Small Liver Fenestrae May Explain the Susceptibility of Rabbits to Atherosclerosis

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Rabbits fed cholesterol rapidly develop high serum cholesterol levels which lead to the development of atherosclerosis. This is related to the retention of cholesterol-rich chylomicron remnants in the circulation. In most animals, such as rats, chylomicron remnants are rapidly removed from the circulation by the liver. The first barrier to this removal is the fenestrated endothelium of liver sinusoids. Measurements made of a large number of sinusoidal fenestrae by scanning electron microscopy have shown the average diameters to be 89 nm in rats and 49 nm in rabbits. We postulate that the small size of endothelial fenestrae in the liver sinusoids of rabbits hinders the egress of chylomicron remnants from the sinusoidal blood, explaining the subsequent development of hypercholesterolemia and atherosclerosis.

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Since early in this century it has been known that rabbits fed cholesterol develop atherosclerosis. This is related to an excess of circulating cholesterol-rich lipoproteins, the chylomicron remnants. Chylomicron remnants are formed by the removal of triglycerides from their parent chylomicrons under the influence of lipoprotein lipase. In rats these remnants are rapidly removed from the circulation by the liver. The uptake of remnants by the hepatocytes may involve recognition of adherent surface proteins such as lipoprotein lipase or specific apolipoproteins, or the lack of specific apolipoproteins. Before the remnants reach the hepatocytes, however, the fenestrated sinusoidal endothelium sieves them from the larger chylomicrons.

The nature of the fenestrated sinusoidal endothelium of the liver, separating the circulating blood from the space of Disse and the hepatocytes, was not fully appreciated until its preservation was improved by perfusion fixation. The dimensions of the fenestrae were seen to be ideal for filtering chylomicrons of different sizes in the rat. That this does, in fact, occur has been substantiated. The first barrier to the removal of remnant chylomicrons from the circulation is the fenestrated sinusoidal endothelium. Since the cholesterol-fed rabbit, unlike the rat, develops hypercholesterolemia due to these remnants, it was decided to examine the diameter of the fenestrae in the two species.

Methods

Animals

Female New Zealand white rabbits of varying ages weighing 1.1 kg to 2.2 kg, and female Sprague-Dawley rats weighing 200 to 450 g, which had been maintained on the usual commercial pellets and water, were starved for 24 hours before experimentation. The animals were anesthetized with parenteral sodium pentobarbitone (Nembutal) before their livers were perfused with fixative in preparation for electron microscopy.

Perfusion Fixation

The transhepatic flow of perfusate was designed to approximate the physiological conditions of portal blood flow. Unlike previous experiments where a constant volume of fixative was infused, the livers in these experiments were perfused at a constant pressure. The physiological portal vein pressures in both rats and rabbits were determined to be between 8 and 10 cm of water, as measured by methods.
described previously. It was found that when the perfusate reservoir was located 15 cm above the portal vein, and the vein was cannulated by a needle of sufficiently large bore (able to deliver a flow which, when not impeded by the liver, was greater than the maximum flow obtainable through the liver), the delivered pressure of the flowing perfusate was within the physiological range. In rats either 14- or 16-gauge needles and in rabbits 8-, 10-, or 12-gauge needles were found to be suitable. The perfusate was maintained at 37°C by a heat exchanger and water bath.

Once anesthetized, the abdominal cavity of the animal was quickly opened to expose the portal vein. The cannula was then inserted and tied in place while the perfusate flow was initiated and the inferior vena cava was cut to prevent venous congestion.

The perfusate consisted of 3 ml of isosmotic sodium cacodylate buffer (pH 7.3) which served to flush the liver of blood, immediately followed by fixative: cacodylate-buffered 1.5% glutaraldehyde. The initial flow rates averaged 130 ml/min for rabbits and 25 ml/min for rats, but these dropped slightly with time, presumably due to "log jams" caused by a few remaining red cells becoming fixed within the sinusoids. The liver immediately blanched and rapidly hardened; perfusion was continued for 3 minutes, by which time the fluid issuing from the inferior vena cava was clear.

For scanning electron microscopy, blocks approximately 5 x 1 x 1 mm were postfixed in buffered 1% osmium tetroxide for 2 hours and dehydrated through an alcohol series. Critical-point drying was achieved in a Polaron E 3000 using carbon dioxide as the drying medium.

