The Hepatic Microvascular System in Health and Its Response to Toxicants

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ABSTRACT

This review briefly summarizes what is known about the dynamic morphology of the hepatic microvascular system that includes all vessels in the liver with a diameter less than 300 μm and various morphological sites within these vessels that regulate the distribution of blood flow. The latter include the various segments of the afferent portal venules and hepatic arterioles, the sinusoids, and central and hepatic venules. Sinusoids are unique exchange vessels lined by fenestrated endothelial cells which have important endocytotic functions and phagocytic Kupffer cells which are important for host defense. These are encircled by extraluminal stellate cells that are specialized pericytes containing fat droplets that store vitamin A. The principle sites for regulating blood flow are in the sinusoidal network with stellate and endothelial cells playing a major role in regulating the diameters of sinusoids and the distribution of blood flow in individual sinusoids, lobules, or segments of lobules. The sinusoidal endothelial cells are a sensitive and early target for several toxicants. For example, as early as 30 minutes after the administration of acetaminophen, the endothelial cells become swollen and begin to lose the ability to endocytose ligands. Within 2 hr, gaps through the cytoplasm appear formed by the destruction and/or coalescence of fenestrae which permit red blood cells to penetrate into the space of Disse. Subsequently, the sinusoid may collapse or disintegrate reducing blood flow. Anat Rec, 291:661–671, 2008.

Key words: liver; hepatic microcirculation; sinusoids; endothelial injury; toxicants

HEPATIC MICROVASCULAR SYSTEM

Overview of the Intrahepatic Circulation

The mammalian liver has a dual blood supply. Approximately 80% of the blood entering the liver is poorly oxygenated venous blood supplied by the portal vein, whereas the remainder is well oxygenated and supplied by the hepatic artery. Within the liver, distributing branches of the portal vein and hepatic artery course in parallel; and, after repeated branching, terminal branches of these vessels (portal venules and hepatic arterioles) supply blood to the hepatic sinusoids contained within hepatic lobules (Figs. 1, 2). The sinusoids are the principal vessels involved in transvascular exchange between the blood and the parenchymal cells. Branches of hepatic arterioles also supply the capsule of the liver as well as the bile ducts, where they feed a peribiliary plexus of capillaries, which in turn, drains into the sinusoids. Portal and arterial blood flowing through the sinusoids is collected in central venules, which in turn drain into hepatic veins through which the blood is returned to the inferior vena cava (Figs. 1, 2).

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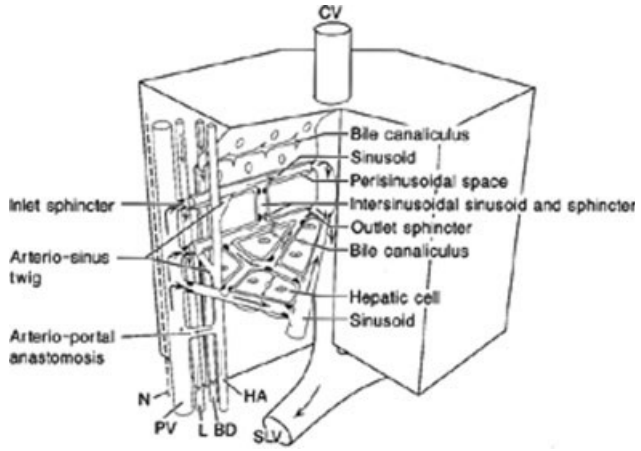

Fig. 1. Hepatic microvasculature as determined by in vivo microscopic studies. PV, portal venule; HA, hepatic arteriole; L, lymphatic; BD, bile ductule; N, nerve; CV, central venule; SLV, sublobular hepatic vein. Arrows indicate direction of flow (McCuskey, 1993).

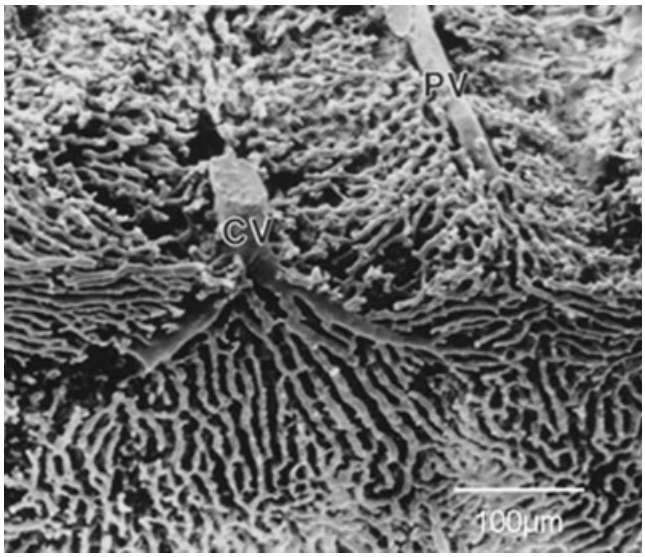

Fig. 2. Vascular cast of the hepatic microvasculature illustrating the tortuous, anastomotic sinusoids adjacent to the portal venule (PV) and the more parallel and larger sinusoids near the central venule (CV) (McCuskey, 1993).

Lymphatic vessels originate as blind-ending capillaries in the connective tissue spaces (portal tracts) associated with the portal veins and hepatic arteries (Fig. 1). The fluid contained in these lymphatics flows toward the hepatic hilus and eventually into the cisternae chyli.

Hepatic Microvascular Functional Units

Several models of the organization of the liver into structural or functional units have been proposed, none of which are mutually exclusive. These include the classic lobule, the acinus, and the portal lobule which are illustrated in Figure 3.

