension\(^29\). A recent small placebo-controlled randomized study showed that simvastatin, a drug that originally developed for hypercholesterolemia and shown to act through the posttranslational modification of endothelial NOS (eNOS), significantly reduced HVPG in cirrhotic patients without altering the blood flow. This suggested that simvastatin improved HVPG by reducing intrahepatic vascular resistance\(^30\).

A number of vasoconstrictor drugs which increase splanchnic vascular tone have been shown to be effective in controlling acute variceal bleeding. The vasopressin analogue, terlipressin, acts on vascular V1 receptors in both the mesenteric and systemic arterial beds to mediate vasoconstriction, and as a result, the drug lowers mesenteric inflow and portal pressure. Terlipressin is generally well-tolerated, but there remains a small incidence of ischaemic events which respond to cessation of the drug\(^31\). This drug reduces the relative risk of mortality from acute variceal bleeding by approximately one third\(^32\). Moreover, terlipressin (plus albumin) is the only treatment shown to prolong short-term survival in type 1 hepatorenal syndrome (HRS)\(^33\). Somatostatin and its analogues octreotide and vaporeotide are splanchnic...
vasoconstrictors which act by inhibiting glucagon secretion and by a local mesenteric vasoconstrictive effect. Although these drugs have a role in the treatment of acute variceal bleeding in combination with endoscopic therapy, they do not reduce mortality in this setting compared to endoscopic therapy alone and are ineffective in HRS.

In summary, NSBBs are widely accepted as the main pharmacotherapy currently available for prevention of variceal bleeding. However, a significant proportion of patient fail to achieve an optimal response or do not tolerate treatment and no other drugs have an established role in the long-term treatment of portal hypertension. Thus there remains a major need to develop more safe and effective treatment options for the treatment of portal hypertension.

NEW CONCEPTS IN RAS PHYSIOLOGY

In recent years it has been shown that the RAS is a much more complex enzymatic pathway than previously thought. It has been long recognized that the RAS plays a central role in cardiovascular and fluid homeostasis via the formation of the potent vasoconstrictor angiotensin II. However, it is now clear that in addition to its vasoactive roles the “classical” axis of the RAS, comprising angiotensin II, angiotensin converting enzyme (ACE) and the angiotensin II type 1 receptor (AT1R), plays a role in the wound healing response to chronic tissue injury and contributes to inflammation, cell proliferation and fibrogenesis. In addition an “alternate” axis of the RAS has been characterized comprising ACE2, a structural homologue of ACE, its peptide product angiotensin-(1-7) and the Mas receptor, which has effects that counterbalance those mediated by the classical axis (Figure 1).

Early studies showed that angiotensin-(1-7) can be generated from angiotensin I by the actions of endopeptidases such as prolyl oligopeptidase and thimet oligopeptidase in tissue and in the circulation by neutral endopeptidase. Whilst the various endopeptidases have been shown to produce angiotensin-(1-7) depending upon their tissue localization and access to substrates, emerging evidence suggests that ACE2 which has a distinct enzyme activity, plays a key role in angiotensin-(1-7) production in several tissues. ACE2 is able to generate angiotensin-(1-7) from angiotensin I indirectly through an intermediary peptide angiotensin-(1-9); however, in comparison, ACE2 has an approximately 400-fold higher substrate preference for angiotensin II which suggests that ACE2 is important not only for production of angiotensin-(1-7) but also in degrading angiotensin II. Recently, Westwood and Chappell described another pathway in which angiotensin-(1-7) is generated directly from angiotensin-(1-12) or via angiotensin I generation.

Angiotensin-(1-7), an effector peptide of the alternate axis of the RAS, is a vasodilator in several vascular beds and has been shown to act mainly via its receptor Mas. However, the existence of a receptor population that is insensitive to blockade with Mas receptor blocker A779 has also been reported. It appears that angiotensin-(1-7), upon binding to its receptor, activates diverse pathways of intracellular signalling, leading to vasodilatation. For example, vasodilatory prostacyclin and/or NO appear to be involved in the response to angiotensin-(1-7) in the regional vascular beds. Therefore appears that angiotensin-(1-7)-stimulated intracellular signaling leading to vasodilatation is depending upon the vascular bed under study and under differing pathophysiological condition.

Most components of the RAS are expressed in the liver, which is the primary source of angiotensinogen synthesis. Recent findings from our laboratory and others suggest that this intrahepatic RAS plays an important role in liver fibrosis since it is markedly upregulated in liver injury and, and blockade of the RAS improves experimental hepatic fibrosis. The alternative axis of the RAS is also expressed in the liver and upregulated in liver disease leading to the generation of angiotensin-(1-7). The major pathway responsible for the generation of angiotensin-(1-7) in the cirrhotic liver is degradation of angiotensin II by ACE2 (Figure 2) confirming previous in vitro findings that ACE2 has the highest substrate preference towards angiotensin II. However there is limited data regarding the possible role of the alternate RAS in liver fibrosis and in modulating intrahepatic blood flow.