The dried blocks were then fractured by snapping between two pairs of forceps and sputter-coated with gold; the fractured surface was then viewed in an International Scientific Instrument 40 scanning electron microscope operated at 30 kV in the secondary electron mode. Photomicrographs were magnified to X 30,000 as calibrated against a grating of known periodicity (Ladd, Burlington, Vermont). Over 1000 fenestrae from the sinusoidal endothelium of each liver were measured to the nearest 0.5 mm (equivalent to 16.7 nm) from randomized scanning electron microscope (SEM) prints.

Results

The distributions of the diameters of 27,500 fenestrae from 17 rabbit livers and of 16,500 fenestrae from 15 rat livers are shown in Figure 1. The smaller size of rabbit fenestrae may also be appreciated in the scanning electron micrographs shown in Figure 2 which compare typical areas of sinusoidal endothelium from a rat and from a rabbit.

The overall mean fenestrae diameter from 17 rabbits was 49 nm (so of the means for individual animals = 10.2). From 15 rats the mean fenestrae diameter was 89 nm (so of the means for individual animals = 7.0). This represents a highly significant difference (t = 12.9, df = 30, p < 0.001). All the rabbits had smaller mean fenestrae diameters than any of the rats.

Discussion

We have shown the endothelial fenestrae of the rabbit liver to be significantly smaller than those of the rat. This confirms a pilot study previously presented in abstract form. Absolute values for the diameters of fenestrae are more difficult to estimate, however, as processing for scanning electron microscopy is known to induce shrinkage artefacts. We estimate that our methods produce linear shrinkage of approximately 10% since our figure for the mean diameter of rat liver endothelial fenestrae (89 nm) is about 10% below the figure of 100 nm reported from transmission electron microscopy after both perfusion fixation with glutaraldehyde and freeze...
Figure 2. Scanning electron micrographs of rat (A) and rabbit (B) sinusoidal endothelia showing numerous fenestrae. Bar represents 1 μm.
fracture.14 This would suggest an absolute value of about 55 nm for the average diameter of fenestrae in the rabbit.

Cholesterol-rich remnants have been shown to be responsible for the hypercholesterolemia in the cholesterol-fed rabbit.1 In such animals, two populations of remnants have been described: "large" remnants which carry most of the circulating cholesterol and which have mean diameters of 98, 88, and 75 nm as determined by negative stain electron microscopy, chemical composition, and analytical ultracentrifugation respectively; and "small" remnants which carry only one-tenth as much cholesterol and have mean diameters of 42, 37, and 41 nm when measured by the same methods. Because the mass of a spherical lipoprotein is a function of its volume (which in turn is a function of the cube of its diameter),8 a greater amount of cholesterol is present in lipoproteins of larger than average diameter in both populations.

The two populations of chylomicron remnants seen in the hypercholesterolemic rabbit may reflect two removal mechanisms. In the rat it has been shown that both the hepatocytes (parenchymal cells) which are separated from the sinusoidal blood by the fenestrated endothelium and, to a lesser extent, the Kupffer cells which lie within the sinusoids, catabolize remnants.25 It has been shown that the rabbit liver is less efficient than that of the rat in removing remnants from the circulation.4 This could be due to the failure or overloading of one or both removal mechanisms.

It seems possible that the remnants might not be able to reach the hepatocytes, either because they are excessively large,8 or because the endothelial filter is too fine,18 or through a combination of these two factors. Another explanation of the rabbit liver's inefficiency in removal of chylomicron remnants is the relative deficiency of liver cell membrane receptor sites specific for their apolipoproteins in the cholesteryl-fed rabbit compared to the cholesterol-fed rat.21 Whether the receptor sites are located on Kupffer cells, other sinusoidal cells, or hepatocytes has not been determined.

These two explanations are not mutually exclusive. It has been suggested that the failure of rats to develop hypercholesterolemia is due to the existence of low affinity, high capacity receptor sites on liver cell membranes.21 It seems possible that such sites may occur on the hepatocyte membranes in both the rat and the rabbit, and that the difference in response to cholesterol feeding is due to the inability of the chylomicron remnants to pass through the fine endothelial fenestrae in the rabbit.

From a broader viewpoint, we suggest that the sieving of chylomicron remnants by the liver may greatly influence lipoprotein metabolism. Not only may the diameter of fenestrae change from species to species as shown in this paper, but also between individuals, and within a single individual under the influence of various physiological and pathological parameters.17, 22-24 The size of chylomicron remnants may also vary, not only between species but within one individual where the amount and type of the dietary fat influences the size and composition of the parent chylomicrons.8, 25-28

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References

Index Terms: hypercholesterolemia • atherosclerosis • Kupffer cells • endothelium • chylomicrons • liver
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