

Vascular Mediators in the Injured Liver

Don C. Rockey

The Intrahepatic Vasculature

The vasculature plays a critical role in multiple physiologic and pathophysiologic processes. A number of recent advances have led to renewed focus on the vasculature in the liver. In patients with chronic liver disease, 3 general vascular beds are of importance. These include the peripheral vasculature, the mesenteric vascular bed, and the intrahepatic microcirculatory unit; abnormalities exist in each. For example, vasodilation and reduced resistance in the peripheral and mesenteric vasculature appear to be a result of increased activity of endothelial cell nitric oxide (NO) synthase (NOS) and enhanced NO production¹ (also see Wiest and Groszmann² for review). In contrast, the intrahepatic microcirculation is characterized by increased resistance, the basis of which appears to be multifactorial. This review focuses primarily on the intrahepatic vasculature and the mediators that affect it.

The intrahepatic microvascular unit is made up of several discrete units, including portal venules, hepatic arterioles, sinusoids, central venules, and lymphatics. Intrahepatic resistance, and thus blood flow, may be modulated at several of these sites.³⁻⁵ Vascular smooth muscle cells in terminal portal venules and hepatic venules have been presumed to be the major sites of pre- and postsinusoidal vascular resistance, whereas the hepatic sinusoids have been compared with capillary beds in other tissues, in which smooth-muscle-like pericytes regulate blood flow.⁶ In the sinusoid, endothelial cells and hepatic stel-

late cells have been identified as the cellular elements most likely to be important in regulation of resistance,^{4,7,8} with most data pointing to stellate cells. Importantly, these two cells are intimately associated with each other and have paracrine effects on one another. For example, NO released by sinusoidal endothelial cells appears to have relaxing effects on stellate cells.

Stellate cells possess extensive long, branching, intersinusoidal, and perisinusoidal cytoplasmic processes⁹ and have been shown to contract *in situ* in the normal sinusoid.¹⁰⁻¹² Stellate cell contractility has been studied extensively in isolated cell systems¹³⁻¹⁵—a number of vasoactive substances have been reported to have effects on stellate cells. Although controversy exists regarding the magnitude of stellate cell contraction in the normal liver, available data emphasize that stellate cells in the injured liver exhibit increased contractility and moreover, the degree of contraction appears to be proportional to the degree of liver injury.^{16,17} The mechanism for enhanced stellate cell contractility is coupled to enhanced expression of smooth muscle proteins and modified signaling pathways after their activation (see below for discussion of the activation process).^{18,19} In aggregate, these data suggest a prominent role for stellate cells in the regulation of intrahepatic resistance.

Cellular components of the intrahepatic vasculature (*i.e.*, endothelial cells, smooth muscle cells, and stellate cells) play an important role in vascular responses, as do the specific vasoactive compounds that exert effects on them. A number of vasoactive mediators have received attention, including vasoconstrictors such as endothelin (ET) and angiotensin II, and relaxing agents such as NO and carbon dioxide.

Not only are hepatic stellate cells thought to be an important functional component of the intrahepatic microcirculatory unit, but a large body of literature has emphasized their role in hepatic fibrogenesis. In this regard, a number of vasoactive agents appear to have potent fibrogenic effects on stellate cells. Thus, this review will additionally review the putative role of vascular mediators in the fibrogenic response to injury.

Specific Vasoactive Mediators Important in Liver Disease

Endothelin

The endothelins, discovered in 1988,²⁰ consist of a family of 3 unique 21 amino acid peptides termed ET-1, ET-2, and ET-3. They bind to 2 known G-protein-cou-

Abbreviations: NO, nitric oxide; NOS, nitric oxide synthase; ET, endothelin; mRNA, messenger RNA; ECE-1, endothelin-converting enzyme-1; TGF- β , transforming growth factor β ; RAAS, renin-angiotensin-aldosterone; ACE, angiotensin-converting enzyme; ecNOS, endothelial cell nitric oxide synthase; nNOS, neuronal nitric oxide synthase; iNOS, inducible nitric oxide synthase; CO, carbon monoxide; HO, heme oxygenase; ANP, atrial natriuretic peptide.

From the Departments of Medicine and Cell Biology and The Liver Center, Duke University Medical Center, Durham, NC.

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Address reprint requests to: Don C. Rockey, M.D., Liver Center Laboratory, Duke University Medical Center, Sands Building, Room 336, Research Drive, Durham, NC 27710. E-mail: dcrockey@acpub.duke.edu; fax: 919-684-4983.