RAS AND INTRAHEPATIC RESISTANCE

As outlined above, in patients with cirrhosis, the development of portal hypertension results from both an increase in the intrahepatic resistance to portal flow and an associated vasodilatation of the mesenteric vascular bed which leads to an increase in mesenteric blood flow. Splanchnic and systemic vasodilatation leads to secondary activation of vasoconstrictor pathways such as the sympathetic nervous system and the RAS in an attempt to maintain systemic vascular filling and blood pressure. However these changes fail to correct the underlying circulatory hemodynamics. There is now increasing evidence that in addition to its well recognized role in the homeostatic response to vasodilatation in cirrhosis the RAS may also play a primary role in the pathogenesis and maintenance of portal hypertension.

Hepatic structural changes such as tissue remodeling and scarring play a central role in increasing hepatic resistance to portal flow in the cirrhotic liver. However, when activated, hepatic stellate cells adopt a contractile myofibroblast phenotype, express the AT1R and have been shown in vitro to contract in response to angiotensin II and other vasoconstrictors such as endothelin-1. The vasoconstriction response to angiotensin II is markedly increased in the perfused cirrhotic liver compared to normal livers, presumably mediated via
Figure 1  Overview of the renin angiotensin system. The effects of the renin angiotensin system (RAS) are determined by the balance between the angiotensin II (Ang II) mediated "classical" axis, depicted in blue, which is vasoconstrictive and the angiotensin-(1-7) [Ang-(1-7)] mediated "alternate" axis, depicted in orange, which is vasodilatory. Both Ang II and Ang-(1-7) can stimulate the Ang II type 2 (AT2) receptor, depicted in green; the effects of which are often analogous to those mediated by the Ang-(1-7) receptor Mas. Recent evidence indicates that a new member, Ang-(1-12), which is cleaved from angiotensinogen, also contributes either indirectly via Ang I or directly to the pool of Ang-(1-7). NEP: Neutral endopeptidase; ACE: Angiotensin converting enzyme; ACE2: Angiotensin converting enzyme 2; AT1 receptor: Angiotensin II type 1 receptor.

Figure 2  Intrahepatic enzymatic pathways of renin angiotensin system peptide production. Schematic representation of the pathways responsible for the generation of vasodilator peptide angiotensin-(1-7) [Ang-(1-7)] in rat liver. The colour of the arrows represents the relative contribution of each pathway for ex vivo formation of Ang-(1-7) from the substrates angiotensin I (Ang I) and angiotensin II (Ang II) in cirrhotic rat liver. The most efficient pathway is represented by blue line, followed by green line. The black line is the least efficient pathway whilst red represents the efficiency less than green but higher than black. The yellow colour represents an efficient pathway to generate Ang-(1-7) directly from Ang II. Although eNOS activity was not measured in this study, the calcium binding protein calmodulin competitively binds eNOS and reduces caveolin binding, thus increasing eNOS activity. There is considerable evidence that another important contributor to elevated vascular tone in the cirrhotic liver is endothelial dysfunction of the hepatic microcirculation which diminishes the response to vasodilators. It has been proposed that the reduced activity of hepatic vascular eNOS with concomitant reduction in NO synthesis impairs intrahepatic vasodilatation and thus, shifts the balance towards vasoconstriction.

Recent findings from our laboratory demonstrated that in-vm perfused cirrhotic rat liver elicited a marked endothelium-dependent vasodilatory effect of exogenous angiotensin-(1-7) on the vasoconstrictive response evoked by angiotensin II (Figure 3). This finding suggests that as in other vascular beds, in the cirrhotic liver angiotensin-(1-7) may cause a vasodilatory response that antagonizes the increase in portal pressure mediated by angiotensin II and other local vasoconstrictors. Although eNOS activity was not measured in this study, it is likely that eNOS activity is upregulated in the cirrhotic liver.
study, the eNOS inhibitor nitro-L-arginine methyl ester, L-NAME, completely abolished eNOS phosphorylation at Ser1177 and the response to angiotensin-(1-7)[59]. Whilst bradykinin via its B2 receptor mediates vasodilation in response to angiotensin-(1-7) in porcine and canine coronary arteries[56,58,74], it increases intrahepatic resistance and portal pressure[75], possibly through acting on B2 receptors on stellate cells. However, angiotensin-(1-7)-induced vasodilation in the cirrhotic liver was not affected by bradykinin B2 receptor blockade[53]. Possible mechanisms for these effects of angiotensin-(1-7) in the cirrhotic rat liver include increased phosphorylation of eNOS Ser1177[59] with simultaneous dephosphorylation at Thr495 and/or effects on calmodulin binding[76]. These findings suggest that it may be possible to reduce intrahepatic resistance and portal pressure by targeting the alternate axis of the RAS in the liver.

