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The Hepatic Sinusoid in Aging and Cirrhosis

Effects on Hepatic Substrate Disposition and Drug Clearance

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Abstract

The fenestrated sinusoidal endothelium ('liver sieve') and space of Disse in the healthy liver do not impede the transfer of most substrates, including drugs and oxygen, from the sinusoidal lumen to the hepatocyte. Plasma components transfer freely in both directions through the endothelial fenestrations and into the space of Disse. The endothelium is attenuated, there is no basement membrane and there is minimum collagen in the space of Disse, thus minimising any barriers to substrate diffusion.

Both cirrhosis and aging are associated with marked structural changes in the sinusoidal endothelium and space of Disse that are likely to influence bulk plasma transfer into the space of Disse, and diffusion through the endothelium and space of Disse. These changes, termed capillarisation and pseudocapillarisation in cirrhosis and aging, respectively, impede the transfer of various substrates. Capillarisation is associated with exclusion of albumin, protein-bound drugs and macromolecules from the space of Disse, and the progressive transformation of flow-limited to barrier-limited distribution of some substrates.

There is evidence that the sinusoidal changes in cirrhosis and aging contribute to hepatocyte hypoxia, thus providing a mechanism for the apparent differential reduction of oxygen-dependent phase I metabolic pathways in these conditions. Structural change and subsequent dysfunction of the liver sieve warrant consideration as a significant factor in the impairment of overall substrate handling and hepatic drug metabolism in cirrhosis and aging.

Hepatic clearance is determined both by the metabolic capacity of the liver and by delivery of substrates to the metabolic sites within the hepatocytes.[1,2] Metabolic capacity is often quantified as the intrinsic clearance (CL_{int}), which is usually considered to be a function of enzyme mass and activity. There are several factors that influence substrate delivery in the healthy liver, including hepatic blood flow, binding between substrates and plasma proteins, transfer of substrates to the hepatocytes and transport across the hepatocyte membrane. These factors may also impact on the delivery of co-substrates such as oxygen, which is required for phase I metabolic pathways.[3]

The relative contribution of each of these factors to the hepatic clearance of any particular substrate will depend on the relative values of individual processes and reactions. For example, when enzyme activity is very low, it becomes the limiting factor for clearance, whereas when metabolic capacity is very high, delivery of substrate becomes limiting. The relative contribution of these processes will differ between substrates and will potentially be influenced by liver disease and aging. In the healthy liver, the influences of each of these various factors on hepatic drug clearance have been drawn together by two well established models.

The well stirred (venous equilibrium) model assumes that the liver is a well stirred compartment and, therefore, that the concentration of the substrate in the hepatic sinusoid is equal to the concentration in the hepatic vein.[4] The hepatic clearance is related to CL_{int}, hepatic blood flow (Q) and protein binding (the unbound fraction, f_u), as shown by equation 1:

$$clearance = \frac{Qf_uCL_{int}}{Q + f_uCL_{int}} \quad \text{(Eq. 1)}$$

The parallel tube (sinusoidal perfusion) model assumes that substrate concentration declines exponentially along the hepatic sinusoid and that the liver is a series of parallel tubes surrounded by hepatocytes.[4] This model, like the well stirred model, relates drug clearance to CL_{int}, Q and f_u, according to equation 2:

$$clearance = Q\left(1 - e^{-\frac{f_uCL_{int}}{Q}}\right) \quad \text{(Eq. 2)}$$

These models assume that there is no significant barrier to the transfer of substrates into the hepatocyte, either at the level of the endothelium or the hepatocyte membrane. Any effects of drug transfer will be included in the intrinsic clearance term and are, therefore, not apparent. More complex models have been developed that take into account barriers to substrate uptake, mainly at the hepatocellular membrane. These have been used widely to describe substrate behaviour in multiple indicator dilution experiments. The models developed by Goresky and colleagues[5,6] compare outflow curves of test substrates and vascular markers in order to generate values for the volumes of distribution, transport, sequestration and protein binding of the test substances. The dispersion model assumes that substrate concentration in the sinusoid is dependent on both convective and dispersive forces[7] and has been used to measure drug transfer across the hepatocellular membrane.[8]

Mechanisms for altered hepatic substrate handling with liver disease and aging are traditionally considered to fall into two groups: the sick cell

theory and the intact hepatocyte theory.[2,3,9,10] The sick cell theory states that there is impaired metabolic capacity (reduced CL_{int}) and includes mechanisms that emphasise reduced gene expression and liver mass.[3,10] The intact hepatocyte theory states that the metabolic capacity of the liver is normal (unchanged CL_{int}), but there is impairment in drug and/or substrate delivery through reduction in blood flow, development of shunts, altered protein binding or altered drug transport.[2,3,9,10]

In our previous reviews of drug metabolism in liver disease[3,10] and aging[11] we discussed how these physiological considerations provide a foundation for understanding the mechanisms that underlie the changes in hepatic drug metabolism that occur with old age and in cirrhosis, specifically:

- reduction in liver mass
- changes in enzyme expression and activity
- reduction in blood flow
- development of shunts in cirrhosis
- changes in protein binding
- altered delivery of the phase I pathway co-substrate, oxygen
- altered transport of drugs across the hepatocyte cell membrane.

