Pathophysiology of Portal Hypertension

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Abstract

The bases of our current knowledge on the physiology of the hepatic portal system are largely owed to the work of three pioneering vascular researchers from the sixteenth and the seventeenth centuries: A. Vesalius, W. Harvey, and F. Glisson. Vesalius is referred to as the founder of modern human anatomy, and in his influential book, *De humani corporis fabrica libri septem*, he elaborated the first anatomical atlas of the hepatic portal venous system (Vesalius 2013). Sir William Harvey laid the foundations of modern cardiovascular research with his *Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus* (Harvey 1931) in which he established the nature of blood circulation. Finally, F. Glisson characterized the gastrointestinal-hepatic vascular system (Child 1955). These physiological descriptions were later complemented with clinical observations. In the eighteenth and nineteenth centuries, Morgagni, Puckelt, Cruveilhier, and Osler were the first to make the connection between common hepatic complications – ascites, splenomegaly, and gastrointestinal bleeding – and obstruction of the portal system (Sandblom 1993). These were the foundations that allowed Gilbert, Villaret, and Thompson to establish an early definition of portal hypertension at the beginning of the twentieth century. In this period, Thompson performed the first direct measurement of portal pressure by laparotomy in some patients (Gilbert and Villaret 1906; Thompson et al. 1937). Considering all these milestones, and paraphrasing Sir Isaac Newton, if hepatologists have seen further, it is by standing on the shoulders of giants.

Nowadays, our understanding of the pathogenesis of portal hypertension has largely improved thanks to the progress in preclinical and clinical research. However, this field is ever-changing and hepatologists are continually identifying novel pathological mechanisms and developing new therapeutic strategies for this clinical condition. Hence, the aim of this chapter is to summarize the current knowledge about this clinical condition.

Glossary of Terms

Cirrhosis Complex pathophysiological process affecting the liver that involves progressive destruction and pathological regeneration of the hepatic parenchyma characterized by the accumulation of extracellular matrix.

Fenestrae Membrane pores present in liver sinusoidal endothelial cells with diameters of ~20–250 nm. The fenestrae are arranged in special structures called sieve plates, which are approximately 0.1 μm in diameter and comprise 20–50 aggregated pores.

Hepatic stellate cell (HSC) Hepatic stellate cells are liver-specific mesenchymal cells located in the space of Disse that can transdifferentiate from a quiescent phenotype into a highly proliferative, contractile, and profibrotic myofibroblast.

Hepatic venous pressure gradient (HVPG) This parameter is defined as the difference between the wedged (WHVP) and the free hepatic venous pressures (FHVP).

Idiopathic portal hypertension A result of various degrees of portal venous injury, with unclear etiology, that predominantly manifest in the pre-sinusoidal region.

Kupffer cells These cells are a large population of resident tissue macrophages that are located in the sinusoidal lumen and in close contact with the endothelial cells and hepatocytes.

Microparticles Small cell-derived vesicles with a diameter comprised between 0.1 – 1 μm that are generated after cell activation or apoptosis. The membrane of the microparticle maintains cell surface molecules from parent cells.

Nonalcoholic steatohepatitis (NASH) A clinical syndrome characterized by a significant hepatic inflammatory response with concurrent fat accumulation in the liver.

Portal hypertension A clinical syndrome characterized by a pathological increase in the hydrostatic pressure – over 6 mmHg – in the portal venous territory.

Portal venous system Vascular system composed by two capillary beds directly interconnected through veins.

Primary biliary cirrhosis Primary biliary cirrhosis is a chronic disease of the liver characterized by a progressive destruction of small bile ducts. This process is usually associated with the development of scarring, fibrosis, and cirrhosis.

Radical oxygen species A group of reactive molecules and free radicals derived from molecular oxygen that act as major cellular mediators involved in cell signaling and cell function. This term encompasses various oxygen species such as superoxide, hydrogen peroxide, hypochlorous acid, and hydroxyl radicals.

Schistosomiasis Clinical condition characterized by the pre-sinusoidal obstruction of portal venules caused by deposition of eggs of parasites belonging to the genus Schistosoma.

Sinusoidal capillarization Structural transformation of the hepatic sinusoids into continuous capillaries characterized by the presence of basement membranes and defenestration of sinusoidal endothelial cells.

Vascular remodeling The long-term structural adaptations that occur in blood vessels to maintain constant flow despite hemodynamic disturbances or vascular abnormalities.

Anatomical and Hemodynamic Characteristics of the Intrahepatic Circulation

The liver receives about 15–25 % of the cardiac output via two sources of blood supply, the hepatic artery that delivers arterial blood and provides 25–30 % of the hepatic blood volume and the portal vein. The hepatic portal venous system carries blood from the esophagus, stomach, intestine, pancreas, and spleen to the liver. With this anatomical arrangement, the concentrations of certain hormones (e.g., substance P, insulin, and glucagon), nutrients, and metabolites are comparatively higher in the hepatic portal circulation than in any other vascular territories (Geller et al. 2009). The blood vessels that connect the capillaries of the gastrointestinal tract with the liver are veins or venules that do not drain directly into the heart, unlike what happens with most capillaries. This type of vascular loop is termed the portal venous system.

The portal vein ranges in size from 5 to 8 cm in length. It is formed by the union (behind the neck of the pancreas) of the superior mesenteric and splanchnic veins, which are the two major tributaries of the portal vein. Anatomical variations include direct communication with the inferior mesenteric vein. The hepatic portal vein also receives other tributaries such as the left gastric vein, the right gastric vein, and the cystic veins. Before reaching the liver, the portal vein enters the free margin of the lesser omentum at the porta hepatis. Then, it divides into right and left terminal branches that further ramify into smaller vessels and subsequently into portal venules that delimit the functional segments of the liver. The portal venules – together with the hepatic arterioles, common bile duct, and lymphatic vessels – run in parallel and form the vascular component of the portal triad. This structure irrigates and drains the hepatic cells through vascular extensions called sinusoids. Hepatocytes exchange digestive end products, toxins, and metabolites with the sinusoidal blood, which is finally collected by the hepatic vein and drained into the inferior vena cava (Fig. 1). Since the portal vein carries mostly deoxygenated blood (pO_2 approximately 40 mmHg compared to 100 mmHg in hepatic artery) and the diameter of the portal vasculature is larger than the hepatic artery in the portal triad, we can infer that the sinusoids mainly contain poorly oxygenated blood (Pinzani and Vizzutti 2010; Geller et al. 2009; Shah and Kamath 2010).

High hepatic/portal vein compliance is another feature that characterizes the intrahepatic circulation in normal livers. The liver and portal circulation constitutes a low resistance system capable of accommodating a large blood volume without substantially altering the values of portal pressure. In contrast, liver disease is associated with increased intrahepatic resistance to portal blood flow and portal hypertension. To understand what variables contribute to these hemodynamic abnormalities, classical equations of fluid mechanics are useful

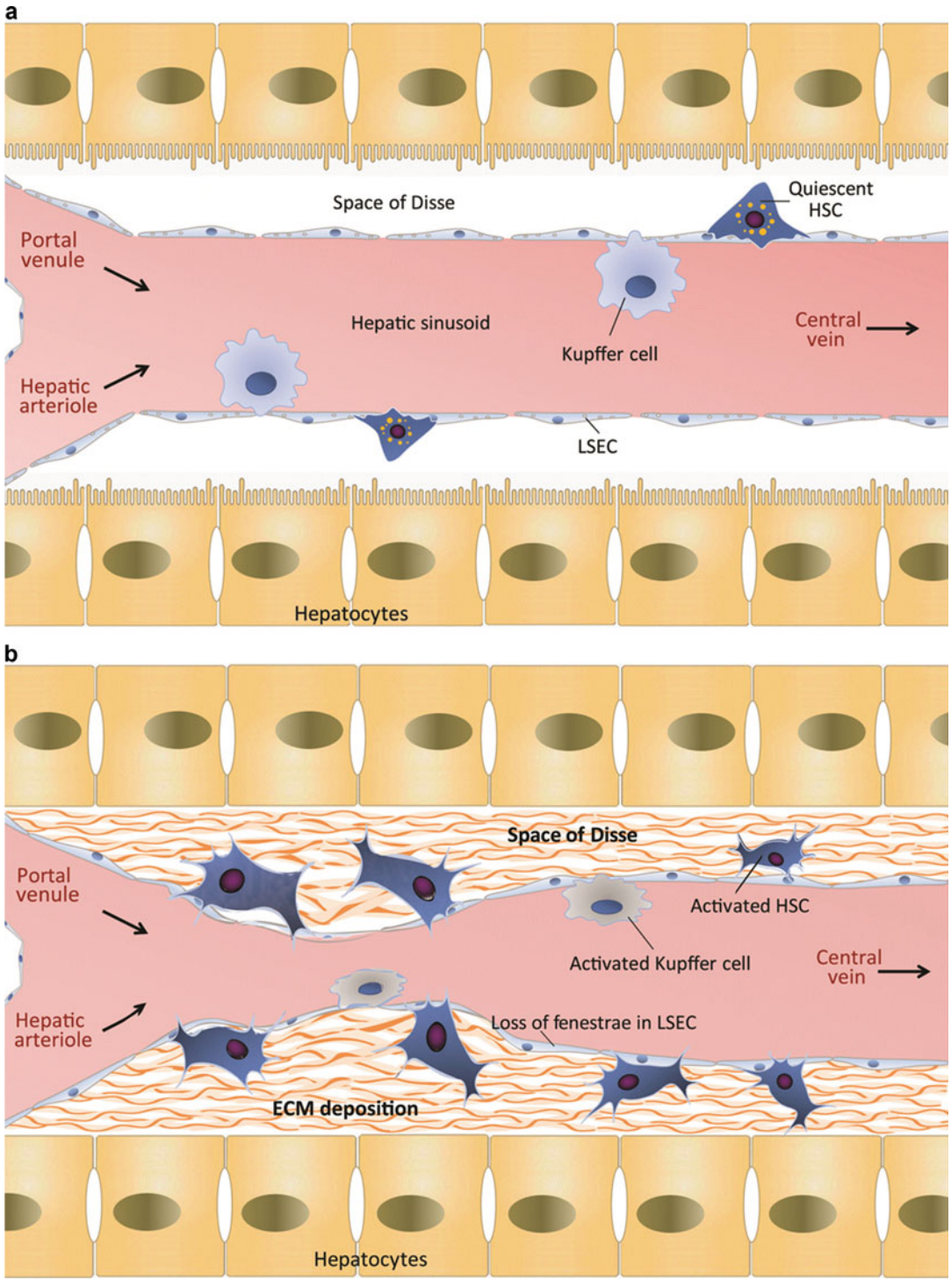

Fig. 1 (continued)

and have been extensively used over time. The blood flow equation is: $Q = \Delta P/R$, in which Q is the blood flow, ΔP the pressure gradient between two points, and R the total vascular resistance. On solving this equation for ΔP ($\Delta P = Q \times R$, which equals the Ohm's law used in electric networks), we see that if Q or R increases, ΔP also does so. R is directly proportional to blood viscosity (μ) and the length (L) of the vasculature and is inversely proportional to its radius to the fourth power (according to the Hagen-Poiseuille equation $R = 8\ \mu L/\pi r^4$). Therefore, twice the length of the vasculature doubles R, while a decrease in the radius of the vessel by half increases the resistance 16-fold. Despite the limitation that these equations are only applicable to Newtonian liquids (blood is a non-Newtonian liquid), we still can infer that both an increment in blood flow and an increment in intrahepatic vasoconstriction should significantly affect portal pressure.