**RAS AND SPLANCHNIC VASODILATATION**

In contrast to intrahepatic hypervascular tone, the systemic and splanchnic circulation in cirrhosis is characterized by vasodilatation and hyporesponsiveness to vasoconstrictors including angiotensin II [65,67,77]. Interestingly, recent studies have shown that systemic levels of the vaso-elastic peptide angiotensin-(1-7) increase as liver fibrosis progresses, whereas angiotensin II levels do not generally rise until cirrhosis is established with concurrent portal hypertension[65,78]. Furthermore, regional levels of the hormone are different from systemic levels such that in cirrhotic patients at transplant, the angiotensin-(1-7)/angiotensin II ratio is elevated in the splanchnic compared to the peripheral circulation, and negatively correlates with systemic vascular resistance[78]. The recent findings suggest that angiotensin-(1-7) might contribute to vasodilation in cirrhosis. Recent work from our laboratory provides support for this hypothesis[79]. We have shown that ACE2 is upregulated in cirrhotic mesenteric vessels and although angiotensin-(1-7) has no effect in the normal mesenteric circulation, it significantly reduces mesenteric vascular contractility in cirrhotic mesenteric beds via activation of the Mas receptor and the release of NO[79].

Studies using isolated vessel preparations from cirrhotic animal models or portal hypertensive rats have led to the concept that vasodilation is also linked to an intrinsic vascular hyporesponsiveness to endogenous vasoconstrictors such angiotensin II, α-adrenergic agonists and endothelin-1[80,82]. This concept is supported by the findings that peripheral vessels are hyporesponsive to angiotensin II, α-adrenergic agonists and endothelin-1 from cirrhotic animals and cirrhotic patients[78,83,94], despite the fact that expression of AT1R and α-adrenergic receptor subtypes 1a and 1b in the peripheral vessels is either normal or upregulated in both cirrhotic animals and patients[79,87,89]. However, previous studies in patients with cirrhosis or in peripheral resistance vessels obtained from such patients reported variable results in this regard[90,91,92], probably attributable to the differences between conditions in different studies. Indeed, small resistance omental vessels from cirrhotic patients had a larger vasoconstriction response to α-adrenergic agonists norepinephrine and methoxamine than similar vessels from healthy controls[89]. The same vessels vasodilated in response to substance P but this was inhibited by blocking NO or prostacyclin synthesis, suggesting that intrinsic hyporeactivity that is present in the peripheral circulation in cirrhosis is related to increased levels of NO.
and prostanoids[83-90]. Hyporeactivity to angiotensin II infusion is also improved after inhibiting systemic NO production using an NO-clamp in cirrhotic patients[88]. This is in keeping with a wealth of literature suggesting that vasodilatory molecules including angiotensin-(1-7) are produced in excess in cirrhosis and that the final in vitro pressor effect is governed by a balance between the pressor and depressor arms of the circulation[90].

There are also data, from both in vitro and in vivo studies, suggesting that vascular hyporeactivity to a range of endogenous pressors is attributed to changes that are downstream of the G-protein coupled receptors[86,88]. Evidence supporting the existence of vascular endothelium/NO independent pathways comes from studies in which endothelium denudation and pharmacological blockade of NOS in isolated vessels from cirrhotic animals and patients failed to improve vascular hyporeactivity to a range of vasoconstrictors[83,84,86,87,97-99]. One of the important NO-independent pathways is an impaired signaling by RhoA and Rho kinase, leading to a decreased phosphorylation of Ca^2+-sensitizing proteins and increased myosin light chain phosphatase activity[87]. Moreover, increased expression of receptor desensitizing proteins, G protein-coupled receptor kinase 2 and β-arrestin-2, have also been implicated in this hyporeactivity to angiotensin II in vessels isolated from cirrhotic patients and rats[87]. It was also shown that mesenteric arteries from portal hypertensive rats had a reduced level of membrane associated RhoA, probably reflecting a diminished activity of RhoA/Rho kinase pathway which in turn results in increased activity of myosin light chain phosphatase and vasodilatation[45,46].

**THERAPIES TARGETING THE RAS IN PORTAL HYPERTENSION**

**Therapeutic potential of the classical RAS**

The evidence from studies in experimental cirrhosis showing the angiotensin II contributes to the variable component of intrahepatic resistance in portal hypertension have provided a rationale for a number of trials examining the effects of ACE inhibitors and angiotensin receptors blockers (ARBs)[2,3,23,87] on portal pressure. Unfortunately many of these studies are small or non-randomized and there is very little long-term data. However Tandon et al.[24] in a recent meta-analysis of individual patient data from three and nine studies that used ACE inhibitors and ARBs, respectively, showed that patients with Child Pugh A cirrhosis receiving ARBs/ACE inhibitors had a similar reduction in HVPG (17%) compared to patients with Pugh A cirrhosis that received NSBB (21%). There was no improvement of HVPG in patients with Child Pugh B/C cirrhosis receiving ARBs. Furthermore, several studies reported that RAS blockade can result in significant hypotension and renal impairment in patients with decompensated (Child Pugh B/C) cirrhosis[25] in whom there is activation of the systemic RAS.

Thus, although ARBs/ACE inhibitors do lower portal pressure in early cirrhotic patients where the activation of RAS may be a predominant pathway responsible for increased intrahepatic tone, they have less effect in late stages of cirrhosis. This probably reflects the fact that the hypotension induced by RAS blockers increases activation of other vasoconstrictive pathways such as the sympathetic nervous system that in turn increase intrahepatic vascular tone[2,8,101-103]. Further studies are needed to clarify whether this class of drugs could be useful in the prevention of variceal bleeding in patients with compensated cirrhosis as an alternative to or possibly even in combination with β-blockers.

