Microcirculation of the Aging Liver: Is Getting Old Like Having Cirrhosis?

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What is known about the liver circulation and microcirculation in elderly individuals? Not much. However, new insights may improve our understanding of age-related changes in hepatic perfusion. In the normal adult liver, sinusoids have a unique structure that allows maximum contact between hepatocytes and the blood perfusing the liver. Unlike capillaries in the heart, lung, or brain, the endothelial cells lining sinusoids are perforated by a multitude of fenestrae 50 to 150 nm in diameter, and have no basal membrane. These fenestrae occupy about 5% to 10% of the endothelial surface (porosity) and are most often arranged in groups for which the name sieve plate was given.[1] The diameter of these fenestrae appears to decrease slightly from zone 1 to zone 3, while their total porosity increases from 6% to 9%.[1] A large extra vascular space, the space of Disse, lies behind the endothelial lining and is continuous with the intercellular space up to the tight junctions of hepatocytes. Normally, only a few collagen bundles can be seen in the space of Disse using electron microscopy (EM) techniques. However, the entire space is not empty and contains several substances forming the extra cellular matrix (*i.e.*, laminin, fibronectin, collagens, hyaluronan, perlecan), which are well identified using immunohistochemical techniques.[2] This matrix acts as a semipermeable gel in which large molecules dissolved in plasma can diffuse according to their molecular weights.[3] Red and white blood cells as well as large lipoproteins (such as chylomicrons, 100 to 1000 nm in diameter) are larger than the fenestrae and do not have access to the space of Disse. In addition, owing to their size difference, blood cells must adjust their shape in order to pass through the sinusoids and flow in single cell rows.[3] A reciprocal adaptation of the endothelial lining is thought to occur during the passage of blood cells, stirring the space of Disse and further improving the exchange of fluid and particles between the blood and hepatocytes.[1] At the same time, the flexibility of the endothelial wall, the result of an absent continuous basal lamina and a loose extra vascular matrix, is necessary to maintain the low sinusoidal pressure gradient (less than 5 mm Hg) observed in normal adult liver.

The filtering effect of fenestrated endothelial cells may also exhibit temporal variations since fenestrae have been shown to contract and dilate under certain conditions.[4] A few years ago, Fraser et al.[5] stressed that perturbation in the porosity of sinusoidal endothelial cells, due to changes in size and/or number of fenestrae or to alterations of the extravascular matrix, may have a profound influence on the metabolism of lipoproteins. In particular, the decreased passage of chylomicron remnants (±50 nm in diameter) through an altered sinusoidal lining may lead to their reduced clearance by the liver and participate in the pathogenesis of atherosclerosis. This factor could at least partially explain their decreased clearance in elderly subjects.[6]

The multiple indicator dilution technique proposed by Goresky[3] provides the best approach to study the unique structure of the normal sinusoidal bed *in vivo* and to evaluate changes caused by anatomical alterations. This technique involves the injection into the portal vein or hepatic artery of a mixture of different indicators and their collection from the hepatic venous outflow. The indicators used for these studies are usually labeled red blood cells (RBCs) and plasma-dissolved substances (such as albumin, sucrose, and water), which are not metabolized or removed by the liver. In the liver, RBCs are confined to the vascular space during the passage through sinusoids, whereas plasma-dissolved substances gain access to the extravascular space through the fenestrae in and between the endothelial cells. Albumin (MW ± 69,000) and sucrose (MW 342) diffuse into extravascular spaces that are inversely related to their molecular weights, and water diffuses into the extravascular and cellular spaces. The distribution (and dilution) of plasma-dissolved substances into spaces larger than those of RBCs produces both a major delay and a decrease in the magnitude of the peak of the outflow of diffusible substances when compared with that of RBCs. In normal adult livers, this distribution is flow limited; indeed, the curve of each diffusible substance can be transformed in such a fashion that it can be superimposed upon the RBC curve. This method yields estimates of the sinusoidal blood volume and of the extravascular distribution volume of diffusible substances, measured

Abbreviations: EM, electron microscopy; RBC, red blood cell; MW, molecular weight.

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Published online in Wiley InterScience (www.interscience.wiley.com).