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pled receptors, termed endothelin A (ET_A) and endothelin B (ET_B) receptors.²¹ ET_A receptors are found predominantly on vascular smooth muscle cells, whereas ET_B receptors are characteristic of endothelial cells (and have a broader distribution including other cell types). Rank order affinities of peptides for the ET_A receptor are ET-1 > ET-2 >>> ET-3, and the affinity of ET-1 for the ET_A receptor is more than 100-fold that of ET-3.²¹ ET_B receptors have equal affinity for endothelin receptor subtypes.²¹ Central dogma suggests that ET_A receptors on smooth muscle cells mediate vasoconstriction and ET_B receptors on endothelial cells mediate vasodilation through NO release. Endothelins are typically produced by endothelial cells and exert paracrine effects on adjacent smooth muscle cells and autocrine effects on themselves (Fig. 1).

Synthesis of ET-1, the major endothelin isotype, is controlled at two major levels (Fig. 1): (1) regulation of precursor ET-1 and (2) processing of immature forms. PreproET-1 (precursor ET-1) messenger RNA (mRNA) expression is stimulated by epinephrine, angiotensin II, vasopressin, shear stress, and cytokines such as interleukin 1.²²⁻²⁴ Proteolytic processing of preproET (approximately 200 amino acid residues) by furin-like enzymes leads to intermediates termed big endothelin (38-41 amino acids). Big endothelins, which have little or no biologic activity, are cleaved at Trp-21-Val/Ile-22 to yield the biologically active, mature peptide. Endothelin-converting enzyme (ECE), a neutral membrane-bound metalloprotease, cleaves big ET-1. Two isoforms of ECE, termed ECE-1 and ECE-2, have been cloned^{25,26}; in addition, ECE-1 undergoes extensive alternative splicing.^{26,27} Finally, binding of mature ET-1 to its cognate receptors leads to classic G protein-coupled receptor-mediated activation cascade (Fig. 2).

The endothelins regulate vascular tone, but are also important in multiple other processes.²⁸ For example, the endothelins play critical roles in cellular growth,²⁹ neural crest development,^{30,31} and wound healing.³²⁻³⁵ Their role in wound healing is particularly noteworthy with regard to fibrogenic liver disease (see below under "Fibrogenesis and Cirrhosis").

The importance of endothelins in liver disease has been emphasized by reports of elevated circulating ET-1/ET-3 levels in patients with cirrhosis.³⁶⁻³⁸ Importantly, the source of endothelin in cirrhotic patients is the injured liver itself.³⁹⁻⁴¹ In the normal liver, ET-1 is produced primarily by sinusoidal endothelial cells (as for endothelial cells in the peripheral vasculature); after injury, however, endothelin is derived largely from stellate cells (Fig. 1), and moreover, the synthesis of ET-1 by sinusoidal endothelial cells is reduced.⁴² The enhanced production of ET-1 in stellate cells after injury is linked to both increased production of precursor ET-1, as