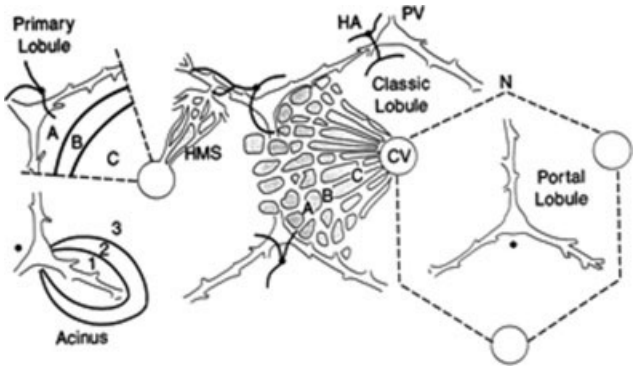

Fig. 3. Contiguous hepatic lobules illustrating the interconnecting network of sinusoids derived from two portal venules (PV). Note that the sinusoids become more parallel as they course toward the central venule (CV), which forms the axis of the classic lobule. Hepatic arterioles (HA) supply blood to sinusoids near the periphery of the lobule, usually by terminating in inlet venules or terminal portal venules. As a result, three zones (1, 2, 3) of differing oxygenation and metabolism have been postulated to compose a hepatic acinus, with its axis being the portal tract (lower left). Several acini would compose the portal lobule (lower right). Each classic lobule contains several cone-shaped subunits having convex surfaces fed by portal and arterial blood at the periphery and its apex at the central venule (upper left). A, B, and C represent hemodynamically equipotential lines in a "primary lobule." A recent modification further subdivides lobules into conical hepatic microcirculatory subunits (HMS), each being supplied by a single inlet venule (McCuskey, 1993).

The classic hepatic lobule is a polygonal structure having as its central axis a central venule, with portal tracts distributed along its peripheral boundary (Kiernan, 1883). The peripheral boundaries of these lobules are poorly defined in most species, including man. In some species, for example, pigs and seals, there is considerably more connective tissue present in the liver and the connective tissue is distributed along the peripheral boundary of classic lobules thus making them very distinct. Considerable sinusoidal anastomoses occur between adjacent lobules, and thus the blood collected by each central venule is supplied by several portal venules. The hepatic acinus (Rappaport et al., 1954; Rappaport, 1973) is a unit having no distinct morphologic boundaries. Its axis is a portal tract and its peripheral boundary is circumscribed by an imaginary line connecting the neighboring terminal hepatic venules (central hepatic venules of the classic lobule), which collect blood from sinusoids. Contained within the acinus are three zones, each having different levels of oxygenation and metabolic function. In yet another model of lobular organization, the lobule is defined by bile drainage. So-called portal lobules (Mall, 1906) have at their center a portal tract, with central veins present around the periphery of each lobule.

Currently, the concept of subunits of the classic lobule forming functional units is the most consistent with existing evidence (Matsumoto and Kawakami, 1982; Ekataksin and Wake, 1997; Ekataksin et al., 1997; Ekataksin and Kaneda, 1999). In this model, each "classic" lobule consists of several "primary lobules". Each primary lobule is cone-shaped, having its convex surface at the periphery of the classic lobule supplied by terminal branches of portal venules and hepatic arterioles, and

its apex at the center of the classic lobule drained by a central (terminal hepatic) venule. These "primary lobules" were renamed as "hepatic microvascular subunits (HMS)" and were demonstrated to consist of a group of sinusoids supplied by a single inlet venule and its associated termination of a branch of the hepatic arteriole from the adjacent portal space. Further confirmation of this HMS concept was obtained studying their development in neonatal livers (McCuskey et al., 2003). Accompanying the HMS are hepatic parenchymal cells and the associated cholangioles and canaliculi. Hepatocellular metabolic gradients also have been demonstrated to conform to this proposed functional-unit concept (Teutsch et al., 1992, 1999).

Hepatic Microvasculature

The hepatic microvascular system comprises all of the intrahepatic vessels having internal diameters less than 300 μm. It thus includes all blood and lymphatic vessels immediately involved in the delivery and removal of fluids to and from the hepatic parenchyma, namely, portal venules, hepatic arterioles, sinusoids, central venules, and lymphatics. The principle sites for regulating blood flow and solute exchange are in the sinusoidal network, which exhibits structural and functional heterogeneity. Perturbations of the hepatic microcirculation in many disease states results in alterations in the perfusion of the sinusoids, hepatic oxygenation, and the exchange processes between the blood contained in the sinusoids and surrounding parenchymal cells (McCuskey, 1993, 1994; Clemens and Zhang, 1999).

The afferent and efferent microvascular connections to the sinusoids within a single hepatic lobule as determined principally by in vivo microscopic studies are illustrated in Figure 1. Most blood enters the sinusoids from portal venules through short inlet venules. Arterial blood enters some of the sinusoids, principally through branches of hepatic arterioles, arterio-sinus twigs which terminate in inlet venules or sinusoids near their origins from inlet or portal venules (Fig. 1; Irwin and MacDonald, 1953; McCuskey, 1966; Bloch, 1970). Occasional direct connections (arterio-portal anastomoses, APA) also have been observed between hepatic arterioles and terminal portal venules (McCuskey, 1966; Bloch, 1970). The frequency of these APAs appears to be species-dependent (Bloch, 1970). Because all of these structures are independently contractile, the sinusoids receive a varying mixture of portal venous and hepatic arterial blood (McCuskey, 1966; Bloch, 1970). Some evidence suggests that between the hilus and periphery of hepatic lobes the fraction of blood delivered to the sinusoids by the hepatic artery differs (Conway et al., 1985). Finally, occasional branches of hepatic arterioles cross the lobule to supply capillaries in the walls of large hepatic veins (Ekataksin, 2000). Blood leaves the sinusoids by flowing into the central venules.

