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Review article

Morphological mechanisms for regulating blood flow through hepatic sinusoids

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Abstract: This review summarizes what is known about the various morphological sites that regulate the distribution of blood flow to and from the sinusoids in the hepatic microvascular system. These sites potentially include the various segments of the afferent portal venules and hepatic arterioles, the sinusoids themselves, and central and hepatic venules. Given the paucity of smooth muscle in the walls of these vessels, various sinusoidal lining cells have been suggested to play a role in regulating the diameters of sinusoids and influencing the distribution and velocity of blood flow in these vessels. While sinusoidal endothelial cells have been demonstrated to be contractile and to exhibit sphincter function, attention has recently focused on the perisinusoidal stellate cell as the cell responsible for controlling the sinusoidal diameter. A very recent study, however, suggested that the principal site of vasoconstriction elicited by ET-1 was the pre-terminal portal venule. This raised the question of whether or not the diameters of sinusoids might decrease due to passive recoil when inflow is reduced or eliminated and intra-sinusoidal pressure falls. In more recent *in vivo* microscopic studies, clamping of the portal vein dramatically reduced sinusoidal blood flow as well as the diameters of sinusoids. The sinusoidal lumens rapidly returned to their initial diameters upon restoration of portal blood flow suggesting that sinusoidal blood pressure normally distends the sinusoidal wall which can recoil when the pressure drops. Stellate cells may be responsible for this reaction given the nature of their attachment to parenchymal cells by obliquely oriented microprojections from the lateral edges of their subendothelial processes. This suggests that care must be exercised when interpreting the mechanism for the reduction of sinusoidal diameters following drug administration without knowledge of changes occurring to the portal venous and hepatic inflow.

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Key words: sinusoid – sphincter – endothelial cell-stellate cell – blood flow – microcirculation–portal venule – hepatic arteriole – central venule

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The hepatic microvascular system comprises 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. Fig. 1 illustrates the afferent and efferent microvascular connections to the sinusoids within a single hepatic lobule.

Most blood enters the sinusoids from portal venules. These inlets are reported to be guarded by sphincters composed of sinusoidal lining cells termed the afferent or inlet sphincters (Fig. 1) (1–4). Arterial blood enters some of the sinusoids, principally through branches of the hepatic arterioles. These vessels, arterio-sinus twigs, terminate in sinusoids near their origins from portal venules (Fig. 1) (3, 4). In addition, occasional direct connections (arterio-portal anastomoses, APA) have been observed within the terminal portal venules (Fig. 1) (3, 4). The frequency of these APAs appears to be species-dependent (4). Since all of these structures are independently contractile, the sinusoids receive a varying mixture of portal venous and hepatic arterial blood (3, 4). Finally, 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 (5).

The organization of the sinusoid network ex-

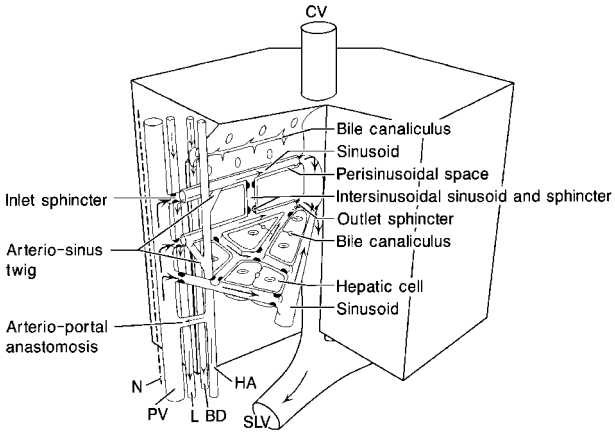

Fig. 1. Hepatic microvasculature as determined by *in vivo* microscopic studies (3, 4, 36–39, 44). PV, portal venule; HA, hepatic arteriole; L, lymphatic; BD, bile ductule; N, nerve; CV, central venule; SLV, sublobular hepatic venule. *Arrows* indicate direction of flow. (From McCuskey (1, 45)).

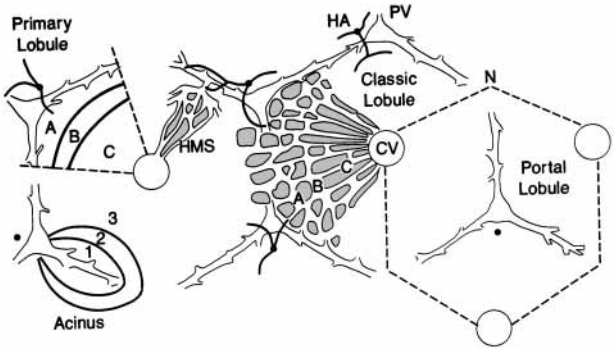

Fig. 2. Contiguous hepatic lobules illustrating the interconnecting network of sinusoids derived from two portal venules (PV). Note that the sinusoids become more parallel as they course toward the central venule (CV), which forms the axis of the classic lobule (of Kiernan (46)) (center). Hepatic arterioles (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 by Rappaport (40, 47) to compose a hepatic acinus, with its axis being the portal tract (lower left). Several acini would compose the portal lobule (lower right) described by Mall (48). Matsumoto and Kawakami (13) proposed that 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." Recently, a modification by Ekataksin et al. (9–12, 49–53) and McCuskey et al. (54) further subdivides lobules into conical hepatic microcirculatory subunits (HMS), each being supplied by a single inlet venule. (From McCuskey (1, 45)).

hibits 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 (terminal hepatic venules) (Fig. 2) (6–8). Short intersinusoidal sinusoids connect adjacent parallel sinusoids (Figs. 1 & 2) (3).