**Alternate RAS-a novel potential target for the treatment of portal hypertension**

Recent animal studies focusing on the alternate RAS have led to the suggestion that new generation antihypertensives developed to target this axis could serve as effective therapeutic agents to treat arterial, pulmonary and portal hypertension[65,86,104,105]. Recent work outlined in this review demonstrates the presence of all of the key components of the alternate RAS in the liver and mesenteric vasculature of both healthy and cirrhotic animals as well as in the liver of healthy and cirrhotic patients[8,10,66,78]. Furthermore circulating angiotensin-(1-7) levels are increased[96,97] in cirrhosis suggesting that it plays an important role in the pathophysiology of hepatic fibrosis and portal hypertension (Figure 4). This evidence linking elevated angiotensin-(1-7) levels to mesenteric and systemic vasodilatation in cirrhosis suggests that blocking the alternate axis could reduce mesenteric flow and thus lower portal pressure. In line with this, Mas receptor blockade would provide an intervention option in portal hypertension[97] as this treatment regime does not appear to compromise the vasodilatory response of angiotensin-(1-7) within the hepatic vasculature in experimental cirrhosis[88]. Further studies are clearly needed examining the effects of Mas blockade and angiotensin-(1-7) on hepatic and mesenteric haemodynamics in experimental cirrhosis in vivo.

**CONCLUSION**

Recent developments in our understanding of the complexities of the RAS and its role in the pathogenesis of chronic liver disease and portal hypertension have opened up new therapeutic possibilities. It is clear that the classical axis of the RAS and its key effector peptide angiotensin II play a central role in hepatic fibrogenesis and in regulating intrahepatic vascular tone in cirrhosis and that despite the mixed results achieved in previous trials, consideration should be given to further prospective studies examining the effects of RAS blockers in patients with compensated cirrhosis. There is also fascinating new evidence showing that there is increased regional production of angiotensin-(1-7) in the mesenteric vascular bed in cirrhosis, and that this vasodilatory
peptide of the alternate axis of the RAS, contributes to mesenteric vasodilatation and the hyperdynamic circulation in cirrhosis. These novel data suggest that ACE2-angiotensin-(1-7)-Mas receptor axis is a potential target for the management of portal hypertension.

REFERENCES

1. Ferrari 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-H2290 [PMID: 16055515 DOI: 10.1152/ajpheart.00618.2005]

2. Bataller R, Brenner DA. Liver fibrosis. J Clin Invest 2005; 115: 209-218 [PMID: 15690074 DOI: 10.1172/JCI20524282]

3. Töx U, Steffen HM. Impact of inhibitors of the Renin-Angiotensin-aldosterone system on liver fibrosis and portal hypertension. Curr Med Chem 2006; 13: 3649-3661 [PMID: 17168728 DOI: 10.2174/092986706779026138]

4. Dickstein K, Kjekshus J. Effects of losartan and captopril on mortality and morbidity in high-risk patients after acute myocardial infarction: the OPTIMAAL randomised trial. Optimal Trial in Myocardial Infarction with Angiotensin II Antagonist Losartan. Lancet 2002; 360: 752-760 [PMID: 12241832 DOI: 10.1016/S0140-6736(02)09891-1]

5. Paizis G, Gilbert RE, Cooper ME, Murthy P, Schembri JM, Wu LL, Rumble JR, Kelly DJ, Tikellis C, Cox A, Smallwood RA, Angus PW. Effect of angiotensin II type 1 receptor blockade on experimental hepatic fibrogenesis. J Hepatol 2001; 35: 376-385 [PMID: 11592599 DOI: 10.1016/S0168-8278(01)00146-5]

6. Parving HH, Persson F, Lewis JB, Lewis EJ, Hollenberg NK. Aliskiren combined with losartan in type 2 diabetes and nephropathy. N Engl J Med 2008; 358: 2433-2444 [PMID: 18525041 DOI: 10.1056/NEJMoa0708379]

7. Pinzani M, Gentilini P. Biology of hepatic stellate cells and their possible relevance in the pathogenesis of portal hypertension in cirrhosis. Semin Liver Dis 1999; 19: 397-410 [PMID: 10643625 DOI: 10.1055/s-2007-1007128]

8. Angus PW. Role of endothelin in systemic and portal resistance in cirrhosis. Gut 2006; 55: 1230-1232 [PMID: 16905694 DOI: 10.1136/gut.2005.088663]

9. Bataller R, Gebele E, Schoonhoven R, Morris T, Lehnert M, Yang L, Brenner DA, Rippe RA. Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. Am J Physiol Gastrointest Liver Physiol 2003; 285: G642-G651 [PMID: 12773299]

10. Bataller R, Gines P, Nicolas JM, Gorbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V, Rodes J. Angiotensin II induces contraction and proliferation of human hepatic stellate cells. Gastroenterology 2000; 118: 1149-1156 [PMID: 10833490 DOI: 10.1056/S0016-5085(00)70368-4]