The organization of the sinusoids network exhibits heterogeneity. Near portal venules and hepatic arterioles, sinusoids are arranged in interconnecting polygonal networks; farther away from the portal venules the sinusoids become organized as parallel vessels that terminate in central venules (Figs. 2, 3; Hase and Brim, 1966; Kardon and Kessel, 1980; Wisse et al., 1983). Short intersinusoidal sinusoids connect adjacent parallel sinusoids (Figs. 1–3; McCuskey, 1966). This heterogeneity is established shortly after birth as the neonate is weaned (McCuskey et al., 2003).

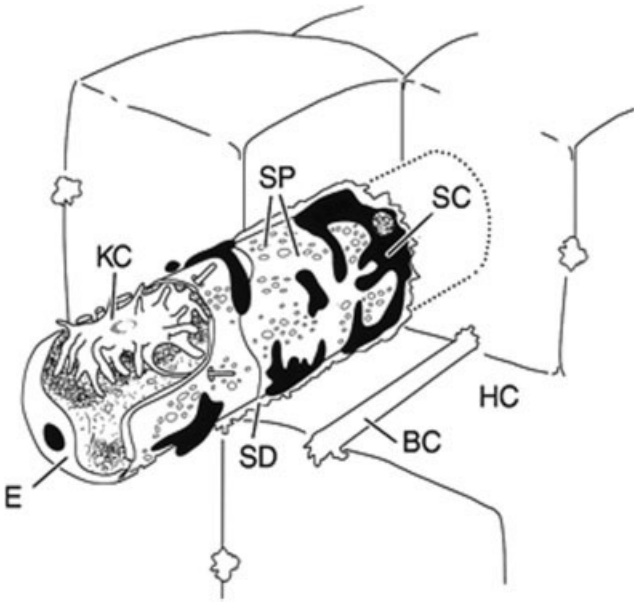

Fig. 4. Sinusoid wall and contiguous hepatic parenchymal cells (HC). E, endothelium; KC, Kupffer cell; SD, space of Disse; SC, stellate cell; SP, sieve plate of fenestrae; BC, bile canaliculus. (McCuskey, 1993).

HEPATIC SINUSOID

The hepatic sinusoid is an unique blood vessel which serves as the principle site of exchange between the blood and the perisinusoidal space of Disse into which project the microvilli of the adjacent hepatic parenchymal cells. The sinusoid is composed of four recognized cells types (Figs. 4–6; reviewed by McCuskey, 1994; Wisse et al., 1996). These are fenestrated endothelial cells and phagocytic Kupffer cells which form the sinusoid lining and are in contact with the blood, extraluminal stellate cells, which are specialized pericytes extending processes throughout the space of Disse, and pit cells which are immunoreactive natural killer (NK) cells that are attached to the luminal surface of the sinusoid and are part of a population of liver associated lymphocytes (LAL; Winnock et al., 1993). Additional cells and cell processes may be present in the space of Disse of some species, most notably, mast cells in the dog (McCuskey, 1989) and adrenergic and peptinergic nerves in most mammalian species except mouse and rat (McCuskey, 1996). The space of Disse is thought by some to function as a lymphatic space that channels plasma to the true lymphatics coursing in the portal tract. Although this hypothesis would help to explain the large efflux of lymph from the liver, it may not be valid because anatomic connections between the Disse's space and the portal tract have not been identified (Niiro and O'Morchoe, 1986; Trutmann and Sasse, 1994). For a review of intrahepatic lymphatics see Trutmann and Sasse (Trutmann and Sasse, 1994).