In this review, we extend these concepts to focus on the effects of the endothelium and space of Disse on substrate transfer. From the pathological perspective, both cirrhosis and aging have in common the development of a thickened and defenestrated sinusoidal endothelium associated with varying amounts of perisinusoidal fibrosis. The change is termed capillarisation in cirrhosis[12-14] and pseudocapillarisation in aging.[15,16] The liver sinusoidal endothelial cell has not been thought to influence drug metabolism in the healthy liver because it is thin, lacks a basal lamina and is fenestrated, and is, therefore, unlikely to impede the transfer of most substrates from the blood in the sinusoid into the extracellular space of Disse. In fact, the sinusoidal endothelium has been called 'the liver sieve' because of these properties.[17] However, the structure of the liver sieve is profoundly altered in aging and cirrhosis and this is likely to influence clearance through effects on drug and substrate delivery.

We review the effects of cirrhosis and aging on the hepatic sinusoidal endothelium and the subsequent effects on substrate handling with special reference to drug clearance. We attempt to estimate the relative contribution of the dysfunctional liver sieve on altered drug metabolism compared with other accepted mechanisms for impaired clearance. This review examines the proposal[3,10,13,18-21] that change in the liver sieve and space of Disse (i.e. capillarisation and pseudocapillarisation) is an important mechanism for impaired hepatic function and drug clearance in cirrhosis and aging.

1. The Liver Sieve and Substrate Transfer

The structures lying between the sinusoidal blood and the hepatocyte cell membrane have not been considered to pose any appreciable barrier to the uptake of most substrates, including drugs and xenobiotics. Indeed it is assumed that the purpose of these structures is to enhance free mixing of the plasma and contents within close proximity to the hepatocyte membrane and its transporter systems (figure 1).[13,17,22]

The hepatic sinusoidal region contains four different types of cells: endothelial cells, Kupffer cells, stellate cells and pit cells. The Kupffer cells are large macrophages that lie within the sinusoidal lumen and are implicated in the clearance of particulate substrates and generation of inflammatory responses. The liver stellate cells are peri-sinusoidal within the extracellular space of Disse and are involved in the synthesis of extracellular matrix and vitamin A storage. Pit cells, within the sinusoidal lumen, are thought to be natural killer lymphocytes.[13,22]

Unlike endothelial cells in other tissues that generate substantial permeability barriers for many substrates,[23] the liver sinusoidal endothelial cells in the healthy state do not impart any permeability barrier to substrate transfer. Liver endothelial cells are extremely attenuated and perforated with pores called fenestrations. Fenestrations are approximately 100nm in diameter and grouped together in clusters called liver sieve plates. Fenestrations occupy between 5% and 10% of the endothelial surface and

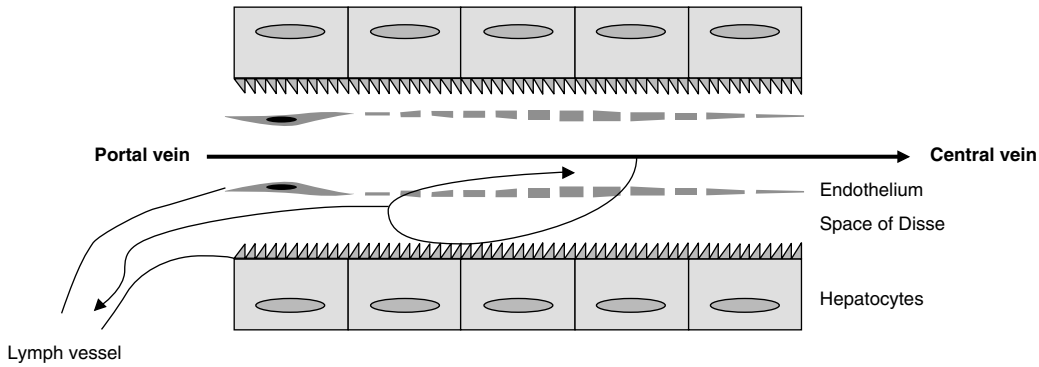

Fig. 1. Blood flow through the liver sinusoid with the production of perisinusoidal plasma, which is in continuum with periportal lymphatics.

there may be a zonal gradation in fenestrations. Specifically, the diameter of the fenestrations decreases from 110.7 ± 0.2nm to 104.8 ± 0.2nm from zone 1 to zone 3 (periportal to perivenous) and the porosity increases from 6% to 8%.[13,17,22]

On the basis of this unique structure, we postulated that the fenestrated endothelium acts like a dynamic filter and coined the term 'the liver sieve' to describe this functionality.[24] Fenestrations permit the passage of plasma and plasma proteins, including albumin and lipoproteins, into the space of Disse and exclude blood cells, platelets and large chylomicrons. It has been suggested that white cells massage fluid and substrates through fenestrations by virtue of the fact that they are relatively rigid and their diameter (8–10μm) is greater than of a typical sinusoid (4–7μm).[17,25] There are many factors that influence the number and size of fenestrations, including hormones, cytokines, sinusoidal pressure, neurotransmitters, alcohol, nicotine and endotoxins,[13,22] indicating that there is bioregulation of fenestrae and dynamic modification by xenobiotics.

