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The sinusoids in cirrhosis

A morphometric study

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Summary. The volume fraction of the sinusoids (Vv), the sinusoidal surface:total volume ratio (Sv), and the sinusoidal length:total volume ratio (Lv) were evaluated morphometrically in twelve biopsy specimens with cirrhosis and in eleven control specimens. The Sv, which is directly related to the radius of the sinusoids, was significantly ($0.01 > P > 0.001$) greater in the cirrhotic group than in the controls. In contrast, the Lv, which is directly related to the length of the sinusoids, and the Vv did not vary significantly in the same groups. These data support the concept that changes of the sinusoids do not contribute to portal hypertension in cirrhosis.

Key words: Liver – Cirrhosis – Sinusoids – Portal hypertension – Morphometry

Introduction[1]

Cirrhosis is a diffuse process characterized by fibrosis and the conversion of the normal liver architecture into structurally abnormal nodules. Changes of the vascular relationships leading to portal hypertension are constant, and possibly essential, features (Anthony et al. 1978). The intrahepatic vessels lie among the nodules, most of which are interconnected by parenchymal bridges, and restrict their growth (Takahashi 1978).

Portal hypertension in cirrhosis is related mainly to obstruction of the postsinusoidal vessels by regenerative nodules (Popper 1977). Proliferation of myofibroblasts and deposition of collagen fibers around the terminal hepatic venules are probably the first events causing impaired blood flow in alcoholic cirrhosis (Nakano et al. 1982). Additional factors that may contribute to portal hypertension in cirrhosis are portal tract scarring, splenomegaly (Popper 1977) and arteriovenous anastomoses in septa, which can also damage the hepatocytes through diversion of blood from the sinusoids (Mitra 1966).

The object of this study was the assessment and statistical analysis of three morphometric variables in biopsy specimens with and without cirrhosis, the volume fraction of the sinusoids (Vv), the sinusoidal surface:total volume ratio (Sv) and the sinusoidal length:total volume ratio (Lv). Its aim was to investigate changes in the sinusoidal architecture that might support the role of sinusoidal distortion in portal hypertension induced by cirrhosis.

Materials and methods

Biopsy specimens. The material examined corresponded to 12 wedge biopsy specimens with cirrhosis, and to 11 control specimens with either chronic persistent hepatitis or mild steatosis. The diagnoses were reaffirmed according to the WHO classification of cirrhosis (Anthony et al. 1978) and the criteria of Bianchi (1983). The diagnosis in each case is given in Table 1.

Three to five sections at 5 μm of the formaldehyde-fixed, paraffin-wax-embedded material were stained with hematoxylin-eosin and examined with a Leitz Orthoplan microscope equipped with a rectangular grid measuring 0.045 mm^2 at 250 magnification. At least five micrographs excluding portal tracts and fibrotic areas were taken of each specimen, at 160 magnification, in order to secure an adequate sample for point-counting.

Morphometric analysis. The Vv was assessed by point-counting using a 25-point grid randomly laid on the micrographs. It was expressed as percentage of the total volume. For each specimen, the prospective number of points to be counted inside the sinusoids (Pn) to ensure a relative standard error (RSE) lower than 5% was roughly calculated (Aherne and Dunnill 1982) from a pilot trial involving 100 points as

$$Pn = (1 - Vv)/RSE^2$$

The RSEs were checked afterwards.

The Sv was calculated by intersection count using a graticule of lines, according to the following formula:

$$Sv = 2PL \quad mm^{-1}$$

where PL was the number of intersections of the lines with the vascular boundary per linear millimeter of the specimen. The combined length of the lines of the graticule, measured by comparison with a stage micrometer, corresponded to 1 mm of the specimen at 250 magnification. For each specimen, the number of graticules (n) to be considered to ensure 95% confidence was assessed as follows:

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1 The DeHoff-Rhines-Underwood system of symbols is adopted

Table 1. Histological diagnosis of the specimens with cirrhosis and of the controls

Specimen	Diagnosis
1	Cirrhosis, micronodular
2	Cirrhosis, micronodular
3	Cirrhosis, micronodular
4	Cirrhosis, micronodular
5	Cirrhosis, micronodular
6	Cirrhosis, micronodular
7	Cirrhosis, micronodular
8	Cirrhosis, micronodular
9	Cirrhosis, micronodular
10	Cirrhosis, micronodular
11	Cirrhosis, mixed micronodular and macronodular
12	Cirrhosis, micronodular
13	Chronic persistent hepatitis
14	Chronic persistent hepatitis
15	Chronic persistent hepatitis
16	Chronic persistent hepatitis
17	Chronic persistent hepatitis
18	Chronic persistent hepatitis, resolving
19	Steatosis, focal, mild
20	Chronic persistent hepatitis
21	Steatosis, mild
22	Normal liver tissue
23	Chronic persistent hepatitis