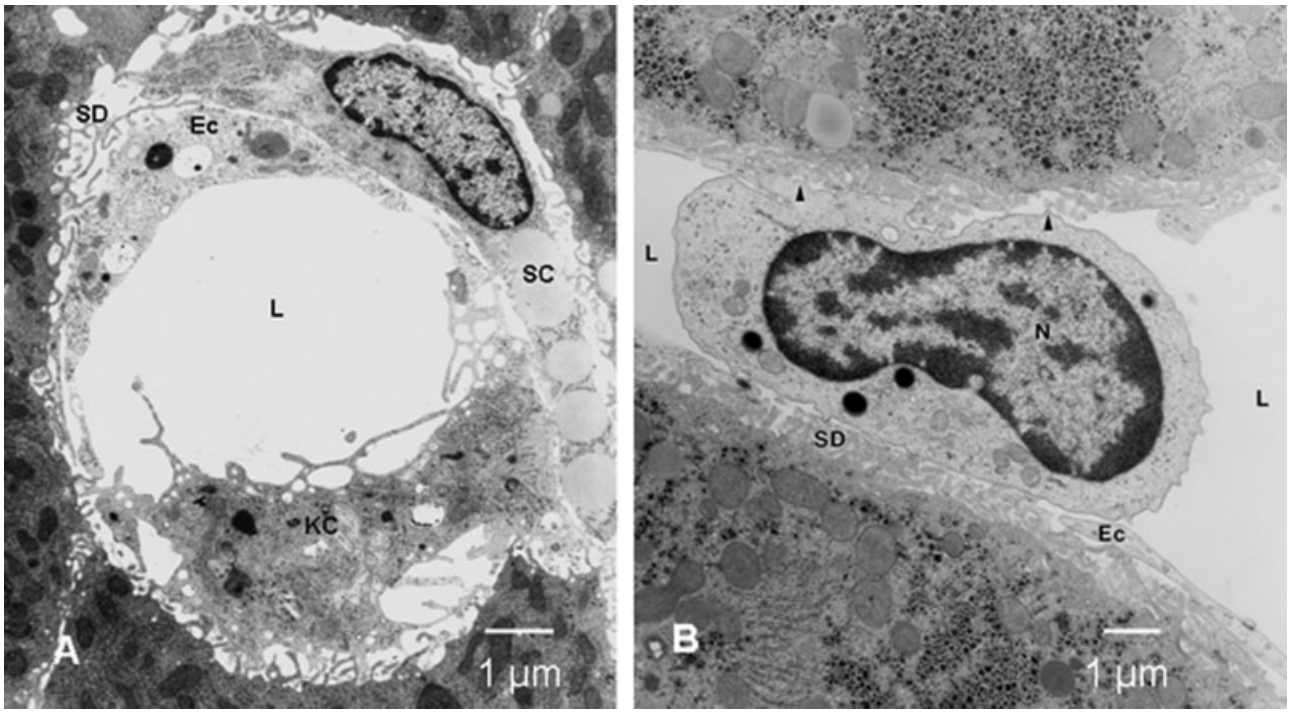

Fig. 5. Transmission electron micrographs of ; (**A**) Sinusoidal endothelium (Ec) with attached Kupffer cell (KC) encasing the sinusoid lumen (L), and perisinusoidal stellate cell (SC) containing fat droplets in space of Disse (SD); and (**B**) Pit cell with typical dense granules. This pit cell is in close contact with the endothelial lining and is seen to contact microvilli of the parenchymal cells (arrowhead). Ec, endothelial cell; f, fenestrae; L, sinusoidal lumen; N, nucleus; SD, space of Disse (Wisse et al., 1996).

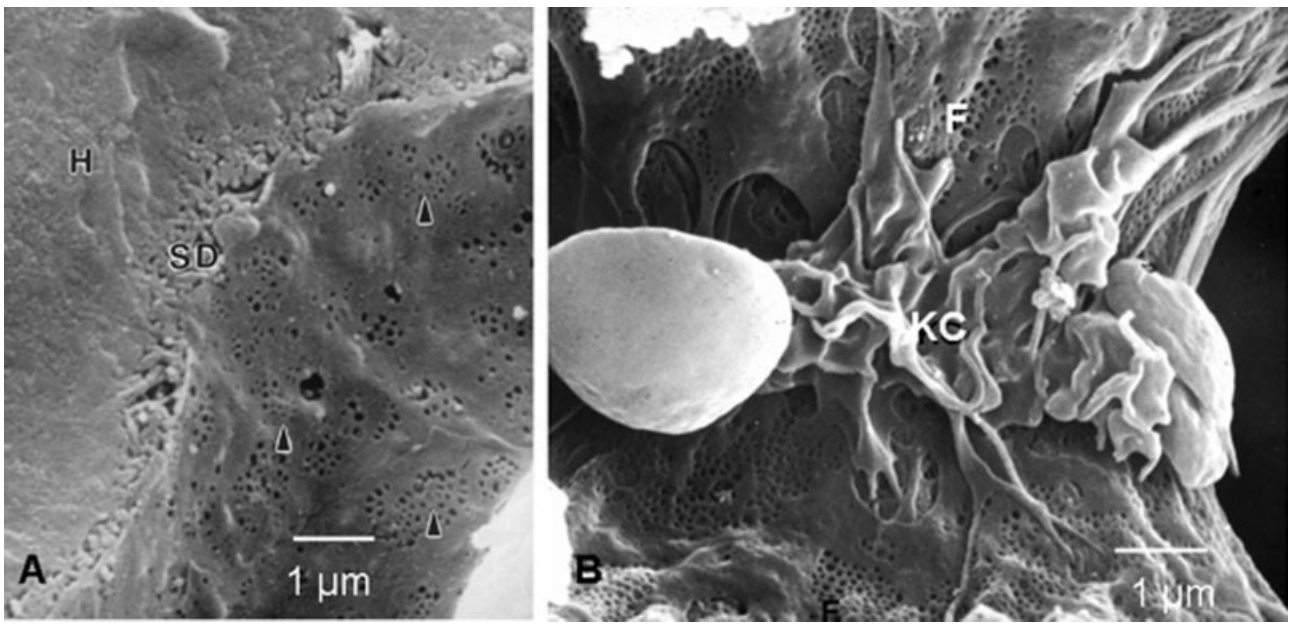

Fig. 6. **A:** Scanning electron micrographs of sinusoid illustrating fenestrae organized in clusters as "sieve" plates (arrows). SD, space of Disse; H, hepatic parenchymal cell (McCuskey, 1993). **B:** Kupffer cell (KC) attached to luminal surface of sinusoidal endothelium by processes that penetrate fenestrae (McCuskey, 1993).

Sinusoidal cells represent approximately 6% of the total liver volume, but account for 30–35% of the total number of liver cells (Blouin, 1977; Blouin et al., 1977). Together, they provide a physical and selective barrier between the blood and the parenchyma that is dynamic and responsive to a wide variety of physical and chemical stimuli. Sinusoidal lining cells have the capacity to divide and proliferate especially when stimulated by immune system modifiers (Bouwens et al., 1986a). Sinusoidal macrophages and NK cells also may be increased in numbers by the respective recruitment and subsequent modification of monocytes and lymphocytes principally of bone marrow origin (Bouwens et al., 1990).

Sinusoidal Endothelial Cells

Sinusoidal endothelial cells in the liver form the basic tubular vessel for transvascular exchange between the blood and the surrounding tissue (Figs. 4–6) and represent approximately 50% of the numbers and volume of sinusoidal cells (Blouin, 1977; Blouin et al., 1977). The morphology of hepatic sinusoidal endothelial cells has been reviewed by several authors (Brouwer et al., 1988; Wisse et al., 1996). These cells are unique to the liver in that their extensive, attenuated cytoplasm contains numerous fenestrae approximately 170 nm in diameter which lack diaphragms and are clustered together in groups known as "sieve plates" (Wisse et al., 1985; Fig. 6). In addition, this specialized endothelium generally lacks a basal lamina during health so that solutes and small particles have direct access to the perisinusoidal space containing processes of fat storing cells and the microvilli of hepatic parenchymal cells.