Ultrastructural examination of the liver in sheep has shown that the space of Disse is continuous with lymphatic vessels found in the portal triads. There are no lymphatic vessels around the central vein.[26] This morphology suggests that plasma flows through the fenestrations into the space of Disse, then upstream along the space of Disse, finally emptying into the lymphatic vessels around the portal vein (figure 1).[26] The hepatic lymph has unusually high levels of plasma proteins (about 80% of plasma concentrations), an observation that supports this concept.[26] The only vessels in the body with a truly discontinuous fenestrated endothelium lacking an underlying basal lamina are the lymphatic vessels and the liver sinusoidal endothelium,[23] suggesting a morphological as well as functional link between lymphatic vessels and liver sinusoidal endothelial cells. A hydrodynamic analysis of flow in the hepatic sinusoids, specifically application of Bernouilli's law to the fenestrations, is also consistent with the concept of retrograde plasma flow along the space of Disse from the zone 3 fenestrations and back into the sinusoidal lumen through the zone 1 fenestrations.[25]

Consistent with the conclusion that there is direct flow of plasma through fenestrations is the pharmacological observation that healthy endothelial cells do not appear to create any permeability barrier for the uptake of small soluble substrates. Extensive investigations of substrate transfer by Goresky and colleagues[6,27-29] using a distributed physiological model, and Roberts and colleagues[1,7,8] using the dispersion model have clearly demonstrated that the barrier to drug transfer lies at the hepatocyte membrane. This also indicates that the extracellular matrix does not impart a significant barrier for drug diffusion.

Even for drugs bound to albumin, endothelial cells are not considered to impede passage into the space of Disse, presumably because the diameter of albumin (3–4nm) is considerably less than that of a typical fenestration (100nm). Albumin has a volume

of distribution somewhat less than the extracellular space; however, this is secondary to the effects of the extracellular matrix rather than partial exclusion by endothelial cells.[6,30]

However, a barrier has been demonstrated at the level of the endothelial cell for the transfer of larger endobiotics. Chylomicrons, which have a diameter of 100–1000nm, are excluded from the space of Disse because they are unable to traverse fenestrations.[13,31] Chylomicron remnants, which are the next smallest lipoprotein, have a diameter of 50–100nm and pass via the fenestrations unimpeded and enter the space of Disse. Here, chylomicron remnants bind various receptors. including the low-density lipoprotein (LDL) receptor, LDL receptor related protein (LRP), apolipoprotein E and heparan sulfate proteoglycan (HSPG), before uptake into and metabolism by the hepatocytes.[13,31]

The hepatic disposition of liposomes has been studied.[32] Liposomes have become established as carriers of drugs by the widespread clinical use of liposomal amphotericin and liposomal daunorubicin. Because of their large diameter, liposomes are largely restricted to the vascular compartment. Romero and colleagues[32] investigated the passage of liposomes across liver endothelial fenestrations. They injected rats intravenously with large fluid liposomes (337nm, enriched with phosphatidylserine), large solid liposomes (298nm, enriched with phosphatidylglycerol), small fluid liposomes (78nm) or small solid liposomes (157nm). Uptake of liposomal radiolabel by hepatocytes was restricted to small liposomes and large fluid liposomes. Large solid liposomes were not taken up by hepatocytes *in vivo*, but were taken up by isolated hepatocytes *in vitro*. Furthermore, phosphatidylserine was not found to alter fenestrations. It was concluded that the liver endothelial cells restrict the passage of liposomes on the basis of size and deformability.[32] This is a clear example where steric hindrance[33] by the healthy liver sieve has a direct influence on the pharmacokinetics of a therapeutic agent.

A similar study has been reported examining the behaviour of colloidal gold granules coated with bovine serum albumin (diameters of 17nm or 79nm). It was found that the uptake by hepatocytes *in vivo* was limited to the smaller particles, consistent with steric hindrance.[34]

2. Capillarisation and Pseudocapillarisation of the Liver Sieve

Cirrhosis is characterised by disruption of normal lobular architecture of the liver by fibrous septa separating areas of nodular regeneration. The fibrous septa bridge the portal triads and central veins and may contain branches from the portal vein, hepatic artery and hepatic vein. Blood vessels within the fibrous septa form 'shunts' that allow between 0% and 65% of blood to bypass the liver.[35] Within the remaining sinusoids, capillarisation of the endothelial cells in cirrhosis was first described by Schaffner and Popper.[12] They reported the development of a continuous endothelial lining separating the sinusoidal lumen from hepatocytes with the deposition of collagen in the space of Disse. Electron microscopy has since confirmed these findings and, in addition, has shown defenestration and reduction in the porosity of the sinusoidal endothelium in humans and rats with cirrhosis (figures 2 and 3).[13,14,36-39]

We have demonstrated that aging in the liver is also associated with ultrastructural changes in the sinusoidal endothelium and space of Disse in rats,[15] humans[16] and in studies of baboons.[40] Aging is not usually considered to be associated with major structural changes in the liver apart from lipofuscin deposition and multinucleate hepatocytes. Fibrotic and/or cirrhotic changes are not a feature of normal aging.[11,41] However, we found that the sinusoidal endothelium is thickened by approximately 50% and there is a reduction in the numbers of fenestrations by approximately 50% in old age (figures 2 and 3).[15,16] This is associated with occasional perisinusoidal collagen deposition and basal lamina formation. Age-related expression of von Willebrand factor and other antigens normally associated with endothelial cells of capillaries was seen in the sinusoidal endothelium, confirming significant age-related changes in the hepatic endothelium.[15,16]