DOI 10.1002/hep.20991

Potential conflict of interest: Nothing to report.

from the displacement of their outflow curves in relation to that of RBCs.[3] In healthy adult livers, the distribution of oxygen has also been shown to be flow limited, contrasting with its barrier-limited distribution in the cerebral capillaries.[7]

In the presence of cirrhosis, three types of alterations of the liver microcirculation are well recognized: collagenization of the space of Disse, formation of a basal lamina beneath the endothelial cells, and, finally, loss of the endothelial fenestrations.[8] These anatomic alterations transform sinusoids into capillary-like channels, limiting the diffusion of plasma-dissolved substances into the space of Disse, thus hindering their direct access to the hepatocyte membrane. This progressive transformation has been evaluated in humans and in experimental models of cirrhosis by using Goresky's technique: in livers with cirrhosis, the diffusion of albumin is markedly decreased and, in some cases, its volume of distribution is close to that of RBCs.[9-13] In such cases, sucrose and water are still able to diffuse into the extra vascular space but their distribution becomes diffusion limited, as occurs in organs with a capillary system such as the coronary circulation. These effects of capillarization were shown to decrease the hepatic removal of protein-bound substances such as indocyanine green, lidocaine, and propranolol.[9-13] At the same time, a marked decrease in oxygen consumption has been consistently reported in livers with cirrhosis and has been mainly attributed to the impaired transfer of oxygen across capillarized sinusoids.[10] In addition, the rigid structure of these new capillarized sinusoids increases the resistance to blood flowing through the liver, contributing to the development of portal hypertension.

Anomalies of the hepatic microcirculation have also been reported in conditions other than cirrhosis. In patients with diabetes, collagenization of the space of Disse was correlated with the presence of diabetic microangiopathy,[14] but changes in endothelial fenestrations have not been reported. In patients with morbid obesity, Marubbio et al.[15] reported collagen deposition in the space of Disse and the presence of electron-dense material resembling basement membranes. Whether these morphological changes are of functional significance, or could play a role in the pathophysiology of nonalcoholic steatohepatitis remains unknown.

Up until now, the major effect of aging on the liver circulation and microcirculation was thought to be a progressive decrease in hepatic blood flow (about 30% to 40%) with a concomitant reduction in liver volume ranging also from 20% to 40%.[16] These changes were associated with an apparent decrease in hepatocyte number while their size was slightly augmented. These alterations were thought to account for the age-related decrease in hepatic drug metabolism.[17] However, the different effect of aging on the two major metabolic pathways, phase I (decreased) and phase II ($\pm$ maintained),[18] led Le Couteur et al.[19] to postulate that aging was associated with an impaired hepatic delivery of oxygen, the "Oxygen Diffusion Barrier Hypothesis" of aging in the liver. Indeed, phase I enzymes are more dependent on oxygen availability than phase II enzymes, while their overall hepatic concentration and/or activity has not been reported to be markedly modified by the aging process. In their original hypothesis, Le Couteur et al.[19] thought the barrier to oxygen diffusion was at the level of the hepatocyte membrane, since most previous EM studies did not show evidence of alterations in the sinusoidal lining with aging.[20,21] In 2001, they reported marked ultrastructural changes in the sinusoidal endothelium of old rats with loss of fenestrae and reduction in the overall porosity of the endothelial lining.[22] A basal lamina could be found in some cases with an increased deposit of collagen and laminin in the space of Disse. Since then, the same group found similar age-related alterations in humans, mice, and nonhuman primates.[23,25] These alterations were called "pseudocapillarization," as the aging endothelium was similar to endothelia of other capillary beds and also resembled those found in capillarized sinusoids with cirrhosis. These physical changes in the sinusoidal lining may well explain the decreased phase I metabolic pathway by limiting the oxygen diffusion in older livers. On the other hand, the reduction in porosity of the endothelial lining should also offer a new limitation to the diffusion of small lipoproteins, such as chylomicron remnants, with a secondary reduction in their hepatic handling. Therefore, it has been hypothesised that a reduced uptake of chylomicron remnants may participate in the pathogenesis of atherosclerosis,[26] as proposed earlier by Fraser et al.[5] However, no study had confirmed this metabolic counterpart of pseudocapillarization in human as well in experimental aging models.