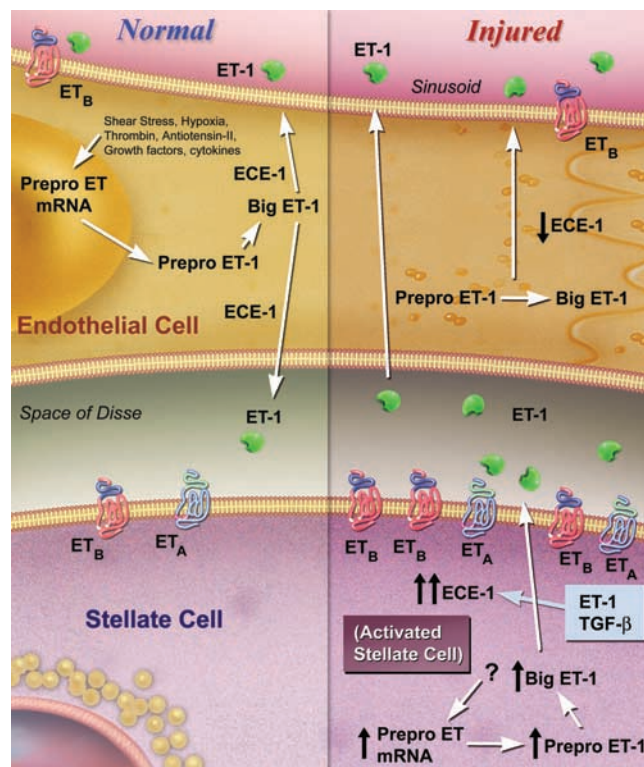


Fig. 1. Endothelin synthesis in normal and injured liver. Under normal conditions, control of endothelin synthesis in the sinusoid mirrors that in the systemic vasculature. Hormones, other vascular mediators, and flow conditions appear to modulate precursor ET-1 synthesis in endothelial cells (including sinusoidal endothelial cells). In this state, proteolytic processing of precursor endothelins leads to production of mature ET-1. ET-1 then has paracrine physiologic effects on neighboring stellate (or smooth muscle) cells. After liver injury, stellate cells undergo "activation" (see the text for details) and synthesis of ET-1 shifts dramatically to activated stellate cells. The mechanism underlying enhanced ET-1 synthesis appears to largely involve up-regulation of ECE-1, the enzyme responsible for conversion of big ET-1 to the mature peptide. In the injured liver, a host of factors, including components in the wounding milieu such as TGF- β , ET-1 itself, and other elements, are likely to modulate ET-1 synthesis. ET-1 in turn has prominent effects on key cellular effectors including stellate cells themselves in an autocrine fashion (see text and Fig. 2 for details), as well as in distant vascular beds such as the lungs.⁹⁶ Regulation of endothelin synthesis is emphasized here because it is best described; however, other vasoactive mediators may ultimately be found to have parallel regulatory pathways.

well as enhanced expression of ECE-1.^{41,42} Recent work indicates that ECE-1 in turn can be regulated by elements found in the injured liver such as transforming growth factor β (TGF- β) and ET-1 itself (the latter in an autocrine loop).⁴³

The effect of endothelins in liver disease is critically tied to their cellular target. Endothelin receptors can be identified on all hepatic cell types, but are most numerous on stellate cells.⁴⁴ Indeed, localization of infused, labeled ET-1 in the liver is consistent with binding to stellate cells.^{15,45} Further, endothelin receptors are up-regulated in the injured liver^{40,46} and specifically in stellate cells¹⁹ (Fig. 1). Thus, in the injured liver, enhanced endothelin receptor