The endothelium of the sinusoids exhibits heterogeneity. The fenestrae are not of uniform size or distribution throughout the length of the sinusoid from its origin at the portal venule to its termination in the central venule. At the periportal end of the sinusoid, the fenestrae are somewhat larger than those located centrilobularly, but their numbers are less which, when combined with the sinusoid having a smaller diameter at the periportal end compared with the centrilobular end, results in a higher centrilobular endothelial porosity (Vidal-Vanaclocha and Barbera-Guillem, 1985; Wisse et al., 1985; Horn et al., 1986). The functional significance of these regional differences is unclear but it is tempting to relate them to the functional metabolic heterogeneity that has been demonstrated for hepatocyte in different regions of the lobule (Jungermann, 1988; Teutsch, 1988; Gumucio et al., 1994; Teutsch et al., 1999) as well as the portal-to-central intralobular oxygen gradient (Lemasters et al., 1981).

The fenestrae constitute only 6–8% of the surface area of the endothelial lining and acts as a selective barrier between the blood and parenchyma acting as a dynamic, selective sieve for particulates such as chylomicron remnants (Wisse et al., 1985; Fraser et al., 1986). Transport of particulates somewhat larger than the size of the fenestrae is postulated to be accomplished by the "forced sieving" and "endothelial massage" concomitant with the passage of blood cells, particularly leukocytes, through the sinusoids and the resulting interaction of these cells with the endothelial wall (Wisse et al., 1985).

The endothelial fenestrae are dynamic structures whose diameters are affected by luminal blood pressure, vasoactive substances, drugs, and toxins as well as disease and ageing (Wisse et al., 1985; Fraser et al., 1986; Le Bail et al., 1990; Oda et al., 1990; Fraser et al., 1995; LeCouteur et al., 2002). The mechanism for active control of the diameters of these fenestrae appears to reside in actin-containing components of the cytoskeleton (Arias, 1990; Braet et al., 1995; Oda et al., 2000, 2001). Additional cytoskeletal components form rings that delineate both the fenestrae and the sieve plates (Braet et al., 1995, 1998). As a result, the fenestrae are thought to regulate the passage of large substances such as chylomicron remnants through the endothelium while allowing free exchange of plasma and large proteins between the blood and the space of Disse. Thus, the sinusoidal endothelial filter influences the fat balance between the liver and other organs, the cholesterol level in the plasma, and the delivery of retinoids to parenchymal and fat storing cells. There is a reduction of the numbers of fenestrae with age as well as diminished endocytosis of AGE molecules (Smedsrød et al., 1997; LeCouteur et al., 2002; Cogger et al., 2003).

Among the variety of substances that are known to be endocytosed by sinusoidal endothelial cells are proteins, glycoproteins, lipoproteins, and glycosaminoglycans (Smedsrød et al., 1994; Wisse et al., 1996), and under certain conditions, larger particulates, which are phagocytosed in the absence of functional Kupffer cells (Steffan et al., 1986). Several receptors to accomplish this have been identified on the cell surface including Fc receptors for immune complexes, transferrin (Tf) receptors, scavenger receptors, mannose, galactose, and apo E and C-lll receptors. Of these, the scavenger and apo-E receptors are particularly abundant on endothelial cells compared with Kupffer cells as are mannose/N-acetyl glucosamine receptors. The former indicate the important role played by the sinusoidal endothelial cells in processing and metabolism of lipoproteins. Recently, they have been demonstrated to play a significant role in the removal of AGE molecules(Smedsrød et al., 1997).

The endothelial cells also are secretory and release interleukin 1, interleukin 6, and interferon (Smedsrød et al., 1994; Wisse et al., 1996). In addition, these cells produce eicosanoids, particularly PGI_2, PGE_2, TXA_2, as well as endothelin and nitric oxide (Wisse et al., 1996). Thus, along with Kupffer cells, the endothelium participates in host defense mechanisms and regulation of sinusoidal blood flow in the liver. Finally, sinusoidal endothelial cells constitutively express the intercellular adhesion molecule, ICAM-1, which along with VCAM-1 is up-regulated by inflammatory stimuli either directly or by mediators released from stimulated Kupffer cells resulting in increased adhesion of leukocytes to the endothelial surfaces (Van Oosten et al., 1995).

Kupffer Cells

Kupffer cells are a component of the sinusoidal wall and play a significant role in the removal of particulates and cells as well as toxic, infective and foreign substances from the portal blood, particularly those of intestinal origin (Wisse et al., 1996). Kupffer cells also are the source of a variety of beneficial, vasoactive, and toxic mediators which are thought to be involved in host

defense mechanisms as well as some disease processes in the liver (Decker, 1990; Wisse et al., 1996). Included among the substances released are eicosanoids, free radicals, cytokines, interferon, platelet activating factor, and lysosomal enzymes.

The morphology of mammalian Kupffer cells, including those in humans, has been described and extensively reviewed (McCuskey and McCuskey, 1990; Wisse et al., 1996). Kupffer cells are macrophages that are anchored to the luminal surface of the sinusoidal endothelium and, thus, are exposed to the blood stream (Figs. 4, 5A, 6B). Occasionally, Kupffer cells also are interdigitated between endothelial cells. However, Kupffer cells are unevenly distributed within hepatic lobules with the majority being found in the periportal region where they are larger and have greater phagocytic activity than Kupffer cells located in the centrilobular region of the lobule (Sleyster and Knook, 1982; McCuskey et al., 1984; Bouwens et al., 1986a).