Blood leaving the sinusoids and flowing into central (terminal hepatic) venules passes through outlet or efferent sphincters composed of sinusoidal lining cells (Fig. 1) (2, 3). Sinusoidal lining cells are also reported to serve as sphincters within the sinusoid network and to regulate the distribution of blood flow in short segments of sinusoids (3).

Studies using three-dimensional reconstruction of sectioned livers, scanning electron microscopic examination of corrosion casts, and *in vivo* microscopy of several species (9–12) support Matsumoto's concept of the functional unit being a conical microvascular subunit of the classic lobule (13) (Fig. 2). These "primary lobules" were renamed "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 (AST) from the adjacent portal space (Fig. 2) (9–11).

Morphological sites for regulating the hepatic microcirculation

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 and hepatic venules (Table 1). These vessels contain several types of contractile cells (Table 2).

Portal venules and central venules contain in their walls limited amounts of smooth muscle relative to their luminal size but are nevertheless con-

Table 1. Potential microvascular sites for regulating sinusoidal blood flow

- Portal venule – pre-terminal, terminal, inlet
- Hepatic arteriole – pre-capillary arteriole, arteriole, AST
- Sinusoid
- Central venule
- Hepatic venule

Table 2. Potential contractile cells

Cell	Vessel
• Smooth muscle cells	Portal venule, hepatic arteriole, Central and hepatic venules
• Fat storing (stellate, Ito) cell	Sinusoid
• Sinusoidal endothelial cell	Sinusoid
• Kupffer cell	Sinusoid

tractile and respond to pharmacologic agents. Hepatic arterioles are more responsive because of a complete investment of smooth muscle and relatively 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 (3, 14, 15).

The sinusoidal lining 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 (2, 3, 14, 15). The relative roles of Kupffer versus endothelial cells in this process is not yet resolved, but both appear to be involved. The participation of perisinusoidal, stellate cells (fat-storing, Ito cells) in regulating sinusoidal diameter has also been reported (16–26), but controversy about their physiological role remains and will be addressed later in this review. All three cell types contain filaments, tubules, and contractile proteins suggestive of contractile activity (27).

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 (28, 29). Transient leukocyte plugging is more frequent in the periportal sinusoids, which are narrower and more tortuous than those in the centrilobular region. The more plastic erythrocytes usually flow easily through such sites unless the lumen is reduced to near zero. Some sinusoids, however, may act as thoroughfare channels and have relative constant rates of blood flow, while others have more intermittent flow (30–32). 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 (33, 34). 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-portal anastomosis so that arterial blood is delivered into the portal venules (3, 4, 35). 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 (3, 4). 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 artery occlusion in healthy anesthetized rats (32). However, arterial inflow to the sinusoids may be more significant in regions near the hepatic hilum (5).

The frequency distribution of the wide variations in blood flow in the sinusoids exhibits a polymodal pattern composed of several Gaussian distributions (30). These wide variations in flow are due to the structural features previously described for sinusoids and are also due to intermittent arterial inflow into the sinusoids (3, 4, 34). Blood pressure in portal and central venules has been measured to be about 6 to 7 cm H_2O and 1.5 to 3.0 cm H_2O, respectively (14, 15). Arterial blood enters the sinusoid at pressures ranging from 12 to 25 cm H_2O (34).

Controversies

As previously mentioned, various sinusoidal lining cells have been suggested to play a role in regulating the diameters of sinusoids and influencing the distribution and velocity of blood flow in these vessels. Evidence for sphincters at the inlets of sinusoids from portal venules and at the outlets of sinusoids into central venules was initially reported by a number of investigators using *in vivo* microscopy (36–39), but others failed to find any evidence for such sphincters (40). 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 (3). More recently, attention has focused on the perisinusoidal stellate cell as the cell responsible for controlling sinusoidal diameter. Several agonists including endothelin-1 (ET-1) were shown to cause contraction of isolated stellate cells in culture and to narrow the lumens of sinusoids in isolated, perfused livers, as well as in livers with intact afferent and efferent vessels (20–26). A recent morphological study, however, suggested that the principal site of vasoconstriction elicited by ET-1 was the pre-terminal portal venule (41). This raised the question of whether or not the diameters of sinusoids might decrease due to passive recoil when inflow is reduced or eliminated and intrasinusoidal pressure falls. Recently, an *in vivo* microscopic study reported that clamping of the portal vein dramatically reduced sinusoidal blood flow in most sinusoids to near zero. Within a few seconds,

this resulted in a 21% reduction in diameters of sinusoids together with their outlets and an 11% reduction in the diameters of central venules (42). The lumens of these vessels rapidly returned to their initial diameters upon restoration of portal blood flow. Thus, it appears that sinusoidal blood pressure normally distends the sinusoidal wall which can recoil when the pressure drops. In the absence of elastic fibers in the Space of Disse (42), it is hypothesized that stellate cells may be responsible for this reaction given the nature of their attachment to parenchymal cells by obliquely oriented microprojections from the lateral edges of their subendothelial processes (43). How this response is modified during liver injury when the stellate cells are frequently activated and have increased contractility is not yet known.

In conclusion, care must be exercised when interpreting the morphological mechanisms for the reduction of sinusoidal diameters following drug administration without prior knowledge of changes occurring to the portal venous and hepatic arteriolar inflow.

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