11. Herath CB, Warner FJ, Lubel JS, Dean RG, Jia Z, Lew RA, Smith AJ, Burrell LM, Angus PW. Upregulation of hepatic angiotensin-converting enzyme 2 (ACE2) and angiotensin-(1-7) levels in experimental biliary fibrosis. J Hepatol 2007; 47: 387-395 [PMID: 17532087 DOI: 10.1016/j.jhep.2007.03.008]

12. Wiest R, Groszmann RJ. Nitric oxide and portal hypertension: its role in the regulation of intrahepatic and splanchnic vascular resistance. Semin Liver Dis 1999; 19: 411-426 [PMID: 10643626 DOI: 10.1055/s-2007-1007129]

13. Albornoz I, Motta A, Alvarez D, Estevez A, Bandi JC, McCormack L, Matera J, Bonofiglio C, Ciardullo M, De Santibanes E, Gimeno M, Gadán A. Nitric oxide synthase activity in the splanchnic vascularity of patients with cirrhosis: relationship with hemodynamic disturbances. J Hepatol 2001; 35: 452-456 [PMID: 11682028 DOI: 10.1016/S0168-8278(01)00168-4]

14. Sarela AJ, Mihaiemer FD, Batten J, Davidson BR, Mathie
28 Groszmann RJ, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; 353: 2254-2261 [DOI: 10.1056/NEJMoa044456]

29 Garcia-Tsao G. Current management of the complications of cirrhosis and portal hypertension: varical hemorrhage, ascites, and spontaneous bacterial peritonitis. *Gastroenterology* 2001; 120: 726-748 [DOI: 11179247 DOI: 10.1016/gastro.2001.22580]

Abraides JG, Albillos A, Bañares R, Turnes J, Gonzalez R, Garcia-Pagan JC, Bosch J. Simvastatin lowers portal pressure in patients with cirrhosis and portal hypertension: a randomised controlled trial. *Gastroenterology* 2009; 136: 1651-1658 [DOI: 1928350 DOI: 10.1053/j.gastro.2009.01.043]

Testro AG, Wongseelashote S, Angus PW, Gow PJ. Long-term outcome of patients treated with terlipressin for types 1 and 2 hepatorenal syndrome. *J Gastroenterol Hepatol* 2008; 23: 1535-1540 [DOI: 17784863 DOI: 10.1111/j.1440-1746.2007.05176.x]

Joannou G, Doust J, Rockey DC. Terlipressin for acute esophageal varical hemorrhage. *Cochrane Database Syst Rev* 2003; (1): CD002147 [DOI: 12535432]

Glud LL, Christensen K, Christensen E, Krag A. Systematic review of randomized trials on vasoconstrictor drugs for hepatorenal syndrome. *Hepatology* 2010; 51: 576-584 [DOI: 19885875 DOI: 10.1002/hep.23286]

Wiest R, Tsai MH, Groszmann RJ. Octreotide potentiates PKC-dependent vasoconstrictors in portal-hypertensive and control rats. *Gastroenterology* 2001; 120: 975-983 [DOI: 11231951 DOI: 10.1053/gast.2001.22529]

Bañares R, Albillos A, Rincón D, Alonso S, González M, Ruiz-del-Arbel L, Salcedo M, Molinero LM. Endoscopic treatment versus endoscopic plus pharmacologic treatment for acute varical bleeding: a meta-analysis. *Hepatology* 2002; 35: 609-615 [DOI: 11680734 DOI: 10.1053/hep.2002.31354]

D’Amico G, Politi F, D’Antoni A, Giannuoli G, Pasta L, Vizziani G, Traina M, Morabito A, Pagliaro L. Second study shows that octreotide may prevent early rebleeding in cirrhosis. *BMJ* 1998; 316: 1320 [DOI: 9554914 DOI: 10.1136/bmj.316.7140.1320]

Pomier-Layrargues G, Paquin SC, Hassoun Z, Lafortune M, Tran A. Octreotide in hepatorenal syndrome: a randomized, double-blind, placebo-controlled, crossover study. *Hepatology* 2003; 38: 236-243 [DOI: 12830007 DOI: 10.1053/jhep.2003.50276]

Lubel JS, Herath CB, Burrell LM, Angus PW. Liver disease and the renin-angiotensin system: recent discoveries and clinical implications. *J Gastroenterol Hepatol* 2008; 23: 1327-1338 [PMID: 18557800 DOI: 10.1111/j.1440-1746.2008.05461.x]

Kagami S, Border WA, Miller DE, Noble NA. Angiotensin II stimulates extracellular matrix protein synthesis through induction of transforming growth factor-beta expression in rat glomerular mesangial cells. *J Clin Invest* 1994; 93: 2431-2437 [DOI: 8200978 DOI: 10.1172/JCI17251]

Marrero MB, Scheffer B, Paxton WG, Heerdlt L, Berk BC, Delafontaine P, Bernstein KE. Direct stimulation of Jak/STAT pathway by the angiotensin II AT1 receptor. *Nature* 1995; 375: 247-250 [DOI: 7746328 DOI: 10.1038/375247a0]