In this issue of HEPATOLOGY, Hilmer et al.[27] examined the distribution of small-labelled chylomicrons ($\pm$50 nm in diameter) in the liver of young and old rats using an adaptation of Goresky's technique. They elegantly demonstrate that in old rat livers, small chylomicrons (not removed by the liver, in contrast to chylomicron remnants) are no longer able to diffuse into the extravascular space, whereas diffusion of sucrose (used as the reference substance) is unaltered. Indeed, in young rat livers, sucrose and small chylomicron outflow dilution curves were almost superimposed (indicating a similar volume of distribution, with a volume ratio about unity), while in old rat livers, the small chylomicrons curve was well ahead of the sucrose one (indicating a smaller volume of distribu-

tion, with a volume ratio significantly lower than unity). In addition, EM studies confirmed in livers of old rats the decrease in endothelial porosity (scanning EM) with an endothelial thickening and basement membrane deposition in sinusoids (transmission EM).

As with every good study, the paper by Hilmer et al. raises several questions. Is defenestration also limiting the diffusion of albumin and albumin-bound substances, as occurs in livers with cirrhosis? Was there any difference in oxygen consumption between young and old rat livers? Is the volume of distribution accessible to small chylomicrons in livers of old rats livers similar to the RBCs, *i.e.*, the vascular space? Does this new barrier have any effect on the perfusion pressure through old sinusoids? Unfortunately, RBCs and albumin were not used in these experiments; their distribution in the old livers would have answered most of these questions, since the level of the new barrier may not be due to the defenestrated endothelium but rather to changes in matrix density. Indeed, we have shown that the extravascular matrix was more important than the endothelial lining integrity in the sieving function of the sinusoidal endothelium of rat livers subjected to extensive cold preservation.[28] The other questions were not addressed in the present paper.

Can these findings in experimental animals be extrapolated to the aged human liver? First, are we sure that pseudocapillarization really occurs in aging human livers? Excellent studies (although "old" ones) performed using transmission EM did not report any significant ultrastructural abnormalities in this population[22,23] and micrographs published recently by McLean et al.[23] are not fully convincing, except for the increased collagen content of the space of Disse. However, scanning EM studies, the best approach to evaluate defenestration, were never performed in old human livers, as done in old animal livers, most probably due to the difficulty in applying this technique, particularly perfusion with fixative, to old human livers. With the advent of liver transplantation, particularly with old organ donors, studies with this important technique should be feasible without having to perfuse the whole liver.[29]

Although the anatomical abnormalities found in cirrhosis, together with their effects on hepatic metabolism, are well established, the anatomical abnormalities found in aging rat livers remain to be corroborated in humans, and their relationship to possible metabolic consequences is still unresolved. There are impressive similarities between both conditions; undoubtedly, liver transplantation using older donors could provide clues to the questions raised. Indeed, because of the shortage of "young" donors, the age limit for liver donation has been raised up to 80 years in certain parts of the world.[30,31] If these livers suffer from pseudocapillarization, the recipients could develop metabolic abnormalities, particularly atherosclerosis, according to the hypothesis proposed by Le Couteur[26] This complication has not yet been reported in recipients of old livers but, most probably, has not been thoroughly examined. Most patients with cirrhosis should have extensive atherosclerosis with their capillarized sinusoids, which does not appear to be the case: if anything, patients with cirrhosis appear to be protected from the cardiovascular complications of atherosclerosis.[32,33] Indeed, the prevalence of ischemic stroke was reduced in advanced liver diseases and the presence and severity of atherosclerotic plaques in carotid and vertebral arteries was similar in patients with cirrhosis and in age-matched controls.[33]

Finally, the mechanism of pseudocapillarization remains to be elucidated. In an intriguing experiment, Modis and Martinez-Hernandez[34] have shown that following implantation of rat liver fragments into quail embryos, the quail capillaries assume a sinusoidal phenotype with fenestrations and absence of basement membrane. These results suggest that hepatocytes modulate the phenotype of endothelial cells, possibly through the secretion of vascular growth factors, which could decrease with age.

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