Classification of Liver Diseases Associated with Portal Hypertension

Portal hypertension is defined as a clinical syndrome characterized by a pathological increase in the hydrostatic pressure – over 6 mmHg – in the portal venous territory. This increase results from an increment in the pressure gradient occurring between the portal vein and the inferior vena cava. The gradients of 10 and 12 mmHg are considered cutoff values for the development of gastroesophageal varices and variceal bleeding, respectively. These complications account for the high morbidity and mortality associated with portal hypertension, and therefore, a gradient of 10 mmHg or higher is considered as clinically significant portal hypertension (Bosch et al. 2009; De Franchis 2010).

Although portal pressure may be measured directly in patients, the invasiveness and difficulty of this approach make this measurement inappropriate in the clinical setting. The gold standard for the measurement of portal pressure and the parameter most commonly used is the hepatic venous pressure gradient (HVPG). This parameter is the difference between the wedged (WHVP) and the free hepatic venous pressures (FHVP). The WHVP is obtained through the placement of a wedged catheter into the hepatic vein, which transmits the pressure from the hepatic sinusoids to the catheter. The values obtained by WHVP are slightly lower than direct portal pressure measurement although this difference is clinically insignificant. Thus, the HVPG provides an accurate estimation of portal pressure (Myers and Taylor 1956; Groszmann and Wongcharatrawee 2004; Bosch et al. 2009). Recently, other noninvasive methods such as transient elastography or magnetic resonance have been used to evaluate portal hypertension (Castera et al. 2012). These noninvasive tools have demonstrated to be useful in patient screening and stratification. However, the inaccuracy of these tools in obese patients or those with ascites and the need for adequate operator training prevent the use of these alternative strategies in daily clinical practice.

Besides its clinical value, the measurement of WHVP, FHVP, and HVPG is useful in the differential diagnosis of portal hypertension syndromes. Taking the liver as a spatial reference, resistance to portal flow may occur in the

Fig. 1 Hepatic sinusoid in normal and cirrhotic livers. In (**a**), a schematic sinusoid representation from a normal liver. Hepatocytes exchange digestive end products, toxins, and metabolites with the sinusoidal blood. This vascular structure, which is lined by fenestrated endothelial cells (*LSEC*), drains blood from the portal triad to the central vein. The characteristic fenestrae of the LSECs contribute to the rapid transport of solutes across the subendothelial space. Kupffer cells are found in the sinusoid while hepatic stellate cells are located in the subendothelial space, named the space of Disse. Hepatic stellate cells (*HSCs*) store retinoids within perinuclear lipid droplets. In (**b**), as fibrosis develops, changes occur within the subendothelial space and within the hepatic sinusoid. These changes include alterations in both cellular morphology and extracellular matrix composition. Activated HSCs lose their retinoid reserve and become the primary source of extracellular matrix. They may also participate in sinusoidal contraction. In addition, there is a loss of hepatocyte microvilli and endothelial fenestrae. Transport across the sinusoidal wall is hence reduced, leading to deterioration of hepatic function. Activation of Kupffer cells accompanies liver injury and contributes to HSC activation

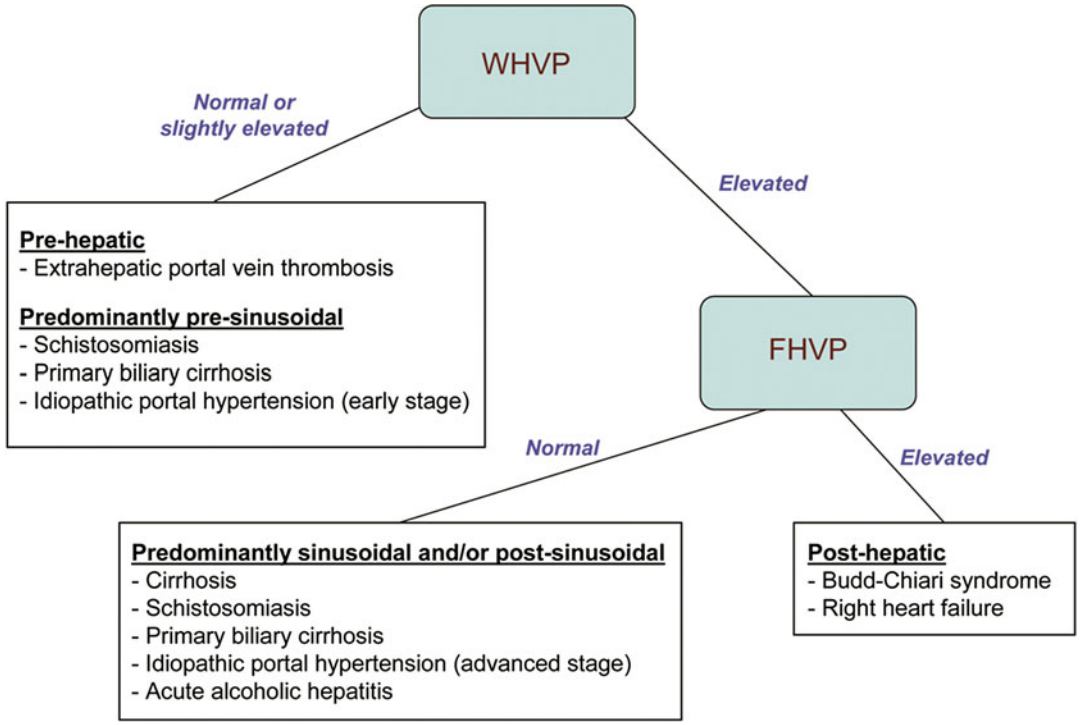

Fig. 2 Most prevalent causes of portal hypertension classified according to the localization of the resistance to portal blood flow. *WHVP* wedged hepatic venous pressure, *FHVP* free hepatic venous pressure

following locations: pre-hepatic, intrahepatic, or post-hepatic areas. Intrahepatic resistance is also subclassified in pre-sinusoidal, sinusoidal, and post-sinusoidal sites. This classification has diagnostic relevance. For example, WHVP values are normal when the resistance to portal blood flow is pre-hepatic or pre-sinusoidal. By contrast, WHVP values exceed the reference values when the increased resistance is mainly intrahepatic (specifically, sinusoidal and/or post-sinusoidal) or post-hepatic. Moreover, high levels of FHVP allow differential diagnosis of post-hepatic portal hypertensive syndromes. Some liver diseases present more than one zone of resistance to blood flow. In these cases, the HVPG measurement would tend to identify the predominant localization to blood resistance. Alcoholic cirrhosis illustrates this heterogeneity since the resistance to portal blood flow in these patients is predominantly sinusoidal, but vascular remodeling of the portal and hepatic vein also contributes to the overall resistance (Wongcharatrawee and Groszmann 2000; Groszmann and Wongcharatrawee 2004). Figure 2 shows the most prevalent causes of portal hypertension classified according to the localization of the resistance to portal blood flow.

Alternatively, portal hypertensive syndromes may be classified according to the presence or absence of cirrhosis. Within the categorization of non-cirrhotic portal hypertension, we may find a group of diseases of varied etiology with a pre-hepatic, intrahepatic, or post-hepatic origin of increased resistance to venous blood flow. The diseases most commonly encountered in this group are idiopathic portal hypertension, extrahepatic portal vein thrombosis and schistosomiasis. Idiopathic portal hypertension is a result of various degrees of portal venous injury, which predominantly manifest in the pre-sinusoidal region. Idiopathic portal hypertension is more common in developing countries, where it accounts for 15–25 % of all causes of portal hypertension, and in Japan where the prevalence reaches up to 30 % (Sarin et al. 2007). The etiology of idiopathic portal hypertension is unclear in most of patients. However, several pathological

mechanisms have been identified in a minority of affected patients. These mechanisms include umbilical/portal pyemia, repeated bacterial infections during infancy, prothrombotic states, exposure to chemicals such as arsenic or vinyl chloride, and hypervitaminosis A (Boyer et al. 1967; Datta et al. 1979; Ludwig et al. 1993). Current animal models of idiopathic portal hypertension are able to reproduce some of the pathophysiological features of this disease. For instance, repeated low doses of heat-killed *E. coli* result in persistently elevated portal pressure and splenomegaly (Omanwar et al. 2004). Extrahepatic portal vein thrombosis has a pre-hepatic/pre-sinusoidal origin caused by portal vein obstruction occurring as a consequence of thrombosis, constriction, or invasion of the portal vein. In this clinical condition, portal hypertension is also associated with splenomegaly, portosystemic collaterals, and gastroesophageal varices. The liver is usually normofunctional, and as a result, portal vein thrombosis is usually asymptomatic until the appearance of an episode of variceal bleeding. The etiology of portal vein thrombosis is diverse and includes: a hypercoagulable state (protein C, antithrombin, or protein S deficiency), hematologic disorders (polycythemia vera or other myeloproliferative disorders), inflammation (diverticulitis, pylephlebitis, inflammatory bowel disease, pancreatitis), sepsis (umbilical vein sepsis, which is the main cause of portal vein thrombosis in children), trauma (splenectomy, abdominal trauma, and surgery), cirrhosis, or malignancy. Multiple etiopathogenic factors may be present in nearly 40–50 % of the patients, although a previous prothrombotic state mainly contributes to the increase in the risk of developing this complication among these patients (Sarin and Wadhawan 2005; Shah and Kamath 2010). The underlying etiology in both idiopathic portal hypertension and extrahepatic portal vein thrombosis is considered to be vascular in origin. In the case of schistosomiasis, the underlying cause of the disease is a parasitic infection triggered by several species of trematodes belonging to the genus Schistosoma. Schistosomiasis is one of the most common causes of non-cirrhotic portal hypertension worldwide. According to data from the World Health Organization, the number of patients reported to have been treated for this disease was 28.1 million in 2011. The pathogenesis of schistosomiasis-associated portal hypertension is a pre-sinusoidal obstruction caused by deposition of the eggs of the parasite worms into the pre-sinusoidal portal venules. Trapped eggs secrete antigens that elicit a strong immune response resulting in granulomatous inflammation, pre-sinusoidal and periportal fibrosis, and progressive obstruction of portal blood flow. In these patients, portal hypertension may also be associated with portal vein thrombosis (Dunn and Kamel 1981; Ross et al. 2002). Murine models of this disease do exist and develop hepatic granulomatous inflammation with a cellular composition and dynamics similar to what is observed in patients (Stavitsky 2004).