Mezzano SA, Ruiz-Ortega M, Egidio J. Angiotensin II and renal fibrosis. *Hypertension* 2001; 38: 635-638 [DOI: 11566946 DOI: 10.1161/01.hyp.00000894234]

Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V, Egidio J. Inflammation and angiotensin II. *Int J Biochem Cell Biol* 2003; 35: 881-900 [DOI: 12676174 DOI: 10.1016/s1357-2725(02)00271-6]

Santos RA, Brosnihan KB, Jacobsen DW, DiCorletto PE, Ferrario CM. Production of angiotensin-(1-7) by human vascular endothelium. *Hypertension* 1992; 19: 156-161 [DOI: 1310484 DOI: 10.1161/01.HYP.19.2_Suppl.I156]

RT. Hepatic and splanchnic nitric oxide activity in patients with cirrhosis. *Lancet* 1995; 346: 1056-1059 [PMID: 8268357 DOI: 10.1007/NejMo2003252]

27 Feu F, Garcia-Pagan JC, Bosch J, Luca A, Teres J, Escorsell A, Rodes J. Relation between portal pressure response to pharmacotherapy and risk of recurrent varical haemorrhage in patients with cirrhosis. *Lancet* 1995; 346: 1056-1059 [PMID: 8268357 DOI: 10.1007/NejMo2003252]
44 Chappell MC, Gomez MN, Pirro NT, Ferrario CM. Release of angiotensin-(1-7) from the rat hindlimb: influence of angiotensin-converting enzyme inhibition. Hypertension 2000; 35: 348-352 [PMID: 10642522 DOI: 10.1161/01.HYP.35.3.348

45 Yamamoto K, Chappell MC, Brosnihan KB, Ferrario CM. In vivo metabolism of angiotensin I by neutral endopeptidase (EC 3.4.24.11) in spontaneously hypertensive rats. Hypertension 1992; 19: 692-696 [PMID: 1317352 DOI: 10.1161/01.HYP.19.6.692]

46 Warner FJ, Smith AI, Hooper NM, Turner AJ, Angiotensin-converting enzyme-2: a molecular and cellular perspective. Cell Mol Life Sci 2004; 61: 2704-2713 [PMID: 15549171 DOI: 10.1007/s00018-004-2420-7]

47 Vickers C, Hales P, Kauksh V, Dick L, Gavin J, Tang J, Godbout K, Parsons T, Barons E, Hsieh F, Acton S, Patane M, Nichols A, Tummino P. Hydrolysis of biological peptides by human angiotensin-converting enzyme-related carboxypeptidase. J Biol Chem 2002; 277: 14838-14843 [PMID: 11815627 DOI: 10.1074/jbc.M200581200]

48 Westwood BM, Chappell MC. Divergent pathways for the angiotensin-(1-12) metabolism in the rat circulation and kidney. Peptides 2012; 35: 190-195 [PMID: 22490446 DOI: 10.1016/j.peptides.2012.03.025]

49 Benter IF, Yousif MH, Anim JT, Cojocel C, Diz DI. Angiotensin-(1-7) prevents development of severe hypertension and end-organ damage in spontaneously hypertensive rats treated with L-NAME. Am J Physiol Heart Circ Physiol 2006; 290: H684-H697 [PMID: 16403946 DOI: 10.1152/ajpheart.00632.2005]

50 Castro CH, Santos RA, Ferreira AJ, Bader M, Alenina N, Almeida AP. Evidence for a functional interaction of the angiotensin-(1-7) receptor Mas with AT1 and AT2 receptors in the mouse heart. Hypertension 2005; 46: 937-942 [PMID: 16157795 DOI: 10.1161/01.HYP.0000178313.04757.8a]

51 Lemos VS, Silva DM, Walther T, Alenina N, Bader M, Santos RA. The endothelium-dependent vaso dilator effect of the nonpeptide Ang(1-7) mimick AVE 0991 is abolished in the aorta of mas-knockout mice. J Cardiovasc Pharmacol 2005; 46: 274-279 [PMID: 16116331 DOI: 10.1097/01.jcp.0000175237.41573.63]

52 Neves LA, Williams AF, Averill DB, Ferrario CM, Walkup MP, Brosnihan KB. Pregnancy enhances the angiotensin (Ang)-(1-7) vasodilator response in mesenteric arteries and end-organ damage in spontaneously hypertensive rats. Hypertension 2003; 41: 3338-3343 [PMID: 12863311 DOI: 10.1161/01.HYP.0000094549.98873.2d]

53 Santos RA, Simoes e Silva AC, Maric C, Silva DM, Machado RP, de Buhr I, Heringer-Walther S, Pinheiro SV, Lopes MT, Bader M, Mendes EP, Lemos VS, Campagnole-Santos MJ, Schulteis HP, Spath R, Walther T. Angiotensin-(1-7) is an endogenous ligand for the G protein-coupled receptor Mas. Proc Natl Acad Sci USA 2003; 100: 8258-8263 [PMID: 12829792 DOI: 10.1073/pnas.1432869100]