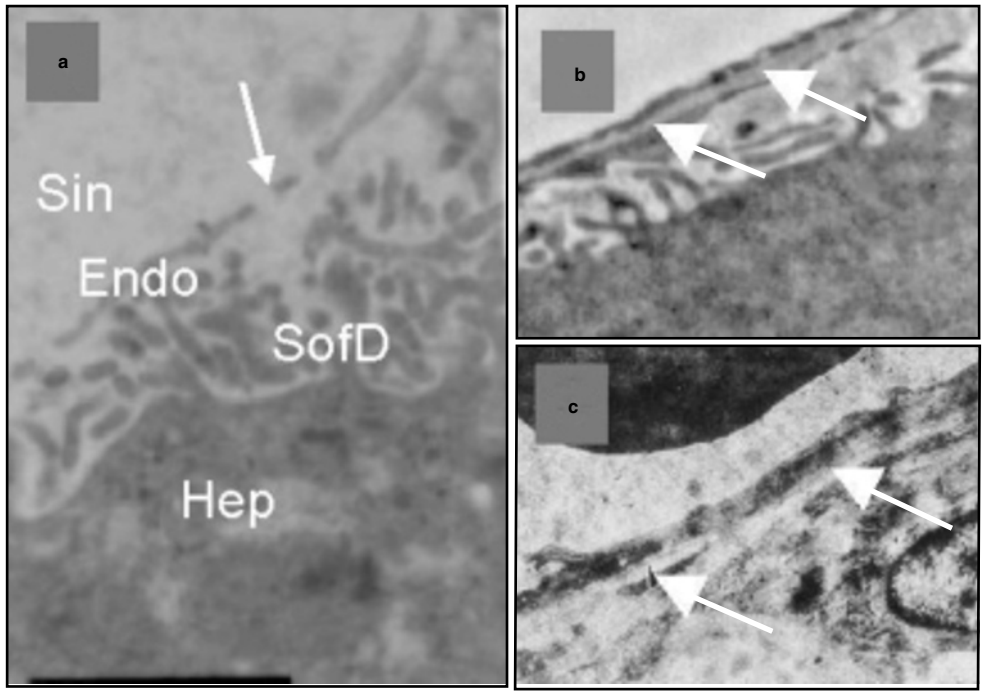

Fig. 2. Transmission electron micrographs of the sinusoidal endothelium and space of Disse in normal (**a**), aged (**b**) and cirrhotic (**c**) rat livers (panel [**c**] reproduced from Mori et al.,[14] with permission). **Endo** = endothelium; **Hep** = hepatocyte; **Sin** = sinusoidal lumen; **SofD** = space of Disse; the arrow in panel (**a**) indicates a fenestration; the arrows in panels (**b**) and (**c**) indicate the basal lamina.

It is of note that the majority of microscopic changes seen in cirrhosis and old age appear to be confined to the perisinusoidal regions of the liver and there are few specific or significant changes to the ultrastructural appearance of the hepatocytes.

3. Effects of Capillarisation on Drug Transfer

The structural changes in the sinusoidal endothelium in cirrhosis and aging are substantial and likely to influence transport of drugs and substrates by a variety of possible mechanisms (figure 4). If it is assumed that there is bulk flow of plasma via the fenestrations into the space of Disse, then defenestration may reduce the hepatic extraction of drugs that undergo flow-limited metabolism (i.e. substrates where the hepatic clearance is influenced primarily by total hepatic blood flow). It is unknown what fraction of drug transfer occurs as a result of bulk flow via fenestrations versus diffusion across the endothelium. Even so, it is plausible that in some cases substrates whose distribution is flow-limited (i.e. substrates where transfer from blood to hepatocytic cytoplasm is unimpeded by any form of permeability barrier) would be expected to take on barrier-limited properties as flow through fenestrations is progressively reduced through defenestration and thickening of the endothelium and a fibrotic space of Disse. Likewise, if it is assumed that drugs and other physiological substrates bound to albumin, and other plasma proteins gain access to the space of Disse via fenestrations, then defenestration will impede clearance of protein-bound substrates. In both of these situations, the reduction in the hepatic extraction of this group of drugs should be directly proportional to the degree of defenestration. For drugs and substrates such as oxygen that diffuse relatively unimpeded across the endothelium and through the space of Disse, thickening of the endothelium and deposition of collagen and other extracellular matrix will progressively impede uptake

by decreasing diffusion. Substrates that reflect each of these scenarios have been investigated, primarily utilising the indicator dilution methodology to assess transfer across the cirrhotic endothelium.[20]

Huet and colleagues[18] studied the effects of capillarisation on the hepatic disposition of albumin and indocyanine green. In 25 humans with alcoholic cirrhosis, the volume of distribution of albumin in the liver varied between values larger than the vascular volume of distribution to the same size as the vascular volume. Specifically, the ratio of volume of distribution of albumin to the volume of distribution of the erythrocytes ($1 + \gamma_{alb}$) ranged from 1.91 to 1.09. This was considered to reflect the severity of cirrhosis and consequent exclusion of albumin from the space of Disse by capillarised endothelium. The hepatic extraction of indocyanine green, a highly extracted indicator that is bound to albumin, correlated with the albumin space. It was concluded that the hepatic extraction of indocyanine green was reduced in cirrhosis because albumin is unable to traverse the defenestrated cirrhotic endothelium. These findings were later replicated in a study of cirrhotic human livers removed at transplantation and isolated and perfused *in vitro*. In these subjects, it was also noted that 21 ± 16% of portal flow was diverted through shunts larger than 15μm in diameter.[42] In cirrhotic rats, Roberts and colleagues subsequently confirmed that the extravascular volume of distribution of albumin correlated directly with the number of fenestrations determined by electron microscopy.[21]

Nuclear scans have been developed to assess functional hepatocyte mass using albumin-bound radiolabels. ^{99m}Tc-diethylenetriaminepentaacetic acid galactosyl human serum albumin (Tc-GSA) binds an asialoglycoprotein receptor found on hepatocytes. Hepatic uptake of Tc-GSA, as measured by nuclear scanning, correlates with hepatocyte number determined histologically in control subjects and those with chronic hepatitis and cirrhosis.[43] In older subjects, functional liver volume