Cirrhotic portal hypertension is the categorization most commonly associated with increased resistance to portal blood flow. The prevalence of cirrhosis worldwide remains unknown since patients with compensated cirrhosis present few or no symptoms. However, some authors have estimated that up to 1 % of the population may present liver cirrhosis (Schuppan and Afdhal 2008). Cirrhosis can be defined as a complex pathophysiological process of the liver that involves progressive destruction and pathological regeneration of the liver characterized by the accumulation of extracellular matrix, mainly collagen I and III. As a result, normofunctional hepatic parenchyma is replaced by fibrotic scars and increased resistance to blood flow. In addition to fibrosis, cirrhotic patients develop other complications that adversely affect the morbidity and mortality such as ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, esophageal variceal bleeding, and hepatic encephalopathy (Moore et al. 2003; Gines et al. 2004). A wide range of diseases and conditions can lead to cirrhosis. Viral hepatitis – by the hepatitis B or C viruses – and alcohol are considered the two most important etiologies of cirrhosis in the Western world, although nonalcoholic steatohepatitis (NASH) is also increasingly being recognized as a common cause. NASH is associated with diabetes, obesity, and metabolic syndrome and is

characterized by a significant hepatic inflammatory response with concurrent fat accumulation in the liver (Farrell and Larter 2006; Larter et al. 2010). In all these diseases, the localization of the increased resistance to portal blood flow is predominantly sinusoidal. Primary biliary cirrhosis and autoimmune hepatitis are also liver diseases that have in origin an exacerbated response of the immune system. The augmented immune response in primary biliary cirrhosis causes chronic inflammation and a progressive destruction of the small intrahepatic bile ducts (Charatcharoenwitthaya and Lindor 2005). In this disease, portal hypertension is predominantly pre-sinusoidal and may precede cirrhosis. The mechanism that triggers inflammation is also unknown in autoimmune hepatitis. However, it has been shown that activation of $CD4^+$ helper T cells triggers the initial pathogenic steps needed for the development of autoimmune hepatitis (Longhi et al. 2006). Other less common causes of cirrhotic portal hypertension are sclerosing cholangitis, biliary atresia, prolonged exposure to drugs and toxins, as well as inherited genetic disorders (i.e., hemochromatosis and Wilson's disease). Several experimental models of cirrhotic portal hypertension in mice and rats have been generated. Among these, common bile duct ligation and carbon tetrachloride administration are the most extensively used (Jimenez et al. 1992; Iredale 2007). Both models present cirrhosis and portal hypertension and are excellent tools to study intrahepatic alterations. Partial portal vein ligation in animal models, mainly in rats, has been used as another strategy to study portal hypertension in the absence of hepatic alterations (Groszmann et al. 1982b). This model is particularly useful in the study of increased pre-hepatic resistance to portal blood flow.

Pathogenesis of Portal Hypertension in Cirrhosis

The therapeutic approaches for cirrhotic patients are limited to liver transplantation. However, chronic graft rejection, the imbalance between the demand and the availability of organs, and lifelong side effects of immunosuppression encourage the development of new therapeutic strategies aimed at improving the treatment of cirrhosis. Therefore, the main goal of both experimental and clinical research has been to understand the pathogenesis of portal hypertension in cirrhosis. Despite the progress made in this field to date, our knowledge of the pathogenesis of portal hypertension is still incomplete. However, nowadays it is now well recognized that most of the diseases causing cirrhotic portal hypertension share common pathological features that can be summarized as follows: (1) the initial step needed for the development of fibrosis and portal hypertension is a sustained and exacerbated hepatic inflammatory response caused by parenchymal injury, (2) an increment in both intrahepatic resistance and splanchnic blood flow causes an increase in portal pressure (see section "Classification of Liver Diseases Associated with Portal Hypertension"), (3) impaired production of vascular mediators contributes to hemodynamic abnormalities in the sinusoidal and in the splanchnic vasculature, and (4) the loss of normal tissue architecture in the liver and splanchnic areas contributes to portal hypertension. In this section, we will describe the dynamic and anatomical mechanisms responsible for the aforementioned pathological features.

Inflammation

Hepatocellular damage triggers an inflammatory response that favors the hepatocellular repair. However, exacerbated and/or persistent inflammatory responses cause tissue damage and eventually lead to liver fibrosis and portal hypertension. In physiological conditions, inflammation involves an activation of many molecular pathways and cellular crosstalk at the site of the injury that result in the following well-known symptoms: increased blood flow, increased vascular permeability, and leukocytes infiltration. This immunological response, namely, acute inflammatory response, is usually of short duration, is localized, has a rapid onset, and is primarily mediated by neutrophils, basophils,

eosinophils, and mononuclear cells. On the other hand, if the inflammatory condition persists and is not resolved within a short time, as in many diseases leading to cirrhosis, the inflammation becomes chronic (Serhan and Savill 2005; Brenner et al. 2013). Anatomically, chronic inflammation is characterized by the replacement of damaged tissue by fibrous connective tissue and a change in the organ angioarchitecture with the occurrence of pathological angiogenesis and lymphangiogenesis (Halin and Detmar 2008). All these changes are associated with a loss of tissue/organ functionality. As opposed to acute inflammation, chronic inflammation is primarily mediated by mononuclear cells (monocytes/macrophages, lymphocytes) and myofibroblast-like cell activation.

To understand the association between inflammation and hepatic fibrosis, researchers have generated experimental models of liver fibrosis with impaired leukocyte activity. Macrophage depletion with gadolinium chloride (Ide et al. 2005) or adenoviral transduction of the liver with a dominant negative mutant form of MCP-1 (Imamura et al. 2005) significantly decreased fibrosis, inflammatory infiltrate, and hepatic stellate cell (HSC) activation in rats treated chronically with thioacetamide or dimethylnitrosamine, respectively. Macrophages are also involved in the recovery phase of the inflammatory process occurring in liver disease. As demonstrated in an experimental model of reversible liver injury, depletion of macrophages in fibrotic mice was associated with lower levels of both fibrosis and myofibroblast-like cell activation. By contrast, macrophage depletion during recovery led to a failure in matrix degradation. These results were obtained in transgenic mice expressing a selective conditional ablation system for macrophages (Duffield et al. 2005). This experimental strategy does not allow discerning between different subpopulations of macrophages, which may be activated during the hepatic inflammatory process. For instance, Kupffer cells are also targeted by this methodological approach. These cells are a large population of resident tissue macrophages that are located in the sinusoidal lumen and in close contact with the endothelial cells. Additionally, Kupffer cells may also physically interact with hepatocytes after migration through the space of Disse (Gressner and Bachem 1994). It is known that certain stimuli such as obesity, alcoholic intake, drugs, or xenobiotics consumption induce the activation of these cells. After activation, Kupffer cells release significant levels of cytokines (e.g., IL-1, IL-6, IL-10, TNF-α), ROS, and eicosanoids. These molecular mediators, together with the secretion of lysosomal enzymes, favor exacerbation of inflammation and tissue injury (Decker 1990; Winwood and Arthur 1993).

Natural killer (NK) cells also play a major role in liver injury. The local lymphocyte population in the liver is enriched in NK cells. These cells are activated during viral infection and target virus-infected hepatocytes promoting either apoptosis or osmotic cell lysis (Ahmad and Alvarez 2004). In addition, NK cells produce IFN-γ, among other cytokines, which activate the expression of CXCL9 in hepatocytes and LSECs. Subsequently, CXCL9 induces recruitment of virus-specific T cells (Crispe 2003). The role of NK cell activation in the regulation of fibrosis has also been demonstrated in experimental models of cirrhosis induced by diethylnitrosamine or CCl_4 treatment. In this setting, NK cell activation by polyinosinic-polycytidylic acid (a toll-like receptor 3 ligand) induces HSC cell death and attenuates the severity of liver fibrosis in mice (Radaeva et al. 2006).

Concerning the role of the adaptative immune response in hepatic fibrogenesis, Novobrantseva et al. studied fibrotic mice deficient in B and T cells ($RAG2^{-/-}$), $CD4^+$ T cells, $CD8^+$ T cells, and gamma-delta T cells. Among these experimental conditions, only $RAG2^{-/-}$ mice showed reduced levels of hepatic collagen deposition. These authors also demonstrated that B cells mediate antibody-independent stimulation of liver fibrosis (Novobrantseva et al. 2005). These results are in agreement with studies demonstrating that hepatic damage induced by CCl_4 is lower in splenectomized rats (Chen et al. 1998). In contrast to the significant role played by macrophages, NK cells, and B cells, neutrophil depletion in BDL cirrhotic animals has no impact on hepatic fibrogenesis (Saito et al. 2003).

The cellular mediators of acute and chronic inflammation substantially differ, being the vasoactive amines and eicosanoids characteristic of acute response and IFN-γ, TNF-α, IL-6, IL1-β, growth factors, radical oxygen species (ROS), and proteases characteristic of the chronic response (Serhan and Savill 2005). Among these mediators, TNF-α, ROS, and eicosanoids are among the factors most studied in chronic liver diseases.

TNF-α is a primary mediator of inflammation in the liver and it is mainly produced by Kupffer cells in pathological conditions (Brenner et al. 2013). TNF-α exerts its mechanism of action through receptor-mediated signal transduction, targeting either TNFR1 or TNFR2. TNFR1 is expressed constitutively by all cell types while TNFR2 is predominantly expressed in activated immune cells (Pennica et al. 1984). In hepatocytes, TNF-α may induce either cell survival (through NF-kB or MAPK activation) or cell death (through caspase, the mitochondrial death pathway, RIP1, or RIP3 activation) (Yamada et al. 1998; Bradham et al. 1998). In experimental models, TNFR1 and TNFR2 gene deficiency is associated with resistance to alcohol-mediated fatty liver and hepatocyte cell death. In this context, alcohol feeding, a condition characterized by low levels of mitochondrial glutathione (GSH), and pharmacological depletion of mGSH sensitize hepatocytes to TNF-α-induced cell death (Colell et al. 1998; Simeonova et al. 2001).

ROS are other major cellular mediators involved in cell signaling and cell function. However, an imbalance between ROS production and intracellular ROS neutralization may affect cellular integrity. When ROS levels exceed cellular antioxidant defenses, oxidative damage develops and this is translated into DNA genotoxicity, protein oxidation and fragmentation, and lipid peroxidation. The presence of oxidative stress has been described in most of the clinical (NASH, HCV, alcoholic liver cirrhosis, hemochromatosis, Wilson's disease, primary biliary cirrhosis, and cholestasis) and experimental conditions (cirrhosis induced by CCl_4, chronic ethanol administration, and bile duct ligation) associated with fibrosis and portal hypertension. In this pathological context, the association between high levels of oxidative stress and a reduction of antioxidant defenses, such as superoxide dismutase and catalase, is also present. ROS is a term that encompasses various oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl), and hydroxyl radicals (OH). The hepatic sources of ROS are diverse and include the leakage of activated oxygen from mitochondria with structural and functional abnormalities, xanthine oxidase, NADPH oxidases, and cytochromes P450 monooxygenases. Among these sources of ROS, NADPH oxidases and SOD are promising candidates to develop new therapies (Poli and Parola 1997; Parola and Robino 2001; De Minicis and Brenner 2007). Transduction of cirrhotic rats with adenovirus encoding for SOD, a critical enzyme that metabolizes $\cdot O_2^-$, resulted in a marked reduction in $\cdot O_2^-$ levels and portal pressure (Lavina et al. 2009). The other target, NADPH oxidase, is present in HSCs (named the non-phagocytic NADPH) and in Kupffer cells (named the phagocytic NADPH). Bataller et al. demonstrated that activated HSCs express NADPH oxidase and generate ROS in an angiotensin II-dependent way. The link between NADPH oxidase activity and fibrosis was further demonstrated in vivo in mice lacking a functional NADPH oxidase. These animals were resistant to liver fibrosis after bile duct ligation. In addition, p47phox deficiency (a regulatory subunit of NADPH oxidase) in Kupffer cells generated resistance to liver injury induced by ethanol and diminished the levels of TNF-α (Bataller et al. 2003). These results show that oxidative stress is an important mediator of inflammatory response following ethanol treatment.