Kupffer cells often present a large irregular surface caused by numerous microvilli, filopodia and lamellopodia extending from the cellular surface (Figs. 5A, 6B; McCuskey and McCuskey, 1990). Attachment to the endothelium appears to be by cytoplasmic processes which often penetrate the endothelial fenestrae to enter the space of Disse where they may come in contact with fat storing cells and, occasionally, parenchymal cells. Other processes frequently extend across the lumen to anchor in the opposite wall of the sinusoid. The endocytotic mechanisms of Kupffer cells have been studied both in situ and, in greater detail, in isolated, cultured cells. Four morphologically recognizable endocytotic mechanisms for Kupffer cells fixed in situ by perfusion have been described: (a) bristle-coated micropinocytosis; (b) pinocytosis veriformis; (c) pinocytosis (fuzzy coated vacuole); and (d) phagocytosis (Wisse and Knook, 1979; Wisse et al., 1996). Of these, the principal endocytotic mechanisms, both in vivo and in vitro are thought to be phagocytosis and bristle-coated micropinocytosis. Phagocytosis of particulates larger than 0.3–0.5 μm (e.g., latex, bacteria, etc.) occurs by hyaloplasmic pseudopodia which extend from the cell surface to engulf the particulate and has been used as a marker to distinguish Kupffer cells from other sinusoidal lining cells under normal conditions(Widmann et al., 1972). However, as noted previously the sinusoidal endothelium also is capable of phagocytosing latex particles if Kupffer cells are injured (Steffan et al., 1986). Bristle-coated micropinocytosis is thought to be responsible for both receptor-mediated and nonreceptor mediated fluid-phase endocytosis. Several receptors have been demonstrated on Kupffer cells including Fc and C3 receptors, N-acetyl-D-galactosamine receptors, N-acetyl-glucosamine/mannose receptors.

Kupffer cells during health have long residence times and slow rates of self-replication augmented by some recruitment and transformation of monocytes. Monocyte recruitment becomes more important during stimulation of Kupffer cell function (e.g., zymozan, BCG, etc.; Deimann and Fahimi, 1979; Bouwens et al., 1984, 1986b; Bouwens and Wisse, 1985).

Stellate Cells

Stellate cells (fat storing cells, Ito cells, or lipocytes) are located external to the sinusoidal endothelium in the space of Disse (Figs. 4, 5A) with a higher frequency in the periportal area than centrilobularly (Wake, 1971, 1974, 1980). These cells contain fat droplets and are the major storage site of retinoids including vitamin A which emits a characteristic, rapidly quenched autofluorescence when excited with ultraviolet light at 328 nm.

The nuclear area of the stellate cell is frequently located in recesses between hepatic parenchymal cells while the thin, multiple cytoplasmic processes of these cells course though the perisinusoidal space and extensively embrace the abluminal surfaces of the endothelium surrounding the sinusoid like a cylindrical basket (Wake et al., 1988). This close relationship of the processes of the stellate cell to the sinusoid wall, the presence of large numbers of cytoplasmic microtubules and microfilaments, and the positive immunostaining of desmin in the rat and α-smooth muscle actin, and the close association of nerve fibers coupled with the demonstration of contractile activity in these cells both in vivo and in vitro, strongly suggests that stellate cells play a role in the local regulation of blood flow through the hepatic sinusoids (Pinznai et al., 1992; Kawada et al., 1993; Zhang et al., 1994; Rockey, 2001).

In health, little or no basal lamina and collagen are associated with the sinusoidal endothelium. As a result, the sinusoid wall is a highly permeable structure that permits continuity of plasma between the blood and the hepatocyte. However, during certain types of liver injury, e.g., cirrhosis, basement membrane material and collagen fibrils accumulate in the perisinusoidal space, resulting in "capillarization" of the sinusoid and impaired transvascular exchange (Le Bail et al., 1990). The perisinusoidal stellate cells are thought to be the cells responsible for the synthesis of this material following their transformation into myofibroblast-like cells having reduced numbers of fat droplets and vitamin A as well as increased capacity to secrete extracellular matrix materials (Gressner, 1994).

Liver Associated Lymphocytes

Pit cells (Wisse et al., 1976) are derived from circulating large granular lymphocytes (LGL; Vanderkerken et al., 1993) which become attached to the sinusoidal wall (Fig. 5B) and which possess natural killer (NK) activity and are part of a population of liver associated lymphocytes (LAL; Kaneda et al., 1983; Bouwens and Wisse, 1992; Winnock et al., 1993). Pit cells contain azurophilic granules which stain for acid phosphatase suggesting that they are lysosomal in nature (Kaneda and Wake, 1983; Bouwens et al., 1987) and characteristic rod-cored vesicles. While the majority of attachments to the sinusoidal wall are to endothelial cells, adhesion to Kupffer cells is not uncommon.

Pit cells have been shown to spontaneously kill tumor cells as well as produce a cytolytic factor which is upregulated by biological response modifiers such as zymosan as well as by interleukin 2 (Bouwens and Wisse, 1992). These substances also induce proliferation of pit cells as does partial hepatectomy perhaps through the activation of Kupffer cells.

MORPHOLOGICAL MECHANISMS FOR REGULATING BLOOD FLOW THROUGH HEPATIC SINUSOIDS

Microvascular Sites

There are several potential morphological sites for regulating blood flow through the sinusoids. These include the various segments of the afferent portal venules and hepatic arterioles, the sinusoids themselves, as well as central venules. These vessels contain several types of contractile cells.