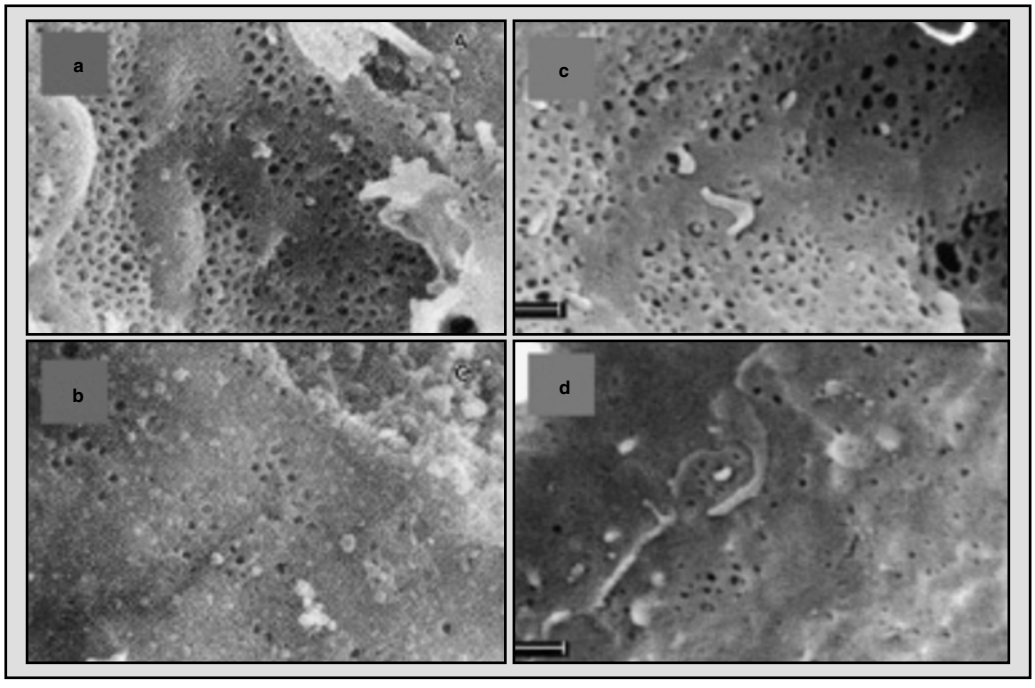

Fig. 3. Scanning electron micrographs of the sinusoidal endothelium in normal (**a**) and cirrhotic (**b**) rat livers and in young (**c**) and aged (**d**) rat livers (panels [**a**] and [**b**] reproduced from Mori et al.,[14] with permission, and panels [**c**] and [**d**] reproduced from Le Couteur et al.,[15] with permission from Hepatology, © Copyright 2001 American Association for the Study of Liver Diseases).

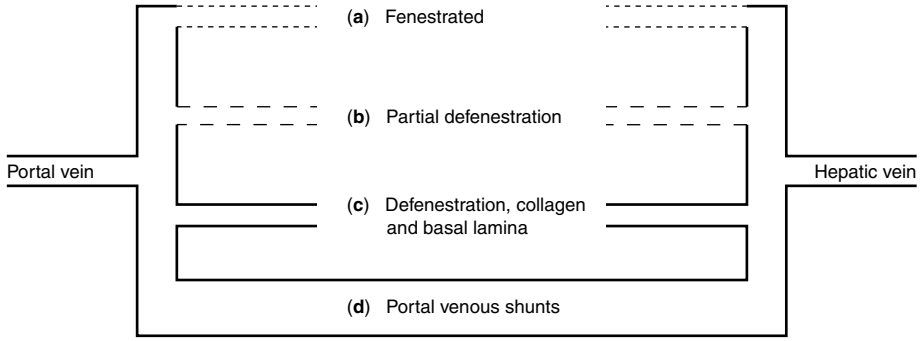

Fig. 4. Sequential mechanisms for altered substrate transfer with capillarisation and/or pseudocapillarisation of the liver sieve. (**a**) Initially, there is unimpeded flow of plasma, solutes, albumin and other macromolecules into the space of Disse; (**b**) partial defenestration reduces the amount of convective flow and transfer is predominantly dependent upon diffusion across the endothelium and space of Disse; (**c**) diffusion is impaired by thickening of the endothelium, development of a basal lamina, modification of matrix within the space of Disse and deposition of collagen; (**d**) large portal-venous and arterio-venous shunts compromise transfer of substrates into hepatocytes.

determined by Tc-GSA decreased more than the liver volume determined by computerised tomography imaging (approximately 45% vs 25% between the ages of 40 and 80 years).[44] The results in subjects with liver disease and older subjects can be explained by decreased access of the albumin-based radiolabel to the space of Disse secondary to defenestration.