Prostanoids and leukotrienes are a structurally heterogeneous group of lipids that also play a major role in inflammation. Prostanoids are generated by the cyclooxygenase isoenzymes – COX1 and COX2 – through the oxidation of arachidonic acid and the production of prostaglandin H2, which is the precursor of prostaglandins and thromboxanes. COX1 is constitutively expressed in many tissues and is associated with

beneficial and cytoprotective effects in the stomach, kidney, and blood vessels. In contrast, COX2 is mostly induced in tissues that are going through an inflammatory process (Crofford 1997; Ricciotti and FitzGerald 2011). COX2 is usually absent in healthy liver but is robustly expressed in the liver in response to endotoxemia, ischemia reperfusion, bile duct ligation, and alcoholic cirrhosis (Suzuki-Yamamoto et al. 1999). Among the prostanoids, thromboxane-2 is one of the most studied in liver disease. Thromboxane-2 stimulates inflammation and leukocyte adhesion in hepatic sinusoids. The treatment of rats with thromboxane inhibitors attenuates these pathological changes occurring during the induction of liver disease. In addition, transgenic expression of COX2 in hepatocytes enhances D-galactosamine-/LPS-induced liver failure (Ricciotti and FitzGerald 2011). Despite this evidence, the therapeutic utility of COX2 inhibition in liver disease is not yet well established. For instance, genetic studies have demonstrated that COX2 protects mice from hepatitis triggered by agonistic anti-FAS antibodies (Li et al. 2009). Leukotrienes are also major products of arachidonic acid metabolism. The key enzymes participating in the conversion of arachidonic acid into leukotrienes are 5-lipoxygenase and 5-lipoxygenase-activating protein (FLAP). Leukotrienes are potent promoters of inflammation through the activation of nuclear factor-κB and the stimulation of cytokine/adipokine secretion (Samuelsson et al. 1987). Several studies have shown that leukotrienes play a significant role during hepatic inflammatory response. 5-Lipoxygenase deficiency in mice decreases steatosis, inflammation, and fibrosis in an Apo $E^{-/-}$ genetic background and in CCl_4-treated mice (Martinez-Clemente et al. 2010a). Accordingly, pharmacological inhibitors for FLAP reduce CCl_4-induced liver injury and inflammatory infiltrate in experimental models of nonalcoholic steatohepatitis (NASH) and nonalcoholic fatty liver disease (NAFLD) (Titos et al. 2005, 2010). Some investigators have proposed a similar role for the 12/15-lipoxygenase pathway in NAFLD (Martinez-Clemente et al. 2010b).

Impaired Production of Vascular Mediators

As mentioned previously, the increase in both the intrahepatic resistance and portal blood flow are the hemodynamic disturbances that cause portal hypertension. The current pharmacological therapies available for the prophylaxis and treatment of variceal bleeding target these two components (Table 1; De Franchis 2010; Bari and Garcia-Tsao 2012). For instance, increased portal blood flow is treated with nonselective β-adrenergic drugs, somatostatin and vasopressin analogs, while the treatment of patients with nitric oxide donors lowers intrahepatic resistance. The demonstrated effectiveness of these drugs is consistent with the relevant role played by their targets in the pathogenesis of portal hypertension. Below, we describe the factors and the pathological processes targeted by these pharmacological treatments.

Catecholamines

Catecholamines such as adrenaline and noradrenaline regulate major and diverse physiological functions through their G-protein-coupled adrenergic receptors α and β. Specifically, noradrenaline can function as a major neurotransmitter in the peripheral sympathetic nervous system, a stress hormone that increases the heart rate through β1 adrenergic receptor activation, and a vasoconstrictor hormone that targets smooth muscle cells through α adrenergic receptor activation. Noradrenaline can also induce splanchnic vasodilation through the activation of the β2 adrenergic receptor (Eisenhofer et al. 2004). This diversity of effects is used as therapeutic strategy for the treatment of patients with clinically significant portal hypertension. This idea was first proposed in 1981 by Lebrec for the prevention of variceal rebleeding and has, since then, become the core of pharmacotherapy in portal hypertension (Lebrec et al. 1981). The effectiveness of this treatment lies in the fact that nonselective β-blockers decrease cardiac output via blockade of β1 adrenergic receptors, which are more abundant in the heart and kidney, and constricts the splanchnic vasculature via blockade of β2 adrenergic receptors, which are more

Table 1 Current pharmacological therapies for the prophylaxis and treatment of variceal bleeding

Therapy stratification	Clinical stages		Therapeutic strategies
Pre-primary prophylaxis	*Cirrhotic patients without gastroesophageal varices*		No specific treatment for portal hypertension Treatment of the underlying liver disease may reduce portal hypertension
Primary prophylaxis	*Low-risk patients*	Small varices without red wale signs or varices occurring in child A or B patients	Nonselective β-blockers (optional)
	High-risk patients	(a) Medium/large varices	Nonselective β-blockers or EBL
		(b) Patients with small varices with red wale signs or child C class	Nonselective β-blockers
Management of acute variceal hemorrhage	*Variceal hemorrhage*		Safe vasoactive drugs starting prior to diagnostic endoscopy (terlipressin, somatostatin, octreotide, vapreotide) and emergency endoscopy therapy at the time of initial diagnostic
Secondary prophylaxis	*Patients who survive an episode of variceal hemorrhage*	(a) Patients who had a TIPS performed during the acute episode	Not require specific therapy for portal hypertension or varices
		(b) Patients who do not have a TIPS performed during the acute episode	The combination of nonselective β-blockers ± isosorbide-5-mononitrate and EBL

EBL endoscopic band ligation

abundant in the digestive track. In addition, cirrhotic patients with portal hypertension exhibit high levels of noradrenaline in blood, which is a reflection of the sympathetic nervous system activation characteristic of this disease (Ruiz-del-Arbol et al. 2005).

An interesting question that illustrates the complexity of the hemodynamic disturbances in portal hypertensive patients is that the magnitude of the activation of the sympathetic nervous system in cirrhotic patients differs according to the vascular territory. For instance, nonselective β-blocker treatment in patients with portal hypertension results in a significant and highly reproducible partial reversion of the circulatory hyperdynamic state. These changes are dose dependent and are significantly associated with the effective dose of the drug in the blood. However, nonselective β-blocker treatment has a much more variable effect on portal pressure, which usually is close to 15 % and is hardly dose dependent (Garcia-Tsao 2001). Therefore, hepatic perfusion is minimally affected by nonselective β-blocker treatment. This fact underscores the contribution of hepato-vascular structural changes, which are unaffected by the conventional pharmacological treatment, to the overall increase in intrahepatic resistance. Another factor that may contribute to the heterogeneous effectiveness of nonselective β-blockers is the reorganization of sympathetic nerve distribution. Several studies have shown the occurrence of this phenomenon in cirrhotic patients and in experimental models of portal hypertension. In cirrhotic livers, the number of sympathetic nerve fibers increases in portal areas and fibrous septa. By contrast, the innervation in regenerative nodules is negligible (Stoyanova and Gulubova 2000; Martell et al. 2010). In addition, portal hypertensive rats show a remarkable regression of sympathetic innervation in the mesenteric vascular bed. The blockade of sensory afferent nerves in portal hypertensive rats prevents both regression of sympathetic innervation and hemodynamic alterations (Coll et al. 2010). These observations reveal the

significant role of the sympathetic nervous system on splanchnic arterial vasodilation.

Vasopressin

Vasopressin (AVP) is a hormone that plays a pathological role in portal hypertension. This neurohypophysial hormone is released in response to changes in both blood pressure and plasma osmolality and regulates water retention and vasoconstriction of blood vessels. These biological effects are mediated by its specific receptors V2 and V1A, respectively. The V1A receptor is located in vascular smooth muscle cells and cardiomyocytes. This receptor modulates vessel vasoconstriction and myocardial function through phospholipase C activation and intracellular calcium mobilization. V2 receptors are distributed on renal collecting duct cells and mediate the well-known antidiuretic effects of AVP through the modulation of intracellular levels of cAMP (Holmes et al. 2003, 2004). Several clinical and preclinical studies have shown that vasopressin is a potent splanchnic vasoconstrictor that reduces portal pressure by decreasing portal and portosystemic collateral blood flow and by lowering the cardiac index (Bosch et al. 1981, 1988). In addition, in situ perfusion experiments in animal models of portal hypertension have shown that vasopressin exerts a direct vasoconstrictive effect on the collateral vasculature (Chan et al. 1999). Despite its effectiveness in decreasing portal pressure, vasopressin treatment is associated with serious side effects, such as bowel necrosis and myocardial infarction. Furthermore, vasopressin has a short half-life and can only be administered as a continuous intravenous infusion. Therefore, its use is restricted to the management of acute variceal bleeding. All these contraindications of vasopressin seem to be corrected, at least in part, by its semisynthetic analog terlipressin. Terlipressin is slowly cleaved to vasopressin by endothelial peptidases, and treatment with this analog has shown similar splanchnic and systemic hemodynamic effects in both experimental models of cirrhosis and cirrhotic patients (Lebrec et al. 1993; Escorsell et al. 1997, 2000; D'Amico et al. 1999). Moreover, terlipressin treatment presents a better biosecurity profile than vasopressin treatment and is, consequently, preferred over vasopressin.

Somatostatin

Somatostatin is a peptide hormone that regulates endocrine systems through its five somatostatin G-protein-coupled receptors, SSTR1 to SSTR5. Somatostatin and its counterpart octreotide suppress the release of the gastrointestinal hormones – insulin and glucagon – and reduce blood flow within the intestine (Ruscica et al. 2013). In 1981 Bosch et al. demonstrated that continuous infusion of somatostatin to cirrhotic patients reduced both wedged hepatic venous pressure and estimated hepatic blood flow (Bosch et al. 1981). Currently, both somatostatin and octreotide are employed in cirrhotic patients to stop active bleeding by gastroesophageal varices (De Franchis 2010). This treatment effectively reduces the splanchnic blood flow and portal pressure. Several lines of evidence suggest that the beneficial effect of somatostatin/octreotide is partially mediated by the inhibition of glucagon release: First, postprandial hyperemia, mediated by vasoactive peptides such as glucagon, aggravates portal hypertension (Albillos et al. 1994). Second, glucagon abolishes the hemodynamic benefits of somatostatin treatment (Pizcueta et al. 1991). However, the rapid onset of effects of somatostatin in cirrhotic patients cannot be exclusively explained by changes on glucagon release, which is a slow response. For instance, cirrhotic patients exhibit a significant decrease in variceal and portal pressure after 2 min of acute treatment with somatostatin. Therefore, it is expected that somatostatin/octreotide may directly affect the splanchnic or the liver vasculature. In agreement with this hypothesis, studies in portal hypertensive rats show that both somatostatin and octreotide enhance the vasoactive properties of other vasoconstrictors such as endothelin-1 in portosystemic collaterals (Reynaert and Geerts 2003).

Nitric Oxide

A pioneering study published by Grosmann et al. in 1982 demonstrated the concept that co-treatment with vasodilators (nitroglycerine)

and splanchnic vasoconstrictors (vasopressin) causes a further reduction in the wedged hepatic venous pressure in cirrhotic patients and in portal hypertensive dogs. This co-treatment did not modify portal blood flow, suggesting that nitroglycerine mainly affects intrahepatic resistance (Groszmann et al. 1982a). Accordingly, cirrhotic patients co-treated with isosorbide mononitrate and nonselective β-blocker exhibited lower portal pressure values than those treated with a nonselective β-blocker alone (Groszmann et al. 1982a; Albillos et al. 1998). Both nitroglycerine and isosorbide mononitrate are nitric oxide (·NO) donors and the lesson that can be learned from these drugs is that ·NO plays a relevant role in the pathogenesis of intrahepatic resistance to portal blood flow.