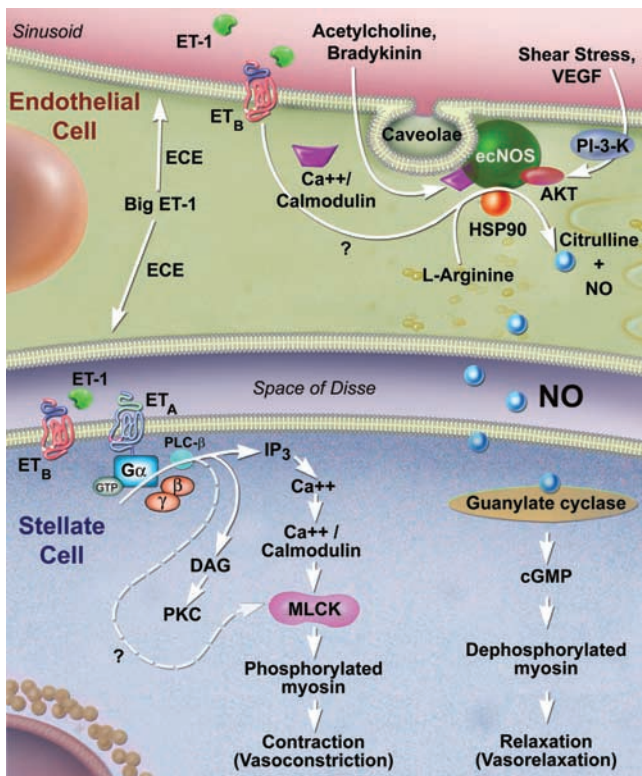


Fig. 2. Signaling pathways in stellate and endothelial cells. Simplified endothelin and NO signaling pathways are shown (and parallel those found in the systemic vasculature). Endothelin-1 binding to its cognate receptors leads to classic G-protein-coupled receptor-mediated activation of phospholipase C- β (PLC- β), cleavage of phosphatidylinositol 4,5-bisphosphate (PIP₂), and subsequent release of the second messengers inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG). In the canonical signaling pathway, IP₃-mediated stimulation of Ca²⁺ release from endoplasmic reticulum stores leads to activation of Ca²⁺/calmodulin-dependent myosin light chain kinase (MLCK), which in turn leads to activation of myosin MgATPase and activation of actomyosin mediated contraction. Non-Ca²⁺-dependent pathways, including the RhoA-rho-kinase, and as yet unknown pathways also appear to be important in stellate cell contractility. On the right, NO is produced after activation of ecNOS. Although NO production has classically been thought to be triggered by changes in intracellular Ca²⁺ and activation of Ca²⁺/calmodulin following stimulation by agonists, ecNOS can be activated and NO synthesis triggered via signaling in a pathway involving phosphatidylinositol-3-OH kinase (PI-3-K) and the serine-threonine kinase, AKT. AKT phosphorylation, stimulated by factors such as shear stress and growth factors, leads to ecNOS phosphorylation and NO production. NO binds to guanylate cyclase and leads to production of cGMP, which in turn leads to dephosphorylation of myosin and cellular relaxation. Finally, it should also be emphasized that signaling pathways may become altered after liver injury.

expression in stellate cells after injury and increased local production of endothelin create an environment that allows abnormal vascular and other physiologic responses.

Angiotensin II

Like the endothelins, angiotensin II has numerous biologic activities.⁴⁷ The best known effect of angiotensin II is smooth muscle cell contraction occurring as a result of binding to two major cognate receptors, known as AT1

and AT2 receptors (which are distinct and share only 32% homology). Known pharmacologic effects of angiotensin II appear to be mediated by the AT1 receptor.

Angiotensin II synthesis proceeds via the action of the integrated renin-angiotensin-aldosterone (RAAS) system. In this pathway, renin converts the inactive plasma protein, angiotensinogen, into angiotensin I. Angiotensin I is then converted to angiotensin II by angiotensin-converting enzyme (ACE). Although the conversion was originally thought to take place only in the lungs, ACE is known to be distributed widely. Angiotensin II has prominent effects on aldosterone synthesis and release, in the central nervous system (on thirst, on antidiuretic hormone), and on vascular smooth muscle cells, including on their contraction, proliferation, and matrix synthesis.

A putative role of angiotensin II in liver is linked to the finding that stellate cells possess AT1 receptors and that it induces cellular contraction and proliferation.⁴⁸ Further, recent evidence suggests that the major cellular source of angiotensin II in the liver is the stellate cell.⁴⁹ Moreover, its synthesis is up-regulated after liver injury, apparently due to pronounced up-regulation of ACE.⁴⁹ Thus, in the injured liver, increased local production of angiotensin II appears to parallel that for endothelin synthesis.

Nitric Oxide

NO is produced from L-arginine by one of 3 NOS isoforms,⁵⁰ encoded by at least 3 different genes,⁵¹⁻⁵³ and falls into two families of enzymes. Endothelial cells (ecNOS) and neurons (nNOS) each contain a distinct "constitutive" NOS, whereas a wide variety of cells are capable of expressing the inducible form (iNOS).⁵⁰

Regulation of NOS expression is complex and involves both pre- and post-translational elements. Many compounds, including cytokines and/or lipopolysaccharide, stimulate transcription of iNOS.⁵⁰ Although nNOS and ecNOS are traditionally thought of as "constitutive" isoforms, levels of ecNOS mRNA and protein may be modulated and production of NO by this isoform subsequently altered. ecNOS mRNA expression is increased by shear stress, and putative shear stress response elements have been identified in this gene's promoter.⁵⁴ ecNOS regulation is further complicated by its regulation at the post-translational level. For example, caveolin-1 in caveolae interacts with ecNOS and governs its activity.⁵⁵⁻⁵⁷ Additionally, protein kinase B (Akt) phosphorylates ecNOS and enhances its ability to generate NO⁵⁸ (Fig. 2).

The biological effects of NO are protean and include vasorelaxation, neurotransmission, and cytotoxicity—in particular toward microbes.⁵⁰ NO has particularly notable effects on the vasculature. Abnormalities in endothe-

lial NO have been described in a number of disorders including atherosclerosis, diabetes, and hypertension.⁵⁰ Mice lacking eNOS exhibit elevated basal blood pressure, emphasizing the critical role of this enzyme in vascular homeostasis.⁵⁹ Likewise, *in vivo* physiologic studies, as well as those examining isolated cells, emphasize that NO clearly modulates intrahepatic resistance^{11,60-62} (also see Wiest and Groszmann² for review).