54 Silva DM, Vianna HR, Cortes SF, Campanogolo-Santos MJ, Santos RA, Lemos VS. Evidence for a new angiotensin-(1-7) receptor subtype in the aorta of Sprague-Dawley rats. Peptides 2007; 28: 702-707 [PMID: 17129638 DOI: 10.1016/j.peptides.2006.10.007]

55 Herath CB, Mak K, Burrell LM, Angus PW. Angiotensin-(1-7) reduces the perfusion pressure response to angiotensin II and methoxamine via an endothelial nitric oxide-mediated pathway in cirrhotic rat liver. Am J Physiol Gastrointest Liver Physiol 2013; 304: G99-G108 [PMID: 23086915 DOI: 10.1152/ajpgi.00163.2012]

56 Brosnihan KB, Li P, Ferrario CM. Angiotensin-(1-7) dilates canine coronary arteries through kinins and nitric oxide. Hypertension 1996; 27: 523-528 [PMID: 8613197 DOI: 10.1161/01.HYP.27.3.525]

57 Ferrario CM, Chappell MC, Tallant EA, Brosnihan KB, Diz DI. Counterregulatory actions of angiotensin-(1-7). Hyper-
RAS and portal hypertension

71 Rocky DC. Vasoactive agents in intrahepatic portal hypertension: implications for therapy. Gastroenterology 2000; 118: 1261-1265 [PMID: 10583501 DOI: 10.1016/S0016-5085(00)00729-9]

72 Shah V, Cao S, Hendrickson H, Yao J, Katusic ZS. Regulation of hepatic eNOS by caveolin and calmodulin after bile duct ligation in rats. Am J Physiol Gastrointest Liver Physiol 2001; 280: G1209-G1216 [PMID: 11352814]

73 Shah V, Toruner M, Haddad F, Cadelina G, Papapetropoulos A, Choo K, Sessa WC, Groszmann RJ. Impaired endothelial nitric oxide synthase activity associated with enhanced caveolin binding in experimental cirrhosis in the rat. Gastroenterology 1999; 117: 1222-1228 [PMID: 10558866 DOI: 10.1016/S0016-5085(99)70408-7]

74 Gorelik G, Carbini LA, Sciclai AG. Angiotensin 1-7 induces bradykinin-mediated relaxation in porcine coronary artery. J Pharmacol Exp Ther 1998; 286: 403-410 [PMID: 9658858]

75 Göli-Pereira L, Nascimento EA, Santos EL, Bracht A, Juliano MA, Pesquero JB, Borges DR, Kouyoumdjian M. Fate of bradykinin on the rat liver when administered by the venous or arterial route. J Gastroenterol Hepatol 2005; 20: 463-473 [PMID: 15740493 DOI: 10.1111/j.1440-1746.2005.03580.x]

76 Fleming I, Fisslthaler B, Dimmeler S, Kemp BE, Busse R. Phosphorylation of Thr(495) regulates Ca2+/-calmodulin-dependent endothelial nitric oxide synthase activity. Circ Res 2001; 88: E68-E75 [PMID: 11397791 DOI: 10.1161/01.hir.010267]

77 Fertiltsch A, Pleiner J, Mittermayer F, Schaller G, Homoncik M, Peck-Radosavljevic M, Wolzt M. Venosconstrictor hyperreactivity can be reversed by antioxidants in patients with advanced alcoholic cirrhosis of the liver and ascites. Crit Care Med 2005; 33: 2028-2033 [PMID: 16148476]

78 Vilas-Boas WW, Ribeiro-Oliveira A, Pereira RM, Ribeiro Rda C, Almeida J, Nadu AP, Simões e Silva AC, dos Santos RA. Relationship between angiotensin-(1-7) and angiotensin II correlates with hemodynamic changes in human liver cirrhosis. World J Gastroenterol 2009; 15: 2512-2519 [PMID: 19469002 DOI: 10.3748/wjg.v15.i15.2512]

79 Grace JA, Jia Z, Herath CB, Burrell LM, Angus PW. Angiotensin-(1-7) is a mesenteric vasodilator in experimental cirrhosis. Hepatology 2010; 52: 919A-1020A

80 Miñano C, García-Tsao G. Clinical pharmacology of portal hypertension. Gastroenterol Clin North Am 2010; 39: 681-695 [PMID: 20951924 DOI: 10.1016/j.gtc.2010.08.015]

81 Schrier RW, Biecker E, Trebicka J, Kohistani AZ, Heller J, Sau erbruch T. Vascular hyporesponsiveness to angiotensin II in rats with CCl4-induced liver cirrhosis. Eur J Clin Invest 2009; 39: 906-913 [PMID: 19522833 DOI: 10.1111/j.1365-2362.2009.02181.x]

82 Kiel JW, Pitts V, Benoit N, Granger DN, Shepherd AP. Reduced vascular sensitivity to norepinephrine in portal-hypertensive rats. Am J Physiol 1985; 248: G192-G195 [PMID: 3970220]