Nitric oxide is a gaseous signaling molecule with radical chemistry properties. This characteristic confers to ·NO the potential to interact in cells with thiols, heme-containing proteins, and other species with unpaired electrons such as $\cdot O_2^-$. In the context of blood vessel reactivity, the best-known target of ·NO is the soluble guanylate cyclase (sGC). The interaction between ·NO and the heme group of sGC activates the generation of 3′,5′-cyclic monophosphate (cGMP) levels that ultimately signal relaxation (Ignarro et al. 1986). Nitric oxide is endogenously produced by the nitric oxide synthase (NOS) isoforms which catalyze the conversion of L-arginine and oxygen into citrulline and ·NO. Three isoforms of NOS have been identified: neuronal NOS (nNOS) (Bredt et al. 1991), inducible NOS (iNOS) (Lowenstein et al. 1992; Lyons et al. 1992), and endothelial NOS (eNOS) (Lamas et al. 1992; Sessa et al. 1992). eNOS expression and ·NO production in endothelial cells are important regulatory mechanisms to maintain vascular tone and to inhibit leukocyte and platelet adherence (Fleming and Busse 2003). The relevance of eNOS in the control of vascular homeostasis is evidenced by the fact that eNOS gene disruption in mice results in a hypertensive phenotype, smooth muscle cell hyperplasia in response to vascular injury, and poor response to angiogenic stimuli (Vallance and Leiper 2002).

Nowadays, there is an agreement that ·NO has a pathological role in liver disease and contributes to both splanchnic vasodilation and increased hepatic resistance. In the context of splanchnic hemodynamics, studies in animal models of portal hypertension and cirrhosis show that mesenteric overproduction of ·NO significantly contributes to splanchnic vasodilation, splanchnic hyperemia, and increased portal venous blood flow. For instance, cirrhotic rats show higher pressor responsiveness to increasing doses of NOS inhibitors than control rats (Claria et al. 1992). NOS inhibition restores the pressor effect of vasoconstrictors in the splanchnic vasculature of cirrhotic rats with ascites (Sieber et al. 1993). Accordingly, the normalization of ·NO production in cirrhotic rats by the administration of low doses of L-N^G-nitroarginine methyl ester (L-NAME) improves systemic hemodynamics (Niederberger et al. 1995, 1996). Similar evidence has also been found in mesenteric preparations of portal hypertensive rats (Sieber and Groszmann 1992) and other experimental models of portal hypertension (Lee et al. 1992; Hartleb et al. 1994).

The molecular mechanism responsible for the ·NO overproduction in cirrhotic portal hypertension has also been intensively investigated. Several studies have observed an increased protein abundance of eNOS and enhanced eNOS activity in arterial vessels of cirrhotic, PVL, and BLD rats compared to control animals (Cahill et al. 1995, 1996; Martin et al. 1996; Morales-Ruiz et al. 1996; Heller et al. 1999; Liu et al. 1999; Stumm et al. 2002). In addition, Theodorakis et al. demonstrated that deletion of the eNOS, rather than the iNOS, gene preferentially protects partial portal vein-ligated rats from portal hypertension (Theodorakis et al. 2003). However, these data were not confirmed by Iwakiri and colleagues who found that partial portal vein-ligated rats maintain their hyperkinetic circulation despite the double deficiency of both the eNOS and iNOS genes (Iwakiri et al. 2002). These results suggest that other compensatory mechanisms may take place in the scenario of dual eNOS and iNOS deficiency. The molecular mechanisms that mediate eNOS overexpression and enhanced eNOS activity in extrahepatic areas are complex, with

some of the potential mechanisms being: increased shear stress, HSP90, altered intracellular eNOS localization, TNF-α, and VEGF. Both TNF-α and VEGF are significantly overexpressed in inflammatory conditions and in response to bacterial infection. In this context, selective intestinal decontamination with norfloxacin partially corrects the hyperdynamic syndrome of cirrhotic patients, suggesting a role of bacterial translocation in eNOS overexpression and activation. As in the animal models, cirrhotic patients show an overproduction of ·NO in different territories such as the systemic vasculature (Guarner et al. 1993; Albillos et al. 1995), the portal vein (Battista et al. 1997; Sarela et al. 1999; Albornoz et al. 2001), the hepatic vein (Battista et al. 1997), and exhaled breath (Matsumoto et al. 1995; Sogni et al. 1995). Moreover, ·NO inhibition treatment in cirrhotic patients corrects arterial hyporesponsiveness to vasoconstrictors and improves the hyperdynamic circulation (Campillo et al. 1995; La Villa et al. 2001; Thiesson et al. 2003).

Nitric oxide is an important regulator of hepatic vascular tone (Mittal et al. 1994; Bauer et al. 1997; Shah et al. 1997; Zhang et al. 1997). Therefore, changes in the hepatic activity of eNOS can lead to an abnormal increase in the resistance to portal blood flow. In the context of cirrhotic portal hypertension, it is of note that there is an overproduction of ·NO in the splanchnic vascular beds, and by contrast, the intrahepatic production of ·NO is diminished. Many authors have described this impaired ·NO production in cirrhotic livers. These studies show that the hepatic deficiency of ·NO affects the response to vasodilators and contributes to a generalized hepatic vasoconstriction. For instance, ·NO production and eNOS protein activity are decreased in perfused cirrhotic livers from CCl_4-treated rats and in isolated endothelial cells from both CCl_4-treated rats and BDL rats (Gupta et al. 1998; Rockey and Chung 1998). Additionally, Sarela et al. showed that the activity of intrahepatic calcium-dependent NOS was lower in cirrhotic patients compared with non-cirrhotic subjects (Sarela et al. 1999). The impaired production of NOS in livers from cirrhotic rats occurs independent of changes in gene expression, which suggest a posttranslational control of eNOS activity (Rockey and Chung 1998). Some studies have revealed diverse molecular mechanisms that contribute to explain this phenomenon. One of these mechanisms is the overexpression of caveolin-1 in cirrhotic livers. Enhanced expression and interaction of caveolin-1 with eNOS reduced NOS activity in livers from CCl_4-treated and BDL rats (Shah et al. 1999, 2001; Hendrickson et al. 2003). Similar to these findings, Yokomory and colleagues demonstrated that liver specimens from cirrhotic patients show an overexpression of caveolin-1 (Yokomori et al. 2002). The link between ROS and impaired ·NO production has also been demonstrated in animal models and cirrhotic patients. Antioxidant treatments effectively reverse the impaired intrahepatic production of ·NO (Ting et al. 1996; Jackson et al. 1998; Taddei et al. 1998; Hernandez-Guerra et al. 2006; Karaa et al. 2006). The administration of ascorbic acid to cirrhotic patients corrects sinusoidal endothelial cell dysfunction and attenuates the postprandial increase in portal blood resistance (Hernandez-Guerra et al. 2006). Another strategy used to decrease hepatic oxidative stress in cirrhotic rats is the overexpression of SOD by gene therapy. Transduction of cirrhotic livers with SOD increases ·NO bioavailability and reduces portal pressure (Lavina et al. 2009). SOD is an enzyme that metabolizes $\cdot O_2^-$. In aqueous solutions, ·NO highly interacts with $\cdot O_2^-$ to produce peroxynitrite ($ONOO^-$), which is more reactive than ·NO and $\cdot O_2^-$ alone. As a result, ·NO is sequestered and inactivated. Besides decreasing the bioavailability of ·NO, oxidative stress also enhances eNOS/caveolin-1 interaction and impairs eNOS activation mediated by the endothelin receptor type B (Karaa et al. 2006). Another factor leading to impaired eNOS activation in cirrhotic livers is Akt activity. Akt is a kinase protein that phosphorylates eNOS on the consensus RxRxxS motif present in its carboxy-terminal end. This specific phosphorylation activates eNOS and enhances ·NO production (Fulton et al. 1999). This impaired Akt activation in cirrhotic livers was first described by Morales-Ruiz et al. (2003). In this study, the administration of an adenoviral vector carrying a constitutively active

mutant of Akt (myr-Akt) increased intrahepatic eNOS activation, normalized portal pressure, decreased superior mesenteric blood flow, and ameliorated arterial hypotension in cirrhotic rats. Several mechanisms seem to contribute to Akt impairment in cirrhotic livers. For instance, one study demonstrated a direct interaction between GRK2, an inhibitor of G-protein-coupled receptor signaling, and Akt uncoupled eNOS activation in experimental models of liver injury. These authors also demonstrated that GRK2 heterozygotic gene deficiency reduces portal hypertension in bile duct-ligated mice (Liu et al. 2005). Other studies have associated Akt impairment with Rho kinase, which is a downstream effector of Rho. In human endothelial cells, Rho kinase activity blocks eNOS phosphorylation through inhibition of Akt (Ming et al. 2002). The in vivo inhibition of Rho kinase leads to Akt activation and cardiovascular protection (Wolfrum et al. 2004). In the context of liver injury, Rho kinase plays a major role in the contractile response of activated HSC (Iizuka et al. 2011). Furthermore, bile duct-ligated rats treated with fasudil – a selective Rho kinase inhibitor – show increased hepatic Akt/eNOS interaction and activation (Anegawa et al. 2008). Besides the mechanisms described above, other treatments capable of activating Akt, such as estrogen (Sakamoto et al. 2005) and simvastatin (Zafra et al. 2004), reduce portal pressure in both cirrhotic rats and patients. From the preceding discussion, it can be predicted that pharmacological activation of Akt may represent a promising strategy for the treatment of cirrhosis and portal hypertension.

Some pharmacological and gene therapy approaches have been designed to specifically deliver ·NO to the liver. These new therapies include the use of liver-targeted ·NO donors, such as the ·NO-releasing derivative of ursodeoxycholic acid NCX-1000 (Fiorucci et al. 2001), and the hepatic gene transfer of nNOS (Yu et al. 2000) and eNOS (Van de Casteele et al. 2002). Although all these methods have successfully reduced portal pressure in cirrhotic animals, their utility in the clinical setting is still unclear. For example, NCX-1000 is ineffective in lowering the HVPG in cirrhotic patients.

Vasoactive Factors That Have Not Yet Been Translated to Clinical Treatment

Several studies in rodents and patients have demonstrated that other vasoactive factors also contribute to the increase in the intrahepatic resistance. The most frequently studied factors are: carbon monoxide, endothelin, thromboxane, leukotrienes, angiotensin, apelin, and cannabinoids. Despite their therapeutic potential, so far none of these targets has been translated to clinical treatment and their use in patients is currently off-label. In this section, we will only describe the role of angiotensin, cannabinoids, and apelin as the other factors have been widely discussed in other reviews.