Sinusoidal endothelial cells produce NO basally⁶³ and increase its production in response to flow.⁶¹ A growing body of work has focused on a cellular defect in NO synthesis in the injured liver. Interestingly, although eNOS mRNA and protein levels are unaltered after liver injury, endothelial-derived NO production is impaired in this situation.⁶³ Given the extensive post-translational modification of endothelial cell NOS, as well as changes in enzyme activity brought about by certain eNOS partners (Fig. 2), it has been postulated that abnormalities in one of these biochemical modifications or protein-protein interactions occurs after injury to sinusoidal endothelial cells. For example, increased binding of caveolin to eNOS in the injured liver is associated with reduced eNOS activity.⁶⁴ The finding that caveolin-1 protein levels were markedly increased in the cirrhotic liver⁶⁴ supports the possibility that such post-translational modifications of eNOS are important in cirrhosis.

Carbon Monoxide

Carbon monoxide (CO) has been shown to stimulate guanylate cyclase and cGMP production, in turn leading to smooth muscle relaxation (*i.e.*, the same mechanism by which NO acts). This finding has suggested that CO, like NO, may play a role in regulation of intrahepatic blood flow. Heme oxygenase (HO) is responsible for breakdown of heme into equimolar amounts of biliverdin, iron, and CO, and therefore plays an important role in CO biology. Three HO isoforms (-1, -2, and -3) have been identified. The most important isoform, HO-1, is ubiquitous and its mRNA and activity are inducible by heme, other metalloporphyrins, transition metals, and stimuli that induce cellular stress. In contrast, HO-2 is present chiefly in the brain and testes and is constitutively expressed. HO-3 has very low activity and its function remains unclear.

A role for CO in the liver has been emphasized by studies showing that endogenously produced CO serves as a vasorelaxant in the hepatic sinusoid.¹¹ Importantly, the major sites of action of CO in the sinusoid appear to colocalize with hepatic stellate cells, and over-expression of CO in the liver reduces intrahepatic vascular resistance.⁶⁵ Further, expression of HO-1 appears to be localized to sinusoidal Kupffer cells.⁶⁶ This finding is

important in the context of potential paracrine effects of CO on stellate and endothelial cells.

Prostaglandins

Prostaglandins, lipid-derived molecules derived from arachidonic acid and cyclooxygenase, have multiple physiologic effects. A number of compounds have been described, including thromboxane (Tx)A₂, prostacyclin (PGI₂), PGE₂, PGF_{2α}, and PGD₂. The prostanoids have been emphasized in many aspects of general vascular biology, suggesting that they are also likely to be important in hepatic vascular homeostasis. Unfortunately, the cell biology surrounding their synthesis, regulation of the pathways responsible for their production, and specific cellular effects in the liver are currently poorly understood.

Catecholamines

Catecholamines exert their effect by binding to a well-defined group of G-protein-coupled receptors classified as α or β . Extensive research indicates that they have profound vascular effects on smooth muscle and in the vasculature; in addition, they have important vascular effects within the liver (see below). However, as with prostanoids, the cell biology of this system in the liver is not well understood. Finally, it is important to emphasize that catecholamines may function as circulating hormones, whereas the majority of other vasoactive compounds (*i.e.*, ET-1, NO, and CO) act in a paracrine or autocrine fashion.

Others

A wide array of vasoactive compounds exert significant effects on multiple circulatory beds; it is likely that they also do so in the liver.⁶⁷ The precise role of many of these compounds, including purinergic agents (adenosine, serotonin, etc.), adrenomedullin, arginine vasopressin, cannabinoids, urotensin-II, atrial natriuretic peptide (ANP), and as yet unidentified factors, is yet to be clearly elucidated in the liver.

A Functional Role for Vasoactive Mediators in Intrahepatic Liver Disease

Portal Hypertension and Intrahepatic Resistance

Portal hypertension develops after chronic liver injury and cirrhosis. Portal pressure is a result of the relationship depicted in Ohm's law: $\Delta P = Q \times R$ where ΔP is the change in pressure along a vessel, Q the flow in the vessel, and R the resistance to that flow. Thus, two major hypotheses have been advanced in an attempt to explain the elevated portal pressure typical of portal hypertension: (1)

increased intrahepatic resistance and (2) increased flow through the splanchnic system via a hyperdynamic circulation. Most forms of liver disease encompass aspects of each. In fact, it is likely that there is interplay between the two systems. First, the splanchnic bed clearly helps regulate portal blood flow. Additionally, the liver is in series with the mesenteric circulation, and vasoactive substances from the mesenteric bed may have effects within the liver. Nonetheless, increased intrahepatic resistance is a consistent and early feature of most forms of liver injury.