Varin and Huet[19] reported the development of a barrier to the transfer of sucrose and water at the sinusoidal endothelium in cirrhotic rats. This was not secondary to the development of large hepatic shunts because these were found to account for only 0.25% of total hepatic flow. In contrast, albumin distribution remained flow-limited although its volume of distribution was reduced to that of the vascular space determined using erythrocytes. The transfer of albumin in other capillary systems appears to be mediated by pores, paracellular flow or, in some cases, transcytosis.[45,46] The hepatic extraction of lidocaine (lignocaine), determined using the indicator-dilution methodology, correlated with the severity of cirrhosis. The presence of an early peak in the lidocaine outflow curves was considered to be evidence of the development of a barrier to uptake at the level of the capillarised endothelium.[19]

The effects of capillarisation on propranolol metabolism in cirrhotic rats were also reported by the same group.[47,48] In cirrhosis, the hepatic extraction of propranolol was reduced from 0.97 ± 0.01 to 0.66 ± 0.14 ($p < 0.01$). Drug metabolising enzyme activity, measured *in vitro* from the same livers, was not significantly reduced (5.3 ± 1.2 L/min/liver in controls vs 3.6 ± 1.9 L/min/liver in cirrhosis),[47] although this may reflect insufficient statistical power. On the other hand, the rate constants for the transfer of propranolol from the sinusoidal lumen into the space of Disse was significantly reduced in cirrhosis (0.75 ± 0.10 s^{-1} in controls vs 0.24 ± 0.13 s^{-1} in cirrhosis, $p < 0.05$).[48] It was concluded that the effect of capillarisation on the transfer of propranolol from the sinusoid into the extracellular space was the major mechanism for the reduction in hepatic clearance of propranolol in cirrhosis.

It has been proposed that the simultaneous measurement of hepatic extraction of sorbitol ($E_{sorbitol}$) and indocyanine green (E_{ICG}) will give an estimate of the degree of capillarisation and vascular shunting.[49] The hepatic extraction of both sorbitol and indocyanine green is very high and, therefore, their hepatic clearance is flow-limited. Sorbitol is not protein-bound and the fraction of hepatic blood flow that is directed through hepatic shunts in cirrhosis is considered to be equal to $1 - E_{sorbitol}$. Indocyanine green is protein-bound and changes in its hepatic extraction will depend on both hepatic shunting and capillarisation of remaining sinusoids. Therefore, the fraction of the sinusoids that is capillarised is $E_{sorbitol} - E_{ICG}$.[49] An analysis of data on $E_{sorbitol}$ and E_{ICG} suggested that there was evidence for both

shunting and capillarisation in cirrhosis.[49] Specifically, a curved relationship was noted between $E_{sorbitol}$ and E_{ICG}. A linear relationship would have been expected if the reduction in extractions of both substrates were secondary only to shunting.

In a subsequent prospective study of 53 human subjects including controls and cirrhotic patients, Ott et al.[50] concluded that changes in $E_{sorbitol}$ and E_{ICG} in cirrhosis are more likely to represent a proportional reduction in the permeability surface area (PS) products of both sorbitol and indocyanine green, rather than differential effects of shunts and capillarisation on each substrate. It was hypothesised that capillarisation may cause functional shunts (i.e. non-transferring sinusoids) and that capillarisation provides the anatomical explanation for the reduction in the PS products of both sorbitol and indocyanine green. However, it was noted that the differential changes between $E_{sorbitol}$ and E_{ICG} could be secondary to technical issues related to analysis of indocyanine green.[50]

Recently, it has been reported that the impairment of hepatic extraction of the albumin-bound bile salt taurocholate correlates with the 'fibrosis index' in cirrhosis rather than defenestration of the endothelium.[21] In addition, the PS product of water correlated with the fibrosis index. Although other explanations were given, it was suggested that fibrosis in the space of Disse could create a rate-limiting step for the hepatocellular uptake of taurocholate and other substrates such as oxygen and water.

Hung et al.[51] reported the effects of cirrhosis on the hepatic disposition of several drugs (atenolol, antipyrine, prazosin, labetolol, propranolol and diltiazem). They found a close relationship between the fibrotic index, PS product at the hepatocellular membrane, lipophilicity of the drug and hepatic extraction. The methodology focused on the hepatocellular transport of these drugs. However, it was conceded that impairment in uptake of drugs without specific membrane transporters may reflect a slower solute diffusivity and longer diffusion path length as a consequence of collagenisation of the space of Disse.[51]

In a recent dynamic computed tomography study of rabbits with hepatic fibrosis, it was reported that the hepatic volume of distribution of the high-molecular-weight marker P840 was significantly smaller than the volume of distribution of the lower-molecular-weight marker iobitridol (22 ± 5% vs 32 ± 7%).[52] The volumes of distribution of the two markers were not significantly different in control rabbits. The effects of hepatic fibrosis on the disposition of P840 were thought to be mediated by capillarisation, although the diameter of P840 (6.35nm) is substantially less than that of a typical fenestration (100nm). Drug metabolism has also been studied in rabbits with the same model of hepatic fibrosis.[53] The livers of these rabbits are capillarised but not cirrhotic and do not have vascular shunts. The systemic clearance of aminopyrine was reduced by 69% and that of indocyanine green reduced by 89% in hepatic fibrosis, and there was a correlation between the two.

We studied the effects of defenestration and capillarisation on the hepatic disposition of chylomicrons and chylomicron remnants.[13,24,54-57] Nicotine, endotoxins and dimethylnitrosamine induce defenestration and are associated with increased susceptibility to dietary cholesterol, indicating that defenestration reduces the capacity of the liver to metabolise dietary fat.[13] Rabbits and chickens have proportionately fewer fenestrations than humans and rodents and are more vulnerable to dietary cholesterol, resulting in hyperlipidaemia and atherosclerosis.[13] In humans, there is a correlation between alcohol consumption, defenestration and hyperlipidaemia, suggesting that the loss of the liver sieve contributes to alcohol-induced hypertriglyceridaemia.[39] Recently, we suggested that the effects of age-related pseudocapillarisation on the hepatic uptake of chylomicron remnants might explain the association between aging, postprandial hyperlipidaemia and, therefore, age-related atherosclerosis and vascular disease.[58]

There have only been a few reports of the effects of age on hepatic sinusoidal permeability and drug metabolism. None have specifically examined the effect of age-related pseudocapillarisation. There

are reports of age-related impairment of hepatic uptake of a few substrates, including dimethadione,[59] glucose[60] and bile salts,[61,62] but these measured the hepatocellular barrier rather than the endothelial barrier.