The vasoconstrictor angiotensin is one of the best characterized in liver dysfunction and significantly contributes to the dynamic component of intrahepatic resistance. The renin-angiotensin-aldosterone axis (RAS) plays a major role in both the regulation of blood pressure and water fluid balance through the vasoconstrictor angiotensin II and the mineralocorticoid aldosterone, respectively. RAS also plays a relevant role in wound healing of chronically injured tissues, inflammation, and fibrogenesis. The functionality of the RAS system depends on the presence of the angiotensin converting enzyme (ACE), angiotensin II, and the angiotensin II type receptor (AT1R). This pathway is named the classical axis (Zhuo et al. 2013). Most components of the classical pathway are expressed in the liver and are markedly upregulated in liver disease. For instance, HSCs express the AT1R and contract after stimulation with angiotensin II. In addition, angiotensin II gene expression is augmented in the cirrhotic liver (Herath et al. 2013). These results, together with the observation that pharmacological inhibition of the RAS improves fibrosis, have led to the clinical use of RAS inhibitors for the treatment of cirrhotic portal hypertension. Several clinical trials have evaluated the usefulness of ACE and AT1R inhibitors. The conclusion of a meta-analysis that considered these clinical trials was that angiotensin receptor blockers and ACE inhibitors effectively decrease HVPG in child A cirrhotic patients to a similar extent as nonselective β-blocker treatment. However, the beneficial

effect of RAS inhibition is lost in child B or C patients (Tandon et al. 2010), suggesting that the RAS is mainly responsible for intrahepatic vasoconstriction in early stages of cirrhosis. Angiotensin (1–7) is another member of the angiotensin family that has vasodilator/antiproliferative properties. Therefore, RAS is a dual system in which vasoconstrictor/proliferative or vasodilator/antiproliferative actions are established by the balance between Ang II and Ang-(1–7) concentrations, respectively. The latter alternative (via angiotensin (1–7) generation) requires the presence of ACE2, angiotensin (1–7), and the Mas receptor, which specifically recognizes angiotensin 1–7 (Zhuo et al. 2013). The relevance of this alternative pathway in portal hypertension is unclear. Nevertheless, cirrhotic animals and patients upregulate elements of the alternative RAS pathway in the mesenteric circulation (Herath et al. 2013). Whether or not angiotensin 1–7 contributes to splanchnic vascular vasodilation in liver disease remains unanswered.

Endocannabinoids have been extensively studied in recent years and have been found to have important local roles in several complications associated with hepatic dysfunction including hemodynamic disturbances, fibroproliferative processes, host defense mechanisms, obesity, and hepatic steatosis (Jorda et al. 2002; Jimenez 2005; Lotersztajn et al. 2005; Kunos and Osei-Hyiaman 2008). Cannabis has been used for medical and recreational purposes from antiquity. Nevertheless, the different targets in the organisms were not identified until the 1980s–1990s with the characterization of the cannabinoid receptors 1 and 2 (CB1 and CB2, respectively) (Matsuda et al. 1990; Munro et al. 1993) and the isolation of anandamide (AEA), the first endocannabinoid known (Devane et al. 1992). The endocannabinoid system is made up of the cannabinoid receptors, their endogenous ligands (endocannabinoids), and the proteins involved in their synthesis and inactivation (Di et al. 2004). The endogenous cannabinoid family includes AEA, 2-arachydonyl glycerol (2-AG), virodhamine, noladin ether, and N-arachidonoyl dopamine. These substances promote their action through CB receptors. Moreover, AEA interacts with the transient receptor potential vanilloid type 1 protein (TRPV1), which is also known as the VR1 receptor. Endocannabinoids are very lipophilic and cannot be stored in vesicles in contrast to what occurs with neurotransmitters. Consequently, the regulation of endocannabinoid signaling is tightly controlled by their synthesis, release, uptake, and degradation. Compelling evidence indicates that the endocannabinoid system plays a major role in numerous pathophysiological processes associated with liver disease. CB1 receptors are crucial mediators in the development of severe complications of cirrhosis, including splanchnic vasodilation, portal hypertension, and cirrhotic cardiomyopathy (Batkai et al. 2001; Ros et al. 2002; Domenicali et al. 2005, 2009; Gaskari et al. 2005; Moezi et al. 2006; Batkai et al. 2007). CB1 receptor blockade has proven to be effective in reducing portal hypertension and cirrhotic cardiomyopathy. Moreover, CB1 stimulation favors fat accumulation and triggers inflammation in NAFLD and alcoholic liver disease and contributes to the progression of the hepatic fibroproliferative processes (Mallat et al. 2011). On the other hand, CB2 receptors mediate antifibrogenic effects and play a major role in the regulation of liver inflammatory response. Overall, these data indicate that activation of CB receptors triggers dual effects; CB1 receptor activation enhances the progression of chronic liver disease to cirrhosis and accentuates some of its complications, whereas CB2 receptors are related to antifibrogenic properties. Therefore, the endocannabinoid system represents a potential therapeutic goal for liver disease. In this regard, the greatest experimental experience has been obtained with CB1 receptor antagonism. Alternatively, selective agonists of the CB2 receptors, which are devoid of psychoactive properties, are currently attracting increasing attention. In fact, fibrotic rats chronically receiving a CB2 receptor agonist show reduced hepatic collagen content, hepatocellular apoptosis, angiogenesis, and cell infiltrate compared to untreated fibrotic rats. In addition, this treatment improved MAP and PP (Munoz-Luque et al. 2008; Reichenbach et al. 2012). This is associated with an attenuated induction of PDGFRβ, α-SMA, MMPs, and

TIMPs; thus, CB2 receptor stimulation stops and/or prevents fibrosis progression in experimental fibrosis. The endogenous cannabinoid system is also of major relevance in the regulation of the immune and host defense mechanisms, most of these effects being mediated by interaction with central and peripheral CB2 receptors (Klein 2005). Actually, stimulation of these receptors attenuates the activation and release of proinflammatory mediators in neurodegenerative inflammatory disorders (Romero-Sandoval et al. 2009; Correa et al. 2010; Chung et al. 2012) and other inflammatory processes associated with liver (Batkai et al. 2012) and cardiac (Wang et al. 2012) reperfusion injury, atherosclerosis (Zhao et al. 2010), inflammatory bowel disease (Wright et al. 2008; Alhouayek et al. 2011), and rheumatoid arthritis (Sumariwalla et al. 2004). In this regard, recent studies have shown significantly diminished mRNA expression of CB1 and CB2 in circulating monocytes of cirrhotic patients. Markedly low CB1 and CB2 mRNA levels were found in peritoneal macrophages of cirrhotic patients with ascites, being almost suppressed when analyzed in patients with peritonitis (Reichenbach et al. 2013). Moreover, LPS reduced CB2 expression in human monocytes resulting in depressed chemotactic activity and therefore impaired host defense response of these cells.

Apelin (AP) is the endogenous ligand of the angiotensin receptor-like 1 (APJ), a G-protein-coupled receptor that has been found to be involved in an array of physiological events, such as water homeostasis (De Mota et al. 2004), regulation of cardiovascular tone (Ishida et al. 2004), and cardiac contractility (Szokodi et al. 2002). AP and its receptor are widely expressed in the central nervous system and in peripheral tissues, especially in endothelial cells but also in leukocytes, enterocytes, adipocytes, and cardiomyocytes (Tatemoto et al. 1998; Kawamata et al. 2001; Horiuchi et al. 2003; Daviaud et al. 2006; Scott et al. 2007). APJ activation leads to inhibition of cAMP production and activation of the Na^+/H^+ exchanger type 1 (NHE1) (Hosoya et al. 2000). Through the former pathway, AP enhances the vascular dilatation after the induction of eNOS, in a molecular cascade leading to extracellular-signal-regulated kinases (ERKs) and P70S6K activation (Masri et al. 2002, 2004). On the other hand, the burst of NHE1 activity in cardiomyocytes leads to a dose-dependent increase in myocardial contractility in vivo and in vitro (Szokodi et al. 2002; Berry et al. 2004). Recent studies have also suggested a role for Apelin in inflammation and angiogenesis since its expression is regulated by TNF-α (Daviaud et al. 2006), and it has been demonstrated that Apelin may trigger vascular sprouting in the absence of VEGF (Cox et al. 2006). Clinical and experimental studies performed in human cirrhotics and rats with CCl_4-induced cirrhosis have shown enhanced circulating levels of AP in this condition (Principe et al. 2008). AP mRNA has shown a fourfold rise only in hepatic tissue but not in the lung, heart, or kidney of cirrhotic rats. These animals also showed hepatic APJ mRNA levels 300 times higher than controls. Apelin was highly expressed by HSC, whereas APJ was overexpressed in the hepatic parenchyma of cirrhotic animals. Moreover, cirrhotic rats chronically treated with an APJ antagonist showed diminished hepatic fibrosis and angiogenesis and improved cardiovascular performance and renal function and less ascites. Human patients also showed a marked increase in AP levels (Principe et al. 2008; Reichenbach et al. 2012). These results were subsequently confirmed in portal hypertensive rats (Tiani et al. 2009) and patients with biliary atresia (Chen et al. 2013) and, for the first time, pointed to the hepatic AP system as a novel therapeutic target in liver diseases. Beyond the beneficial effects that APJ blockade has demonstrated, the disruption of the APJ signaling pathway using specific inhibitors could also interfere with the progression of chronic liver disease. In this regard, it has been demonstrated that AP is upregulated in HSCs of patients with cirrhosis and behaves as a paracrine mediator of fibrogenesis-related gene induction in a cell line derived from human HSCs (Principe et al. 2008; Melgar-Lesmes et al. 2010). Concerning the AP receptor, it has been shown

that tissue expression of APJ is overexpressed in the liver of cirrhotic patients (Melgar-Lesmes et al. 2011). This activation occurs mainly in HSCs but also in hepatocytes surrounded by fibrotic septae. Hypoxia and LPS seem to be involved in this phenomenon (Eyries et al. 2008). The basis to integrate the existent information on the role of the hepatic AP system in chronic liver disease should, therefore, consider two main components: the regulation of AP/APJ expression and the effects of APJ activation on fibrogenesis and angiogenesis. On one hand, hypoxia and inflammation induce the hepatic expression of APJ. On the other hand, the activation of APJ mediates the induction of profibrogenic genes (Melgar-Lesmes et al. 2011), the proliferation of HSCs, and the release of proangiogenic factors. Consequently, the hepatic AP system represents a link between chronic inflammation and the subsequent fibrogenic and angiogenic processes occurring in liver cirrhosis.

Other Bioactive Products Contributing to Hemodynamic Disturbances

Recently, researchers have described a novel bioactive product, microparticles, with vasoactive properties in blood of cirrhotic patients (Rautou et al. 2012). Microparticles are small cell-derived vesicles with a diameter between 0.1 and 1 μm and are presumably derived from cell activation or apoptosis. The membrane of the microparticle maintains cell surface molecules from parent cells that enable the identification of their cellular origin through the use of specific antibodies (Mause and Weber 2010). The severity of cirrhosis is positively associated with the plasma concentrations of microparticles derived mostly from leukocytes, endothelial cells, and hepatocytes. Rautou et al. showed that circulating microparticles from patients with advanced cirrhosis impair ex vivo arterial contraction to phenylephrine in vessels of control and cirrhotic rats and decrease the mean arterial pressure in rats. This effect was absent when microparticles from Child-Pugh A cirrhotic patients or healthy subjects were tested (Rautou et al. 2012). The degree of contribution of this mechanism to portal hypertension and its therapeutic potential is an active field of research.

Loss of Normal Tissue Architecture in Liver and Extrahepatic Areas

As mentioned above, increased resistance to intrahepatic portal blood flow and increased mesenteric blood flow are the hemodynamic components that cause portal hypertension. Both increases can be corrected to some degree by pharmacological treatment. However, these classical pharmacological strategies can only reduce intrahepatic resistance to some extent. Significant evidence supports the hypothesis that the reversal of long-term structural changes (i.e., angiogenesis, endothelial capillarization, extracellular matrix accumulation, portosystemic shunts, vascular remodeling), in both liver and splanchnic areas, may further improve the efficacy of the pharmacological treatments. The mechanisms involved in the development of long-term vascular changes are still not completely understood. However, such information would be of potential benefit for the treatment of patients with portal hypertension.