With regard to the increased intrahepatic resistance typical of chronic liver injury, both fixed (*i.e.*, extracellular matrix, regenerative nodules, and vascular thrombosis) and modulable elements (*i.e.*, endothelial cells and stellate cells) play a role.^{3,4} Although the resistance fraction that each the modulable and fixed elements contribute is difficult to precisely quantitate, it has been shown that portal pressure can be reduced by 20% to 30% with pharmacologic agents that reduce intrahepatic resistance.⁶⁷ Of the modulable cellular elements, stellate cells are highly contractile and thus represent the most dynamically modifiable component. (Some controversy exists regarding the major resistance site(s) within the normal liver because methods to measure intrahepatic blood flow *in vivo* are unable to differentiate passive recoil of sinusoids due to upstream preterminal portal venular smooth muscle contraction from direct sinusoidal effects.) Nonetheless, stellate cell contractility is increased in the injured compared with the normal liver, emphasizing their prominent potential role in the genesis of intrahepatic resistance.¹⁷ Many vasoactive compounds have effects on stellate cells and therefore modulate intrahepatic resistance (Table 1). To date, however, available data suggest that ET-1 and NO not only have the most prominent effects on isolated stellate cells, but also that they have the most profound effects on intrahepatic resistance and blood flow in the liver *in vivo*.^{14,16,17,68,69}

During liver injury, a remarkable paradigm is currently evolving: the proposed model revolves around the concept that a shift in the balance of vasoactive substances occurs after liver injury and alters intrahepatic resistance (Fig. 3). For example, in normal liver, vasoconstrictors (*i.e.*, ET-1) and vasodilators (*i.e.*, NO) are “balanced.” However, in the injured liver, an imbalance occurs; in the model highlighted, ET-1 synthesis is increased and NO production decreased, leading to an “endothelialopathy” within the liver. In the context of exaggerated stellate cell contractility (due to up-regulation of contractile proteins and a hyper-responsive contractile signaling cascade; see Reynaert et al.⁷⁰ for review of cellular aspects of stellate cell contractility), intrahepatic (sinusoidal) resistance becomes increased.⁶²⁻⁶⁴

Table 1. Agents With Reported Effects on Stellate Cell Contraction

Compound	Magnitude
Contraction	
Endothelin (1, 2, 3)	++++
Angiotensin II	++
Thrombin	+
Vasopressin	+
Prostaglandin F _{2α}	+
U46619 (Thromboxane A ₂)	+
Substance P	+
Serum	+++
PAF	+
Adenosine	+
Relaxation	
Nitric oxide (NO)	++++
Carbon monoxide (CO)	+
PGE ₂	+
Lipo-PGE ₁	+
Adrenomedullin	+

NOTE. The magnitude of contraction is set on a relative scale, based on the combined available literature.

Abbreviations: PAF, platelet activating factor; PGE₂, prostaglandin E₂.

Although abundant data support the proposed endothelialopathy involving endothelin and NO (Fig. 3), other vasoactive compounds are likely to play a role. For example, prostanoids have effects on cultured stellate cells,⁷¹ and studies in whole animals emphasize their potential contribution.⁷² In this work, when cirrhotic livers were exposed to indomethacin, the vasoconstrictive response to methoxamine was exaggerated, suggesting the presence of COX-derived vasoconstrictors. Additionally, angiotensin II stimulates stellate cell contractility⁴⁸ and results in increased intrahepatic resistance when perfused into the liver *in vivo*.^{17,73} Likewise, catecholamines, somatostatin, ANP, arginine vasopressin, adrenomedullin, and other compounds have prominent effects on stellate cells and/or in the intact liver, and therefore may be important modulators of intrahepatic resistance.⁷⁴⁻⁷⁷ Further study is expected to more clearly define their functional roles in the normal and injured liver.

Therapeutic Implications. Despite the complexity of the described dysregulation of endothelin and NO in the injured liver, this perturbed system is an extremely attractive target for therapeutic intervention in portal hypertension. For example, NO replacement or endothelin antagonism in the injured liver leads to a reduction in portal pressure.^{17,78-80} Future studies will test these approaches in the injured liver.

