Endothelial Dysfunction in Advanced Liver Disease

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This review takes a focused bedside to bench back to bedside approach to assess the issue of endothelial dysfunction in advanced liver disease. We start with a patient presentation of an approximately 50-year-old man with a history of hepatitis C virus infection that had been diagnosed about a decade ago, and who previously presented acutely with several episodes of hematemesis and melena. His history was remarkable for the discovery of hepatitis C virus 10 years ago. At that time, he had a normal physical examination and had no more than 2-fold elevation in AST and ALT, and the rest of his laboratory test results were normal. He drank occasional alcohol. He underwent a liver biopsy, which revealed stage 2 fibrosis and grade 2 inflammation (based on the Metavir staging and grading system). At this time, he was treated with standard pegylated interferon and ribavirin, but unfortunately, he was a nonresponder and he was at that time told that there was nothing more that could be done. On examination, he had witnessed hematemesis and a blood pressure of 90/40 mm/Hg, and his heart rate was 120 bpm. He was jaundiced, and he had ascites. His laboratory tests revealed a hematocrit of 25%, an INR 1.9, bilirubin 6.2 mg/dL and platelets 92,000/mm^3. Endoscopy revealed an actively bleeding esophageal varix, which was banded and stopped the bleeding. He subsequently spiked fever and was found to have spontaneous bacterial peritonitis. He was effectively treated with antibiotics, lactulose, and was discharged on the 8th hospital day. Unfortunately, he returned with recurrent bleeding 2 weeks later, developed sepsis and died after a prolonged hospital course.

This patient had cirrhosis with subsequent portal hypertension, a disease that we would like to understand, and ultimately prevent. Thus, this review will highlight the current understanding of vascular biology and in advanced liver disease (ie, cirrhosis). Although many vascular disorders occur in patients with cirrhosis, the major vascular disease occurring as a result of cirrhosis is portal hypertension, with its attendant devastating morbidity and mortality. Thus, we will focus here on the cell and molecular aspects of intrahepatic portal hypertension, highlighting the critical features involved.

INTRAHEPATIC CIRCULATORY UNIT

Cirrhosis leads to vascular abnormalities in essentially all of the vascular beds (Figure 1). In patients with cirrhosis, there is intense vasoconstriction in the liver and the kidneys, whereas in the other vascular beds, there is vasodilation. All of these vascular abnormalities are associated with substantial clinical disease. Here, we will focus on the liver and its vasoconstrictive phenotype.

The intrahepatic circulatory unit consists largely of 3 major microvascular components including the terminal portal venule and hepatic arteriole, the sinusoids (corresponding to the capillary bed) and the terminal hepatic venule. At the sinusoidal level, the major cellular components include sinusoidal endothelial cells (SECs) and stellate cells (also known as lipocytes or Ito cells), each of which seem to have an important role in the regulation of sinusoidal blood flow.[1–3] From an ultrastructural standpoint, stellate cells possess long and extensive cytoplasmic processes that essentially encircle many if not all SECs.[4,5] This anatomic relationship in the sinusoid suggests that stellate cells function as liver-specific pericytes, the latter being cells that control capillary blood flow in a wide variety of tissues.[6,7] Furthermore, the close anatomic relationship of SECs to stellate cells suggests a (functional) paracrine relationship between the 2 cell types.

Portal pressure is proportional to resistance and flow according to the hydraulic equivalent of Ohm's law: $\Delta P = Q \times R$, where ΔP is the change in pressure along a vessel, Q is the flow in the vessel and R is the resistance to flow. Elevated portal pressure typical of liver injury and portal hypertension seems to include both increased intrahepatic resistance as well as increased flow through the splanchnic system (ie, a hyperdynamic circulation). The level of increased resistance to flow varies with specific types of liver disease and may occur pre-, intra- or postsinusoidally. Importantly, most forms of liver injury result in "sinusoidal" portal hypertension. Emerging data suggest that changes in portal pressure, even early in the liver injury process, are an important predictor of clinical outcome, making portal hypertension thus highly significant.[8–11]

SINUSOIDAL PORTAL HYPERTENSION

Vascular homeostasis is controlled by the balance of vasoconstriction and vasorelaxation; each of these biological processes is controlled by a specific set of cells and molecules. In terms of the processes that mediate vasoconstriction, vasoconstrictors, such as endothelin 1, which are typically produced locally by the endothelium itself and have paracrine on smooth muscle cells, cause phosphorylation of myosin and activation of the actin-myosin contractile apparatus in smooth muscle cells, and thereby lead to smooth muscle cell contraction, narrowing of vascular structures and an increase in resistance to blood flow. Of note, many vasoconstrictors have been identified, including epinephrine, angiotensin II and others. On the vasorelaxation side, the endothelium also produces a variety of vasorelaxing molecules (such as, but not limited to, nitric oxide [NO]). NO stimulates cyclic GMP and leads to dephosphorylation myosin and inactivation of the actin-myosin contractile apparatus in smooth muscle cells, leading to vasorelaxation. Some molecules may be produced in an autocrine fashion, effecting endothelial cells and/or smooth muscle cells. In the liver and sinusoid, this physiology is prominent.