Long-Term Vascular Structural Changes in Liver

The hepatic sinusoid is a specific capillary network, physically separated from hepatocytes by the space of Disse through which blood from the hepatic artery and from the portal vein circulates. The hepatic sinusoid is made up of four different populations of cells: endothelial cells (LSEC), which represent 20 % of total hepatic cells; Kupffer cells, which are resident hepatic macrophages; HSC; and pit cells, which are resident liver NK cells. Hepatocytes contain microvilli on their sinusoidal surface, and these microvilli expand into the space of Disse to increment the exchange surface. The space of Disse is constituted by proteins and other plasma components that have been sieved by LSECs from the sinusoidal circulation (Braet and Wisse 2002).

Sinusoidal Capillarization

Thanks to the pioneering work done by Wisse on the ultrastructure of LSECs (Wisse et al. 1983, 1985), we currently know that the hepatic sinusoid differs from other capillaries in the body

because this vascular structure lacks a basal membrane. In addition, the LSEC that form the sinusoids contain fenestrations (fenestrae) with diameters of ~20–250 nm. The fenestrae are arranged in special structures called sieve plates, which are approximately 0.1 μm in diameter and comprise 20–50 aggregated pores. These differential characteristics of LSECs enable an efficient exchange of metabolites between blood and hepatocytes. The lack of a basement membrane in the sinusoids allows direct interaction between LSECs and hepatocytes, further increasing the transport efficiency of the system. The mechanism of fenestration formation in LSEC is not entirely clear. The most complete model describing the process of fenestra formation is the "sieve-raft model," proposed by Svistounov et al., that describes an inverse association between the occurrence of membrane rafts and sieve plates in LSEC (Svistounov et al. 2012). Specifically, the fenestration of LSEC occurs when some areas of the plasma membrane, devoid of membrane stabilizers such as rafts or actin, invaginate. Due to the thinness of the cytoplasmic extensions of LSECs, these invaginations turn into fenestrations. This theory is also consistent with previous observations pointing to VEGF as a potent stimulator of fenestration in LSECs (Yokomori et al. 2003). Several studies have highlighted the relevance of the changes occurring in these special structures of LSEC in liver disease. For instance, preclinical studies have demonstrated that LSEC undergo defenestration before the development of fibrosis and in the context of alcohol liver disease (Fig. 1). Accordingly, exposure to alcohol changes the cell membrane fluidity in both in vitro and in vivo models of alcohol exposure (Dey and Cederbaum 2006). Moreover, liver cirrhosis is associated with increased caveolin-1 expression in LSECs. The relevance of this finding lies in the fact that the abundance of caveolin-1 is directly correlated in cells with the presence of lipid rafts (Shah et al. 1999). Therefore, there are reasons to consider that the sieve-raft theory may also explain the defenestration occurring in liver diseases. This structural change, together with the development of a basement membrane in sinusoids, is termed capillarization (Bhunchet and Fujieda 1993; Mori et al. 1993). Furthermore, platelet-derived growth factor (PDGF) signaling through Ephrin-2 and neuropilin-1 stimulates the HSC coverage of sinusoids in vivo (Semela et al. 2008). All these morphological changes of sinusoids increment the intrahepatic resistance to portal blood flow and cause hepatocellular necrosis.

Hepatic Stellate Cell Activation

HSCs are vitamin A-storing cells in physiological conditions that have a regular distribution along the space of Disse (Wake et al. 1988). These cells present some neural markers, such as n-CAM (Knittel et al. 1996), and interact directly with the central nervous system through contact with neuronal terminations (Lafon et al. 1989). Recently, researchers have demonstrated that HSCs may also differentiate from hematopoietic stem cells and be recruited from bone marrow after liver injury (Miyata et al. 2008). Therefore, we may have a population with a heterogeneous origin, especially in pathological conditions.

In liver disease, HSCs contract in vitro in response to vasoconstrictors, such as ET 1, Ang II, or vasopressin and relax in response to vasodilators, such as ·NO (Marra and Pinzani 2002). However, in vivo contraction remains elusive as only indirect evidences are available to date. Thus, whether or not this contraction is physiologically relevant is still controversial. Three-dimensional reconstruction of HSCs in porcine liver, using Golgi's silver staining, has shown that cellular protrusions of the HSC surround the sinusoidal endothelial cells (Wake et al. 1988). These cellular protrusions present actin filaments, suggesting contractibility and supporting the hypothesis that HSCs act like hepatic-specific pericytes involved in sinusoidal contraction during liver injury. According to this hypothesis, the role of HSC in cirrhotic portal hypertension is central given that an excessive contraction of these cells due to impaired production of vascular mediators, which is characteristic of liver injury, leads to an increase in the intrahepatic vascular resistance.

HSCs are also responsible for long-term structural hepatic changes because this cell type, when

activated by TGF-β and/or cytokines, is a major contributor to extracellular matrix (ECM) generation in injured liver. ECM production by HSCs is a physiological repair response. However, when this process is deregulated by different mechanisms, which can include chronic injury, or excessive inflammatory response, the healthy functional tissue is replaced by fibrotic nonfunctional scars constituted mainly by ECM. This pathological regeneration impairs normal functioning of the liver and disrupts the hepatic vascular architecture, resulting in increased vascular resistance (Shibayama and Nakata 1992).

Activation of HSCs by TGF-β leads to the expression of α-SMA, which is a marker of transdifferentiation into myofibroblasts. TGF-β is a superfamily of homologous growth factors that transduces its signal by binding to specific serine/threonine kinase membrane receptors. A type II TGF-β receptor dimer binds the TGF-β ligand and recruits a type I receptor dimer forming a heterotetrameric complex with the ligand (Wrana et al. 1992). TGF-β receptor types I and II have high affinity for TGF-β1 and low affinity for TGF-β2. Additionally, another TGF-β receptor, TGF-β receptor type III, can be distinguished according to its high affinity for both TGF-β1 and -β2. The receptor/ligand complex internalizes via clathrin-coated pits into early endosomes that contain an accessory protein named SARA (Smad anchor for receptor activation). SARA is an FYVE domain containing a scaffolding protein that interacts with the MH2 domain of inactive Smads, targeting them to early endosomes and promoting Smad phosphorylation at C-terminal serines (Moustakas et al. 2002; Javelaud and Mauviel 2004; Massague et al. 2005). Eight different members of the Smad family have been identified in mammals. Based on their function, the Smads are classified as receptor-activated (R-) Smads (Smad1, Smad2, Smad3, Smad5, and Smad8), common-partner (Co-) Smads (Smad4), or inhibitory (I-) Smads (Smad6 and Smad7). Smad2 and Smad3 are specific mediators of TGF-β/activin pathways, whereas Smad1, Smad5, and Smad8 are involved in bone morphogenetic protein (BMP) signaling (Javelaud and Mauviel 2004; Massague et al. 2005). BMP is another member of the TGF-β superfamily. The R-Smads then dissociate from the receptor complex to form oligomers at different stochiometries: heterotrimers with two R-Smads and one Smad4 (Smad3) or heterodimers consisting of an R-Smad (Smad2) and a Co-Smad. These complexes translocate to the nucleus and function as transcriptional regulators of target genes through interactions with other transcription factors, corepressors, and coactivators. This diversity of interactions confers cell type-dependent diverse ligand responses.

The modulation of all the players in this signaling pathway has an effect on HSC-mediated liver fibrosis. One example is the repression of TGF-β receptor type II by the overexpression of SKI-like oncogene, which decreased fibrosis in rat liver through downregulation of MMP-2 in HSCs (Marquez-Aguirre et al. 2009). In a similar way, the adenoviral delivery of an antisense TGF-β oligonucleotide inhibits profibrotic activities in HSCs (Arias et al. 2002). Different researchers have also studied the role of the Smad family in the context of cirrhotic portal hypertension. For instance, Smad7 overexpression prevents the activation of HSCs and reduces liver fibrosis in rats (Dooley et al. 2003). Smad3 activation, which occurs in HSCs during liver fibrosis, is correlated with enhanced transcription of COL1A2 and PAI-1 genes. In Smad3-deficient cirrhotic mice, liver fibrosis decreases by 50 %, although HSCs maintain α-SMA expression. These results suggest that Smad3 is not necessary for HSC transdifferentiation but mediates, at least in part, liver fibrosis (Schnabl et al. 2001). There are other factors that can indirectly influence TGF-β responses in cells. It has recently been described that NOGO-B, a member of the reticulon (Rtn) family of proteins, potentiates TGF-β-induced Smad-2 phosphorylation. This effect has an impact on portal hypertension, given that NOGO-B deficiency decreases portal hypertension in bile duct-ligated mice (Zhang et al. 2011).

All of the above suggests that targeting TGF-β, specifically on HSCs, could be a useful tool to reduce portal hypertension in cirrhosis.

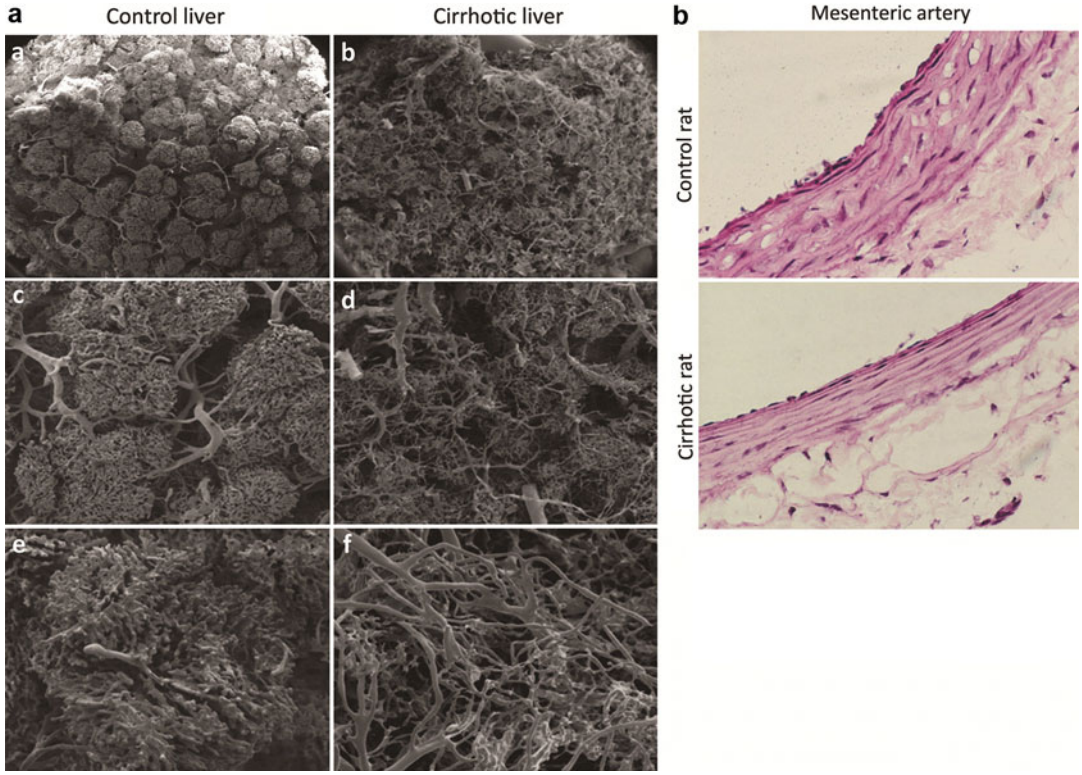

Fig. 3 *Long-term vascular structural changes in liver and extrahepatic vasculature.* In (**A**), representative scanning electron microscopic images of livers of control and cirrhotic rats. Casts from control animals show a quiescent sinusoidal vasculature organized in nodular pattern (*panels a*, *c*, and *e*). In contrast, the sinusoids of cirrhotic rats appeared irregular, disrupted, bulging, and saccular (*panels b*, *d*, and *f*). Original magnifications ×50 (*panels a* and *b*), ×150 (*panels c* and *d*), and ×500 (*panels e* and *f*). In (**B**), photomicrographs of representative cross sections of mesenteric artery from a control rat and a cirrhotic rat with ascites. Note the marked reduction in wall thickness and the diminution in the number of nuclei in the cirrhotic vessel (H&E staining; original magnifications ×200)

Pathological Angiogenesis

Angiogenesis is the primary mechanism of new vessel formation in postnatal stages and is defined as the formation of new capillaries from preexisting blood vessels. This complex mechanism is the result of several coordinated steps involving endothelial cells: the activation of endothelial signaling pathways by angiogenic factors (i.e., vascular endothelial growth factor, angiopoietins, fibroblast growth factor, integrins), release of proteases to digest the basement membrane, cell migration, cell proliferation, tubular morphogenesis, formation of new vascular sprouts, and recruitment of pericytes into the new vascular structures. Angiogenesis is restricted to reproduction and wound healing in healthy adults. In the context of wound healing, angiogenesis may be excessive in several clinical conditions and may lead to a major source of complications, such as abnormal tissue perfusion, an increase of vascular permeability, tissue inflammation, and tumor growth.