4. Effects of Capillarisation and Pseudocapillarisation on Oxygen Delivery

In the healthy liver, the distribution of oxygen is flow-limited and there is no barrier to hepatocyte uptake. This has been confirmed using isotopic oxygen with the multiple indicator dilution method,[63] and carbon monoxide as a surrogate for oxygen disposition with a wash-in method.[64] In both cases, the outflow curves of the gases were found to be superposable upon those of the extravascular markers and water (figure 5), confirming the absence of any significant permeability barrier to the uptake of oxygen.

Once within the hepatocyte, the dependence of enzymatic reactions on oxygen is defined by the Michaelis constant of oxygen (K_mO_2), the oxygen concentration at which enzymatic function is half-maximal.[65] All enzymatic processes have a dependence on oxygen because of their requirement for energy, however amongst those with the highest oxygen dependencies are the phase I cytochrome P450 pathways because they utilise oxygen directly as a co-substrate.[3] This has been widely investigated *in vitro*[65] and has also been confirmed in the intact liver, where a hypoxic threshold has been identified for propranolol clearance.[66] By contrast, phase II conjugative pathways have reduced oxygen requirements and remain functional at lower oxygen concentrations than those that are required to maintain phase I pathways.[3]

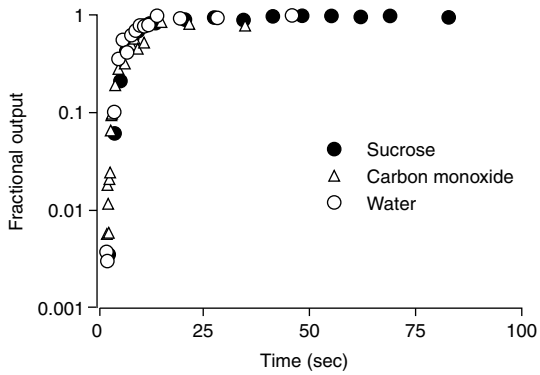

Fig. 5. The linear superposition principle applied to the carbon monoxide outflow curves from wash-in experiments performed in the perfused rat liver. The outflow curves for water and carbon monoxide are superposed on the outflow curve for sucrose after the timepoints are corrected for time zero and then multiplied by the ratio of the volume of distribution of sucrose to the volume of distribution of water and carbon monoxide, respectively. This confirms that there is no barrier to the uptake of gases such as carbon monoxide or oxygen in the healthy liver (reproduced from Le Couteur et al.,[64] with permission).

In our original review of hepatic drug metabolism and liver disease,[3] we noted a trend for the clearance of drugs that undergo metabolism via phase I pathways to be reduced in cirrhosis and for phase II pathways to be maintained. More recent studies indicate that this trend is maintained until liver injury is end-stage.[10,67,68] We proposed that one explanation for the differential impairment of phase I metabolism in cirrhosis is impaired oxygen delivery into the hepatocytes secondary to the effects of capillarisation on oxygen transfer. This has become known as the Oxygen Limitation Theory of drug metabolism in cirrhosis.[3] The theory is particularly important because of the therapeutic implication that improved oxygen delivery (for example through the use of selective hepatic arterial vasodilators) may improve hepatic function.[69]

The differential effects of cirrhosis on drug metabolism have been more recently studied. In a cirrhotic rat model, *p*-nitrophenol glucuronidation, *p*-nitrophenol sulfation and propranolol oxidation were compared. Glucuronidation was not reduced in cirrhotic rats, whereas a reduction in sulfation and oxidation was confirmed using an intact liver methodology.[70] In cirrhotic humans, the metabolism of theophylline, a phase I substrate, was found to be significantly reduced compared with that of intravenous paracetamol (acetaminophen), a phase II substrate.[71]

Oxygen uptake and consumption is markedly reduced in cirrhosis.[48,51,66,72] This is consistent with oxygen limitation, but may have many other explanations such as shunts, impaired mitochondria and

oxygen steal.[72] Furthermore, oxygen supplementation has been shown to improve various aspects of hepatic metabolism and drug metabolism in cirrhosis. We have shown that cirrhosis of the liver in rats is associated with reduced levels of high-energy phosphate metabolites such as adenosine triphosphate, indicative of intrahepatocyte hypoxia. This intracellular hypoxia was corrected by short-term oxygen supplementation.[73,74] Clearance of theophylline, which undergoes oxygen-dependent phase I metabolism, was normalised by oxygen supplementation in the cirrhotic rat.[75] In cirrhotic humans, oxygen supplementation corrected the clearance of theophylline, a phase I substrate, but not that of paracetamol, which is primarily a phase II substrate.[71] Augmentation of highly oxygenated hepatic arterial blood flow to the perfused cirrhotic rat liver improved the intrinsic clearance of propranolol, a phase I substrate.[72]

However, it remains unknown whether capillarisation impairs oxygen transfer from blood to the hepatocytes. In capillary systems found in non-hepatic tissues (e.g. heart,[76] hindlimb[77] and placenta[78]), a significant barrier to oxygen transfer can be measured at the level of the capillary wall. As yet, the effects of capillarisation or pseudocapillarisation of the liver on oxygen transfer have not been reported. However, the capillarised sinusoid is similar in structure to that of capillaries in these other tissues, therefore the effects on oxygen transfer are likely to be the same.