83 Newby DE, Jalan R, Masumori S, Hayes PC, Boon NA, Webb DJ. Peripheral vascular tone in patients with cirrhosis: role of the renin-angiotensin and sympathetic nervous systems. Cardiovasc Res 1998; 38: 221-228 [PMID: 9683925 DOI: 10.1016/S0008-6369(98)00008-X]

84 Neef M, Biecker E, Heller J, Schepke M, Nischalke HD, Wolf M, Spengler U, Reichen J, Sauerbruch T. Portal hypertension is associated with increased mRNA levels of vasopressor G-protein-coupled receptors in human hepatic arteries. Eur J Clin Invest 2013; 33: 249-255 [PMID: 12641544 DOI: 10.1046/j.1365-2362.2003.0131.x]

85 Vaughn RB, Angus PW, Angus JA. Enhanced vascular responses to noradrenaline in isolated omental arteries from patients with advanced cirrhosis: evidence against intrinsic vascular hyporeactivity in cirrhosis. Gut 2004; 53: 1544-1545 [PMID: 15361610]

86 Langer DA, Shah VH. Nitric oxide and portal hypertension: interface of vasoreactivity and angiogenesis. J Hepatol 2006; 44: 209-216 [PMID: 16297493 DOI: 10.1016/j.jhep.2005.10.004]

87 Shah V, Cellular and molecular basis of portal hypertension. Clin Liver Dis 2001; 5: 629-644 [PMID: 11565134 DOI: 10.1016/S1089-3261(05)70185-9]

88 Vaughan RB, Angus PW, Angus JA. Vasoconstrictor responses are normal but prostanoid-mediated vasodilation is enhanced in human cirrhotic mesenteric arteries. J Gastroenterol Hepatol 2005; 20: 1158-1164 [PMID: 16045562 DOI: 10.1111/j.1440-1746.2005.03946.x]

89 Wiest R, Groszmann RJ. The paradox of nitric oxide in cirrhosis and portal hypertension: too much, not enough. Hepatology 2002; 35: 476-491 [PMID: 11826425 DOI: 10.1035/j.hepat.2002.01342]

90 Colle IO, De Vriese AS, Van Vlietberghke HR, Lameire NH, De Vos MM. Vascular hyporesponsiveness in the mesenteric artery of anaesthetized rats with cirrhosis and portal hypertension: an in-vivo study. Eur J Gastroenterol Hepatol 2004; 16: 139-145 [PMID: 15079986 DOI: 10.1097/00042737-200402000-00004]

91 Mathie RT, Ralevic V, Burnstock G. Portal vascular responsiveness to sympathetic stimulation and nitric oxide in cirrhotic rats. J Hepatol 1996; 25: 90-97 [PMID: 8836907 DOI: 10.1016/S0168-8278(96)80333-3]

92 Schepke M, Heller J, Paschke S, Thomas J, Wolf M, Neef M, Malago M, Molderings GJ, Spengler U, Sauerbruch T. Contractile hyporesponsiveness of hepatic arteries in humans with cirrhosis: evidence for a receptor-specific mechanism. Hepatology 2001; 34: 884-888 [PMID: 11679958 DOI: 10.1033/j.hepatic.2001.26794]

93 Zhang HY, Shirasawa Y, Chen X, Yu H, Benoit N. Impaired agonist-dependent myosin phosphorylation and decreased RhoA in rat portal hypertensive mesenteric vascular bed. Am J Physiol Gastrointest Liver Physiol 2005; 288: G603-G608 [PMID: 15153995 DOI: 10.1152/ajpgi.00116.2004]

94 Alam J, Bass NM, Bacchetti P, Gee L, Rockey DC. He-
patic tissue endothelin-1 levels in chronic liver disease correlate with disease severity and ascites. *Am J Gastroenterol* 2000; 95: 199-203 [PMID: 10638583 DOI: 10.1111/j.1572-0241.2000.01684.x].

102 **Gracia-Sancho J**, Laviña B, Rodríguez-Vilarrupla A, García-Calderó H, Bosch J, García-Pagán JC. Enhanced vasoconstrictor prostanoid production by sinusoidal endothelial cells increases portal perfusion pressure in cirrhotic rat livers. *J Hepatol* 2007; 47: 220-227 [PMID: 17459512 DOI: 10.1016/j.jhep.2007.03.014]

103 **Guturu P**, Shah V. New insights into the pathobiology of portal hypertension. *Hepatol Res* 2009; 39: 1016-1019 [PMID: 19796039 DOI: 10.1111/j.1872-0241.2009.00533.x]

104 **Ferreira AJ**, Raizada MK. Are we poised to target ACE2 for the next generation of antihypertensives? *J Mol Med (Berl)* 2008; 86: 685-690 [PMID: 18449520 DOI: 10.1007/s00109-008-0339-x]

105 **Shenoy V**, Qi Y, Katovich MJ, Raizada MK. ACE2, a promising therapeutic target for pulmonary hypertension. *Curr Opin Pharmacol* 2011; 11: 150-155 [PMID: 21215698 DOI: 10.1016/j.coph.2010.12.002]