In liver disease, angiogenesis is evident during the progression of cirrhotic portal hypertension caused by hepatitis C, biliary cirrhosis, autoimmune hepatitis, or alcohol toxicity. The new hepatic vessels are located within the scar areas and form portosystemic and arterio-portal anastomoses (Mazzanti et al. 1997; El Assal et al. 1998; Rosmorduc et al. 1999; Battista et al. 2001; Corpechot et al. 2002; Medina et al. 2003; Tugues et al. 2007; Fig. 3a). Several preclinical studies in

experimental models have shown that antiangiogenic drugs effectively decrease liver fibrosis and portal hypertension. These studies used different antiangiogenic strategies: blockage of endothelial cell proliferation with TNP-470, specific inhibitors of angiogenic factor receptors such as anti-VEGF receptors antibodies (VEGFRs) or the Tie2 receptor antagonist, and treatment with multitarget inhibitors with a broad selectivity for receptor tyrosine kinases (Wang et al. 2000; Yoshiji et al. 2003; Taura et al. 2008). Sunitinib and sorafenib belong to the last group of antiangiogenics and have in common the characteristic that both drugs inhibit VEGFR and PDGFR receptors. The inhibition of PDGFR has an additional therapeutic interest considering that PDGF is a potent mitogenic, profibrogenic, and chemotactic factor for HSC. When activated, HSCs overexpress the PDGFR receptor-β (PDGFR-β) that plays a key role in the progression of fibrosis (Pinzani et al. 1989; Wong et al. 1994). The abovementioned studies demonstrate that inhibition of angiogenesis is an efficient strategy to block liver fibrosis. However, some discrepancies with respect to this statement exist. One study showed that angiogenesis blockade using αVβ3 inhibitors actually worsened experimental fibrosis (Patsenker et al. 2009). This result warns of the need to carefully select the antiangiogenic therapy to be used to achieve the desired therapeutic effect. Safety is also a major issue on antiangiogenic treatment, especially if we consider its potential translation to patients with clinical portal hypertension and liver fibrosis. Although antiangiogenic agents are clinically used in the treatment of angiogenesis-related diseases, some studies have warned about the occurrence of side effects and the development of resistance to antiangiogenic treatment. The placental growth factor (PlGF) is another angiogenic factor that belongs to the VEGF family and acts as a specific ligand for VEGFR1. Unlike VEGF, PlGF plays a negligible role in physiological angiogenesis. However, studies in transgenic mice have revealed that the angiogenic activity of PlGF is restricted to pathological conditions. In contrast to VEGF inhibitors, anti-PlGF antibodies reduce pathological angiogenesis in most disease models without affecting healthy blood vessels, resulting in no major side effects in mice and humans (Fischer et al. 2007; Lassen et al. 2009; Riisbro et al. 2009; Van de Veire et al. 2010). In experimental models of mice with portal hypertension and liver fibrosis, gene deficiency or pharmacology inhibition of Plgf normalized the splanchnic and hepatic angioarchitecture in mice with liver fibrosis. The absence of Plgf activity also decreased fibrosis, portal pressure, and inflammatory infiltrate. All these beneficial effects were obtained in a context of a good safety profile (Van Steenkiste et al. 2009. 2011). These studies agree with the concept that antiangiogenesis treatment must be selected according to its therapeutic efficiency and biosecurity.

The beneficial effect of antiangiogenic treatments in preclinical studies encourages the development of clinical trials. However, the functional crosstalk between liver angiogenesis and fibrosis should first be clarified. The first unanswered question is why antiangiogenic treatment reduces portal pressure and liver fibrosis. One would expect angiogenesis to be an important component of the hepatic healing response. Furthermore, considering the principles of hemodynamics, an increased number of blood vessels should reduce intrahepatic resistance. However, the studies mentioned above contradict this reasoning. Some investigators have speculated that inflammation is the process linking angiogenesis and fibrogenesis. In fibrotic livers, pathological angiogenesis would result in a proinflammatory neovasculature which develops mainly within the scar area. These new vessels would contribute to the perpetuation and the amplification of the inflammatory state due to the expression of adhesion molecules and cytokines, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). As a result, inflammatory infiltrate would accumulate around the proinflammatory vasculature and would further enhance the development of fibrosis (Tugues et al. 2007; Van Steenkiste et al. 2011). Angiogenesis may also stimulate the extension of fibrosis because new vessels could increase the supply of inflammatory cells, oxygen, and nutrients within the scar. Concerning the potential

improvement in hepatic hemodynamics attributed to angiogenesis, it should be considered that the neovasculature in injured tissue may generate an immature-dysregulated microvascular bed that may change the distribution of blood flow and impair vascular reactivity (Ezaki et al. 2001; Baluk et al. 2005). Therefore, a normalization of liver vasculature would be preferred in order to achieve good liver perfusion instead of disorganized and chaotic vascular growth.

Long-Term Vascular Structural Changes in Extrahepatic Tissue

Long-term structural changes of the angioarchitecture also occur in the splanchnic area during portal hypertension and cirrhosis. This statement is supported by several evidences. For instance, prominent persistent abnormalities in the microangioarchitecture of the gastric mucosa occur in rats with partial portal vein ligation, which match the hypertrophic gastropathy observed in cirrhotic patients (Albillos et al. 1992). Portal hypertension is accompanied by a significant increase in ·NO-dependent angiogenesis in vivo (Sumanovski et al. 1999; Sieber et al. 2001). An extensive neovascularization, visualized by intravital microscopy, occurs in the hepatic arterial system of partial portal veinligated rats (Yokoyama et al. 2001). Increased angiogenesis and permeability have been reported in the peritoneal circulation of rats with portal hypertension and cirrhosis (Geerts et al. 2006). Ascites of cirrhotic patients behaves as a powerful inducer of angiogenesis through the induction of the PI3k/Akt signaling pathway in human endothelial cells (Morales-Ruiz et al. 2005). This latter work raises the possibility that ascites would further stimulate splanchnic angiogenesis in patients with decompensated cirrhosis, and this, in turn, would aggravate splanchnic hyperemia.

The development of portosystemic collateral vessels prone to bleeding is one of the major causes of complications in portal hypertension. Our understanding of the mechanisms underlying the formation of collateral vessels has evolved over the last decades. Initially, some studies proposed that collateral vessels arise from the passive dilatation of preexisting venous channels as a consequence of increased intrahepatic resistance and portal venous inflow. Currently, recent investigations suggest that collateral formation is not only induced by changes in the blood flow but also by structural vascular changes induced by proangiogenic factors. In this context, the treatment of portal hypertensive rats with neutralizing antibody to VEGF receptor 2, SU5416, rapamycin (a VEGF signaling pathway inhibitor), and imatinib (an inhibitor of abl, c-kit, and PDGF receptor activity) significantly reduced portosystemic collateral formation and attenuated the hyperdynamic splanchnic circulation (Fernandez et al. 2004, 2005, 2007). In concordance with these studies, PlGF deficiency in portal hypertensive mice with or without liver fibrosis decreased splanchnic angiogenesis and reduced portosystemic shunting, as well as mesenteric artery flow (Van Steenkiste et al. 2009). All these studies suggest the contribution of active molecular signaling on the formation of collaterals to counteract the increased intrahepatic resistance. However, research in this field is still in its infancy, and more efforts are needed to define the ultrastructural changes that occur during vascular shunting, the respective contribution of the hemodynamic and molecular signaling to the formation of collaterals, and the degree of crosstalk between these two mechanisms. These efforts will pay off to guide the search for effective therapies in the management of variceal bleeding.

Another structural change, namely, vascular remodeling, is responsible for long-term structural changes in the extrahepatic vasculature during portal hypertension and cirrhosis. Vascular remodeling is defined as the long-term structural adaptations that occur in blood vessels to maintain constant flow despite hemodynamic disturbances or vascular abnormalities. These adaptations include changes in vascular diameter, in the media cross-sectional area, or in the media/lumen ratio (Rudic and Sessa 1999). In cirrhotic rats, both resistance and conducting blood vessels exhibited fewer layers of smooth muscle cells compared with control rats. These structural changes in the composition of the media resulted in a significant decrease of media thickness (Fig. 3b). In the same study, these authors

demonstrated that inhibition of NOS in cirrhotic rats blocked the development of this vascular remodeling. The hemodynamic consequence of this treatment was an increase in mean arterial pressure and in peripheral resistance in cirrhotic animals (Fernandez-Varo et al. 2003). This study suggests that the increased endothelium-derived ·NO observed in extrahepatic vessels during cirrhotic portal hypertension causes long-term vascular changes. This phenomenon may further contribute to the unresponsiveness of endogenous vasoconstrictors described in the splanchnic area of portal hypertensive patients. The extrahepatic lymphatic vasculature of cirrhotic rats also shows this vascular remodeling in response to vascular ·NO overproduction (Ribera et al. 2013). Both studies establish a ·NO mechanism responsible for extrahepatic vascular remodeling affecting all types of vasculature during cirrhotic portal hypertension. To date, the therapeutic implications of this pathological model have not been studied in patients. Nonetheless, the potential clinical implications of such studies would be of great interest.

Future Directions

In recent decades, our understanding of the pathogenesis of portal hypertension has advanced significantly. Hepatologists have identified novel pathological mechanisms and have developed new research lines addressed to correct structural changes in tissue architecture and in hemodynamic abnormalities. Despite the knowledge gained, the clinical management of patients with severe portal hypertension is still deficient. We still have to face many unresolved questions concerning both pathogenesis and therapeutics. Can we use pharmacological treatments to normalize hepatic and splanchnic tissular architecture? Do we have the necessary tools to undertake targeted drug delivery – in cells or tissues of interest – in the near future? Can we find biomarkers for the complications related to portal hypertension such as portal vein thrombosis and variceal rupture? Concerning the last question, the identification of the biological mechanisms leading to these complications is a pending issue. It is expected that the accumulation of knowledge, together with further efforts to answer unresolved questions, will eventually lead to the development of safer and more effective therapies for portal hypertension and its complications.

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Cross-References

- Anatomy and Physiology of the Hepatic Circulation
- Assessment and Management of Portal Hypertension
- Essentials of Angiogenesis
- Hemodynamics: An Introduction
- Physiology and Pathophysiology of Microcirculation
- Vascular Endothelial Function

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