In a review of aging and hepatic drug metabolism,[11] we noted that there was also selective impairment of phase I pathways in old age. This observation was originally noted in male rats and was thought to indicate a species-specific effect of male hormones on cytochrome P450 expression in the rat. This trend has been observed many times in humans,[11,79,80] and, unlike in male rats, is not associated with major changes in the expression or *in vitro* activity of cytochrome P450 enzymes.[81] Although changes in hepatic blood flow and liver mass have been identified as contributors to age-related changes in hepatic drug metabolism,[82] these mechanisms cannot fully explain the differentiation between phase I and phase II pathways that are seen *in vivo*.[11,80] Therefore, we postulated that aging is also associated with impaired oxygen delivery – the Oxygen Diffusion Barrier Hypothesis of aging in the liver.[11] Hepatic oxygen uptake is generally reduced with age,[11,60,83,84] and there is reduction in high-energy phosphate metabolites, consistent with intrahepatocyte hypoxia.[15] The effects of oxygen supplementation on drug metabolism in old age are not known.

5. Comparison of Mechanisms for Impaired Hepatic Drug Metabolism

There are significant structural changes in the endothelium and space of Disse in cirrhosis and aging that are likely to impair the uptake of drugs and oxygen through impaired plasma flow secondary to defenestration and through development of an increased diffusion barrier secondary to deposition of collagen and thickening of the endothelium. Whether these structural changes will have any impact on hepatic drug clearance will depend on concomitant changes in hepatic blood flow, protein binding, liver mass and enzyme expression and activity. This can, to some extent, be resolved by comparing the rate constants for hepatic blood flow, drug transport and CL_{int} in health and disease – with the caveat that estimates of these parameters will be profoundly affected by methodology, substrate and species.

Hepatic blood flow in the healthy liver is approximately 0.02–0.03 L/sec/kg of liver tissue, of which one-third is via the hepatic artery and two-thirds via the portal vein.[85] The rate of transport of substrates between the sinusoid and the hepatocyte (PS product) can also be calculated in units of L/sec/kg, which allows comparison of transport rates, blood flow rates and enzymatic elimination (intrinsic clearance). In perfused rat livers, the PS product for propranolol across the endothelium was approximately 0.1 L/sec/kg in healthy livers and 0.02 L/sec/kg in cirrhotic livers (calculated from Gariepy et al.[48]). The PS product for the hepatocellular uptake of six drugs in the perfused rat liver varied between 0.4 and 1.5 L/sec/kg in the healthy liver and was

reduced by about 50% in cirrhotic livers.[51] The PS product for the transfer of carbon monoxide was 0.21 L/sec/kg in the healthy liver[64] and was reduced to 0.01 L/sec/kg in the more usual (i.e. 'capillarised') networks seen in the rat hindlimb[77] and human placenta.[78]

CL_{int} and *in vitro* enzyme activity have been estimated for numerous substrates. For highly extracted substrates such as propranolol, CL_{int} is extremely high. For example, it is in the order of 5 L/sec/kg when estimated from extracts of healthy rat livers.[47] In perfused rat livers, CL_{int} of propranolol, determined using the parallel tube model, is approximately 1.5 L/sec/kg in normal livers and 0.5 L/sec/kg in cirrhotic livers.[72] In microsomes, CL_{int} varies between 0.002 and 0.4 L/sec/kg, depending on substrate.[1]

Comparison of these various clearances suggests that under certain circumstances, particularly where there is capillarisation or pseudocapillarisation of the sinusoid, the liver sieve may become the rate-limiting step for hepatic drug metabolism through effects on drug transfer. These analyses do not give any indication of the effects of the alterations in the liver sieve on oxygen delivery and subsequent effects on phase I enzyme activity.

6. Conclusion

In health, the liver sinusoidal endothelial cells and space of Disse do not impede the transfer of most substrates from the sinusoidal lumen to the hepatocyte. Plasma may flow freely from the sinusoidal lumen, through the endothelial fenestrations in and out of the space of Disse. The 'massaging' action of blood cells appears to assist this exchange. However, the transfer of blood cells, the largest of lipoproteins such as chylomicrons, and large rigid liposomes are restricted on the basis of size.

Both aging and cirrhosis are associated with marked changes in the sinusoidal endothelium and space of Disse. These changes, termed capillarisation and pseudocapillarisation, respectively, are likely to impede the transfer of substrates, including drugs, albumin and oxygen, from blood to the hepatocyte. There is some evidence for this proposal in cirrhosis, involving albumin, sucrose, oxygen, propranolol and some other drugs. Specifically, defenestration is associated with exclusion of albumin and protein-bound drugs from the space of Disse, and the progressive transformation of flow-limited to barrier-limited distribution of soluble substrates. There has been very limited research with respect to aging and substrate transport in the liver; however, on the basis of structural similarities, it is probable that the same functional impairment will occur.

There is also some evidence that the liver sieve in cirrhosis and aging might contribute to intrahepatocyte hypoxia, thus providing an alternative mechanism for the apparent differential reduction of oxygen-dependent phase I metabolic pathways in these conditions.

Structural change and subsequent dysfunction of the liver sieve should be considered a significant factor in the pathogenesis of the impairment of hepatic drug metabolism in cirrhosis and aging, through impediments to drug transport and oxygen diffusion. These structural and functional changes in the liver sinusoidal endothelium may have an important impact on the metabolism of other substrates, including lipoproteins and disease-producing toxins.[13,58,86]

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