In virtually all forms of intrahepatic liver disease, there is a predictable and measurable increase in intrahepatic resistance.

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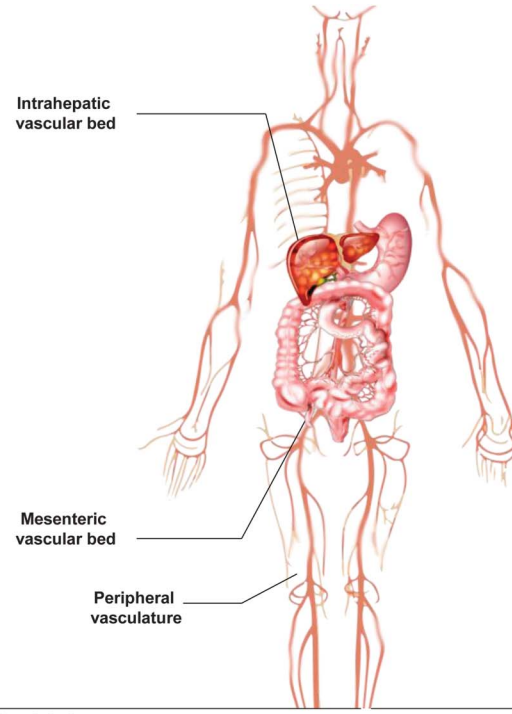

Molecular biology	Physiology
Intrahepatic vascular bed	
• Intrahepatic vascular bed • Increased gene and peptide expression of vasoconstrictors (E T-1, Ang II) • Reduced eNOS phosphorylation, activity and NO production	• Increased resistance • Increased pressure • Reduced portal venous blood flow
Mesenteric vascular bed	
• Reduced gene and peptide expression of vasoconstrictors (E T-1, Ang II) • Increased eNOS phosphorylation, activity and NO production • Decreased Rho kinase activity • Increased expression of angiogenic factors (VEG F, PDGF, PIGF)	• Arterial vasodilation • Arterial wall thinning • Decreased response to vasoconstrictors • Increased portal venous blood flow
Peripheral vasculature	
• Increased eNOS phosphorylation, activity and NO production	• Reduced resistance • Decreased arterial response to vasoconstrictors • Autonomic dysfunction • Increased cardiac output • Increased blood flow
Pulmonary vasculature (typical, not "portopulmonary changes")	
• Increased ET-1 gene and peptide production • Increased ET-B receptor expression • Increased eNOS phosphorylation, activity and NO production	• Pulmonary small vessel dilation • Extensive shunting, with left sided hypo-oxygenation • Increased cardiac output

FIGURE 1. Vascular beds in portal hypertension. Shown are prominent vascular beds and their associated pathophysiology. The intrahepatic vascular bed is typified by an increase in resistance to flow. Liver injury leads to abnormal endothelial function, with a reduction in the production of vasodilators by sinusoidal endothelial cells such as NO; concomitantly, there is an increase in the synthesis of vasoconstrictors such as ET-1 and Ang II, by other cells in the sinusoid (see also Figure 2). The mesenteric vascular bed is characterized by vasodilation by reduced resistance caused by upregulation of vasodilators such as NO, leading to increased flow to the portal vein. The net result of this physiology is an increase in intrahepatic resistance and portal blood flow from the splanchnic circulation, leading to increased portal pressure and portal hypertension. In peripheral vascular beds, increased eNOS activity and NO production typically lead to reduced resistance, low systemic pressure and a hyperdynamic state typical of patients with cirrhosis. Abnormalities also exist in other vascular beds such as the pulmonary, brain, renal and likely even others. Ang II, angiotensin II; eNOS, endothelial cell nitric oxide synthase; ET-1, endothelin 1; NO, nitric oxide. From *Journal of Hepatology* Vol. 61, 912–924, Figure 1.

Early in the disease process, cellular elements are likely to play an important role in regulation of intrahepatic resistance. We have focused on cells in the sinusoid, the functional vascular unit of the liver. The sinusoid is made up of a unique fenestrated endothelium,[2,12,13] which is encircled in close physical proximity by hepatic stellate cells that have long cytoplasmic processes,[5] and encircle and enwrap the endothelium (Figure 2). This is important because this anatomy parallels that found in tissue pericytes, cells known to regulate capillary blood flow.[6] There are other unique features of