The adrenergic system, in particular the α -adrenergic component, appears to play an important role in controlling intrahepatic vascular resistance in humans. In cirrhotic patients, continuous α -adrenergic blockade with prazosin reduced portal pressure^{81,82} and the addition of

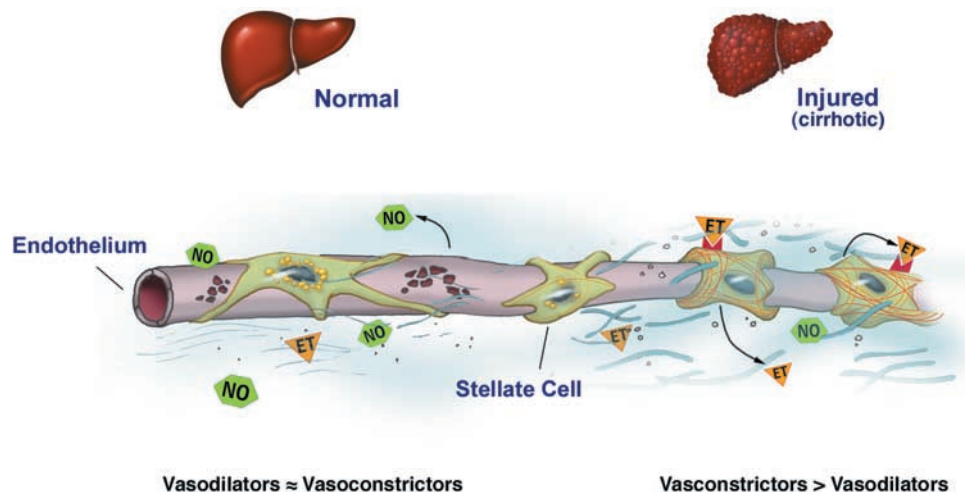


Fig. 3. An endothelialopathy in liver disease contributes to intrahepatic portal hypertension. In the normal sinusoid (left), quiescent stellate cells produce little or no ET-1, whereas NO production by the endothelium is normal. However, after liver injury (right), stellate cells become activated and produce increased quantities of ET-1; moreover, NO production by sinusoidal endothelial cells is reduced. In addition, after stellate cell activation, expression of smooth muscle proteins is increased and signaling pathways are potentiated. The net effect is enhanced stellate cell contractility and sinusoidal constriction (shown on the right) with an increase in resistance to sinusoidal blood flow. Much of the available data emphasize the importance of ET-1 and NO, and for simplicity, these compounds are highlighted. Nonetheless, other vascular mediators (*i.e.*, angiotensin II, prostanoids, and CO) may also play a role in the endothelialopathy found in liver injury and cirrhosis. Finally, endothelial dysfunction may also be present in the nonsinusoidal intrahepatic vascular compartment.

propranolol enhanced the decrease in portal pressure.⁸¹ Although the cellular target of prazosin was not identified, hepatic blood flow was increased in these studies, suggesting that the mechanism for the reduction in portal pressure was a reduction in intrahepatic resistance.

Given data indicating that angiotensin II induces stellate cell (and smooth muscle cell) contractility, the RAAS is an attractive therapeutic target in intrahepatic portal hypertension. However, studies in humans with cirrhosis in which angiotensin II signaling has been blocked have been met with mixed results⁸³⁻⁸⁵; in particular, angiotensin II receptor antagonists adversely affected glomerular filtration rate and caused systemic hypotension in cirrhotic patients⁸⁵; therefore, caution is required with these agents. Indeed, because of potential systemic adverse effects, some have suggested that cirrhotic patients not be treated with these compounds.

Fibrogenesis and Cirrhosis

Hepatic fibrogenesis is the liver's wound healing response to injury and leads to cirrhosis. The principal effector of fibrogenesis is the stellate cell, which after injury, undergoes transformation from a resting cell to an activated, myofibroblast-like cell during a process termed "activation." Not only is activation associated with increased expression of smooth muscle proteins, that in turn mediates cellular contractility (as above), but this process is also characterized by increased production of extracellular matrix proteins, enhanced cellular proliferation, and exaggerated expression of a number of growth factors and their receptors, all of which contribute to the wounding response.