Portal venules and central venules contain limited amounts of smooth muscle relative to their luminal size but nevertheless are contractile and respond to pharmacologic agents. Hepatic arterioles are more responsive because of a complete investment of smooth muscle and relative small lumens. The principal site of regulation of blood flow through the sinusoids, however, is thought to reside in the sinusoid itself, where the major blood pressure drop occurs in the liver (Nakata et al., 1961; McCuskey, 1966). In vitro primary culture studies and in vivo microscopic studies of the hepatic microcirculation also have identified the sinusoid as a principal site within the normal and injured livers (Zhang et al., 1994; Suematsu et al., 1995; Clemens and Zhang, 1999).

Sinusoidal endothelial and Kupffer cells are responsive to a wide variety of pharmacodynamic substances. By contracting (or swelling), they may selectively reduce the patency of the sinusoid lumen, thereby altering the rate and distribution of blood flow (McCuskey, 1966; Nakata, 1967). Evidence for sphincters at the inlet of sinusoids from portal venules and at the outlets of sinusoids into central venules was initially reported by several investigators using in vivo microscopy (Irwin and MacDonald, 1953; Bloch, 1955; Knisely et al., 1957), but others failed to find any evidence for such sphincters (Rappaport, 1973). Most methods, including electron microscopy, have failed to demonstrate either smooth muscle fibers or other contractile cells at these locations in healthy animals. Sinusoidal endothelial cells were subsequently identified by high resolution in vivo microscopy to act like sphincters by swelling or contracting in response to vasoactive substances, thereby narrowing the sinusoidal lumen and limiting blood flow (McCuskey, 1966). The relative role of Kupffer versus endothelial cells in this process is not resolved, but both appear to be involved.

The hepatic stellate cell are thought to be responsible for controlling sinusoidal diameter. Hepatic stellate cells possess extensively long, branching cytoplasmic processes, surrounded the sinusoidal wall (Wake, 1980) similar to pericytes in other tissues and organs. Hepatic stellate cells exhibit contractile properties, which have been demonstrated in vitro primary culture system (Pinznai et al., 1992; Kawada et al., 1993; Rockey et al., 1993) and in the perfused liver (Zhang et al., 1994; Suematsu et al., 1995). Both their anatomical location and contractility or relaxation in response to various vasoactive substances have led to the proposal that hepatic stellate cells serve to regulate the diameters and blood flow at the sinusoidal level.

As a result of these structures, blood flow through individual sinusoids is variable. At sites where the lumen is narrowed by the bulging, nuclear regions of sinusoidal lining cells, flow may be impeded by leukocytes that transiently plug the vessel and obstruct flow (Wisse et al., 1985). Transient leukocyte plugging is more frequent in the periportal sinusoids, which are narrower and more torturous than those in the centrilobular region. The more plastic erythrocytes usually flow easily through such sites unless the lumen is reduced or near zero. Some sinusoids, however, may act as thoroughfare channels, and have relative constant rates of blood flow, while others have more intermittent flow (Koo, 1987). This may depend not only on the distribution of intrasinusoidal sphincter cells but also on the distribution of arterio-sinus twigs (AST) and the contribution of arterial blood flowing to individual sinusoids. For example, arterial blood flowing into an individual sinusoid through a dilated AST may increase the rate of sinusoidal blood flow (Rappaport, 1977). Because of the delivery of arterial blood at higher pressure, some arterial blood may even reverse the entry of portal blood into the sinusoids. As a result, the AST in concert with the initial segment of the sinusoid in which it terminates may form a "functional" arterio-potal anastomosis so that arterial blood is delivered into the portal venules (McCuskey, 1966; McCuskey et al., 1983). In the anesthetized healthy animal, however, terminal branches of the hepatic arteriole containing flow are seen infrequently so that most blood delivered to the sinusoids is derived from the portal venules (McCuskey et al., 1983). Consistent with this is the in vivo microscopic observation that the velocity of flow in sinusoids as well as portal and central venules located near the capsule of the liver is not significantly altered by hepatic arterial occlusion in healthy anesthetized rats (Koo et al., 1976). However, arterial inflow to the sinusoids may more significant in regions near the hepatic hilum (Conway et al., 1985).

The frequency distribution of the wide variations in blood flow in the sinusoids exhibits a polymodal pattern composed of several Gaussian distributions (Koo, 1987). These wide variations in flow are due to the structural features previously described for the sinusoids and are also due to intermittent arterial inflow into the sinusoids (McCuskey, 1966; Rappaport, 1977). Blood pressure in portal and central venules has been measured to be approximately 6 to 7 cm H_2O and 1.5 to 3.0 cm H_2O, respectively (Nakata et al., 1961; Nakata, 1967). Arterial blood enters the sinusoid at pressures ranging from 12 to 25 cm H_2O (Rappaport, 1977).

Innervation

Aminergic, peptidergic, and cholinergic nerves are contained in the portal tracts which affect both intrahepatic blood flow as well as hepatic metabolism (McCuskey, 1996, 2004). The role of neural elements in regulating blood flow through the hepatic sinusoids, solute exchange, and parenchymal function is incompletely understood. This is due in part to limited investigation in only a few species whose hepatic innervation may differ significantly from humans as recently reviewed by McCuskey (2004). For example, most experimental studies have used rats and mice having livers with little or no intralobular innervation. In contrast, most other mammals, including humans, have aminergic and peptidergic nerves extending from perivascular plexus in the portal space into the lobule where they course in the Disse's space in close relationship to stellate cells and