Recent investigation has emphasized a putative role for several vasoactive mediators in the pathogenesis of hepatic fibrogenesis. For example, exposure of isolated cultured stellate cells to ET-1 led to stimulation of smooth muscle α actin, proliferation, and extracellular matrix protein synthesis.^{39,41} Further supporting the concept that endothelin is important in fibrogenesis are *in vivo* studies showing a reduction in the fibrogenic response with endothelin antagonism.^{86,87} These data are even more compelling given data in experimental models of fibrogenesis demonstrating increases in ET_A and ET_B receptor density on stellate cells after injury.^{19,40}

In a variety of diseases leading to parenchymal wound healing, including the liver, angiotensin II appears to be important in fibrogenesis.^{88,89} In the liver, angiotensin II, like ET-1, stimulated stellate cell proliferation and smooth muscle α actin expression, important features of activation.⁴⁸ Additionally, emerging data suggest that the angiotensin system may mirror the endothelin system in that compounds in the wound healing milieu (such as TGF- β) stimulate production of angiotensin II, and that angiotensin II is produced by activated stellate cells after liver injury in an autocrine fashion.⁴⁹

The role of adrenergic innervation and/or circulating catecholamines in fibrogenesis is under investigation. Given the close approximation of neural structures to stellate (and endothelial) cells, it is possible, and even likely, that catecholamines play a role in fibrogenesis.

The role of NO in hepatic fibrogenesis is controversial; NO production appears to vary, both temporally and spa-

tially, during liver injury. In *in vivo* experimental models, NO is present after acute administration of hepatotoxins, presumably as a result of iNOS induction.⁹⁰ In contrast, ecNOS-dependent NO production is reduced after chronic injury and fibrogenesis.^{63,64} However, it is unclear whether a lack of NO is important in this setting.

Therapeutic Implications. The cell and molecular biology of endothelin biosynthesis and its effects on stellate cells suggest that endothelin receptor antagonism should be an effective antifibrotic approach. However, it is noteworthy that receptor specificity is likely to be an important issue with targeting in fibrogenesis. On one hand, ET_A blockade in an *in vivo* rat model of fibrogenesis led to decreased hepatic collagen expression, as well as reduced serum fibrosis markers⁸⁷ and a mixed receptor antagonist had similar overall effects.⁸⁶ On the other hand, it has been shown in culture systems that ET_B receptor binding in stellate cells may inhibit stellate cell proliferation.⁹¹ Additionally, ET_B receptors in sinusoidal endothelial cells signal to NO and thus may be protective for the hepatic microvasculature.⁹² Therefore, further studies are required to clarify the roles of the two separate endothelin receptors in fibrogenesis.

As with endothelin, therapies aimed at angiotensin II may also emerge as therapeutic options. Indeed, inhibition of angiotensin II *in vivo* reduced hepatic fibrogenesis and TGF- β 1 expression in animal models.^{89,93} Although the mechanism of angiotensin II antagonism appears to be linked to stellate cells, further work is required to understand the cell biology of angiotensin II synthesis, regulation, and signaling in the injured liver. Finally, in patients with portal hypertension, inhibition of angiotensin II could have untoward systemic effects (see above).

The adrenergic system may also serve to be an important therapeutic target in fibrogenesis. In an *in vivo* model of liver injury and fibrogenesis (induced by CCl₄), six-hydroxydopamine, a toxin that destroys noradrenergic fibers, decreased fibrosis by 60%.⁹⁴ Further, the α 1-adrenergic receptor antagonist, prazosin, reduced fibrosis significantly.⁹⁴

Importantly, two recent studies have shown that NO, when supplemented during liver injury, ameliorates fibrogenesis.^{80,95} However, the mechanisms underlying this effect remain unknown.

Summary

Vasoactive mediators have important effects on sinusoidal cells (endothelial and stellate) and are important not only because they modulate intrahepatic resistance, but also because they modulate fibrogenesis (and it is likely that other critical roles for these mediators will emerge). Endothelin, angiotensin, and NO are currently

the most attractive therapeutic targets. Recent preclinical data suggest that strategies focused on these compounds in the treatment of portal hypertension and fibrosing liver disease will soon emerge. An important underlying theme concerning vascular mediators in liver disease is that they play dual roles: in portal hypertension and in fibrogenesis. A critical caveat in targeting vascular systems in patients with advanced chronic liver disease, and particularly those with portal hypertension, is that vasoactive compounds, antagonists, or synthesis inhibitors will almost certainly have significant systemic hemodynamic effects. Therefore, ideal therapeutic agents will need to be targeted specifically to the liver.

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