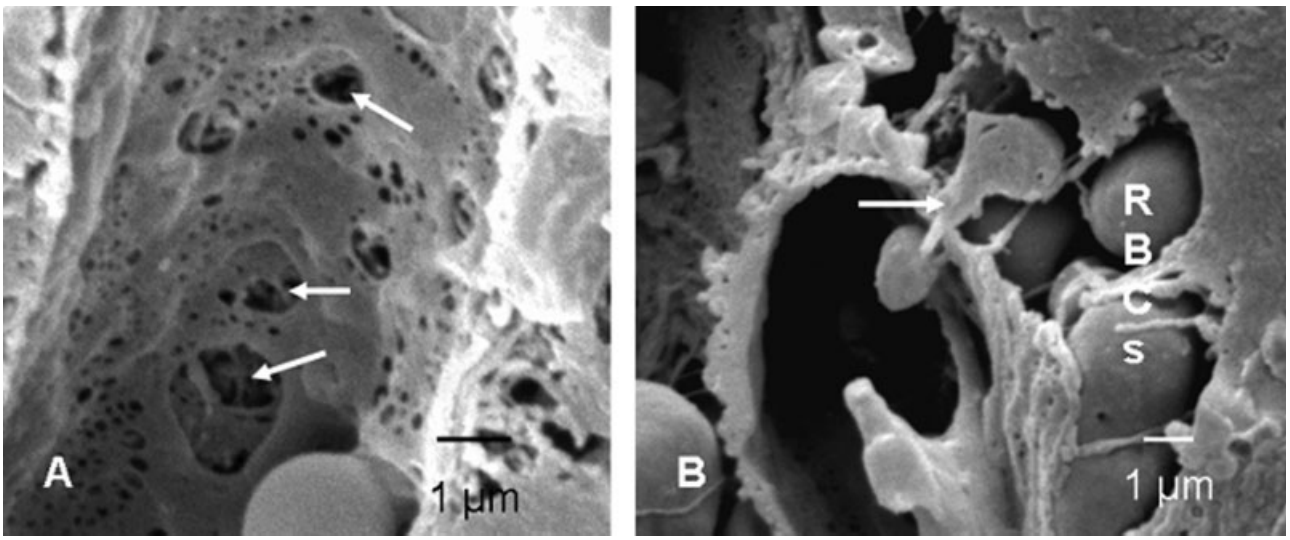

Fig. 7. Scanning electron micrographs of sinusoids from liver of mouse 6 hr after ingestion of acetaminophen. **A,B:** Gaps form in the sinusoidal endothelium (A, arrows) through which erythrocytes (RBCs) penetrate into the space of Disse (B).

hepatic parenchymal cells. While these fibers extend throughout the lobule, they predominate in the periportal region. Cholinergic innervation, however, appears to be restricted to structures in the portal space and immediately adjacent hepatic parenchymal cells. Neuropeptides have been colocalized with neurotransmitters in both adrenergic and cholinergic nerves. Neuropeptide Y (NPY) has been colocalized in aminergic nerves supplying all segments of the hepatic-portal venous and the hepatic arterial and biliary systems. Nerve fibers immunoreactive for substance P (SP) and somatostatin (SOM) follow a similar distribution. Intralobular distribution of all of these nerve fibers is species dependent and similar to that reported for aminergic fibers. Vasoactive intestinal peptide (VIP) and calcitonin gene-related peptide (CGRP) are reported to coexist in cholinergic and sensory afferent nerves innervating portal veins and hepatic arteries and their branches, but not the other vascular segments or the bile ducts. Nitrergic nerves immunoreactive for neuronal nitric oxide (nNOS) are located in the portal tract where nNOS colocalizes with both NPY and CGRP containing fibers.

RESPONSES OF THE HEPATIC SINUSOID TO TOXICANTS

Hepatic sinusoidal endothelial cells (SEC) are a target for toxicity to several toxicants including acetaminophen alone and in combination with alcohol, pyrrolizidine alkaloids, lipopolysaccharide alone and in combination with galactosamine (DeLeve et al., 1997, 1999, 2003; Ito et al., 2003b, 2004b, 2005b; McCuskey et al., 2005; McCuskey, 2006). The basic response of the SEC to these toxicants is quite similar. For example, SEC are a sensitive , direct target for early toxicity to acetaminophen (paracetamol, APAP) and this toxicity is exacerbated following a single and multiple week-end type alcoholic binge(s) (Ito et al., 2003b, 2004a; McCuskey et al., 2005; McCuskey, 2006). SEC become swollen and begin to loss the ability to endocytose ligands for the scavenger receptor, as early as 30 minutes after the administration of APAP. Gaps through the SEC (Fig. 7) appear to be formed by the destruction and/or coalescence of fenestrae and are seen as early as 2 hr after the administration of APAP which is before any evidence of injury to parenchymal cells. The gaps permit red blood cells to penetrate into the space of Disse (Fig. 7B). Subsequently, the sinusoid may collapse or disintegrate reducing blood flow. The gaps are larger and more frequent in ethanol binged animals subsequently treated with APAP. Similar gaps are seen in the early stages of hepatic veno-occlusive disease induced by pyrrolizidine alkaloids and following treatment with galactosamine-endotoxin. Administration of a NO donor or a MMP-2 and MMP-9 inhibitor minimizes endothelial injury and red blood cell penetration into the space of Disse (Ito et al., 2003a, 2005a). The injury is exacerbated when an inhibitor of eNOS is administered and minimized when iNOS is inhibited suggesting a protective role for constitutive NO derived from SEC. Both NO and MMPs are known to affect the cytoskeleton of SEC, which in turn affects the formation and maintenance of the fenestrae suggesting that these toxicants affect the cytoskeleton. Free radicals such as superoxide released as a result of oxidative stress have been proposed to be a common factor in SEC injury (Deaciuc et al., 1999).

CONCLUSIONS

The liver contains a unique and complex microvascular system whose structure is well suited to the many functions that it must subserve. While considerable knowledge has been gained about these structural–functional relationships, much has yet to be learned about the significance of the structural and functional heterogeneity of this unique system and the mechanism involved during both health and disease.

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