Table 2. Mean volume fraction (Vv), volume:surface ratio (Sv) and length:volume ratio (Lv) in 12 biopsy specimens with cirrhosis and in 11 without cirrhosis

Specimen	Vv (%)	Sv (mm^{-1})	Lv (mm^{-2})
1	21.5	29.2	77
2	33.5	25.8	90
3	12.5	20.8	33
4	17.0	19.8	34
5	17.0	22.2	89
6	20.5	25.4	42
7	20.0	31.8	76
8	16.0	21.8	61
9	7.0	11.0	36
10	11.5	18.4	57
11	23.0	45.8	94
12	43.0	45.6	78
13	18.5	25.8	59
14	16.5	15.8	40
15	11.5	18.6	41
16	13.5	17.8	37
17	21.5	17.4	32
18	22.0	10.3	68
19	19.0	20.9	84
20	15.0	9.8	64
21	12.5	12.3	100
22	24.5	20.4	136
23	13.0	7.4	41

$$n=(s/0.05\,PL)^2 \qquad \text{(a)}$$

where s was the standard deviation of the trial sample of each specimen, and PL the mean number of intersections. Again, significance was checked afterwards.

The Lv was measured in the rectangular grid as

$$Lv=2P/A \quad mm^{-2}$$

where P was the number of profiles of the sinusoids, and A the unit area. The number of rectangular grids to be examined was again assessed with the formula (a).

Finally, the differences in the means of the Vv, Sv and Lv between the two groups of biopsy specimens were compared by the standard errors of the means.

Results

Table 2 shows the Vv, Sv and Lv of each specimen of the cirrhotic and non-cirrhotic groups of biopsies. The means were 20.2%, 26.5 mm^{-1}, and 63.9 mm^{-2} for the cirrhotic group; and 17.0%, 16.0 mm^{-1}, and 63.8 mm^{-2} for the non-cirrhotic group, respectively. The paired means of each variable were compared. Those of the Sv series showed a statistically significant ($0.01>P>0.001$) difference. The Vv and Lv means did not differ significantly in the same groups of specimens.

Discussion

Impairment of sinusoidal flow is a controversial factor of portal hypertension. The microcirculation could be reduced by margination of leukocytes and capillarization of the sinusoids, which may acquire a continuous basement membrane (Popper 1977). A positive correlation has been found (Orrego et al. 1981; Blendis et al. 1982) between the amount of collagen in the Disse space, measured on an arbitrary morphological scale, and intrahepatic pressure. The volume of the Ito cells, which are likely to induce intralobular fibrosis in cirrhosis, increases (Ryoo and Buschmann 1983). Moreover, the surface area of the hepatocytes, measured as cell area divided by the number of nuclei, correlates with intrahepatic pressure (Blendis et al. 1982). In 1984, Krogsgaard and coworkers claimed that distortion of the liver architecture in cirrhosis is a major factor of the increase in portal pressure. They failed to demonstrate, however, any correlation between portal pressure and the following variables: (1) degenerative changes in liver tissue, (2) mean volume of the hepatocytes, calculated as the number of nuclear profiles per unit area, and (3) relative sinusoidal vascular volume, measured by point-counting. Nor did the latter two variables correlate.

Our results showed that the mean surface of the sinusoids was significantly greater in cirrhotic than in non-cirrhotic liver specimens. In contrast, the volume fractions of the sinusoidal lumina and

their mean lengths did not differ significantly in the same groups.

The shape of the sinusoid, as that of any vessel, can be geometrically likened to a cylinder, the curved surface (S) of which is

$$S=2\pi rh \quad \text{(b)}$$

where r is the radius and h the altitude of the cylinder. The sinusoid being a long cylinder, its curved surface roughly equates to its total surface. Our data supported the concept that the r of the sinusoids was greater in cirrhotic than in non-cirrhotic specimens, since the Sv, which is directly related to S, increased significantly, and the concomitant Lv, a function of h, was steady.

Poiseuille's law on the flow of viscous fluids,

$$P[2]-P[1]=\frac{VL}{Kr^4t} \quad \text{(c)}$$

relates the differential pressure ($P[2]-P[1]$) directly to the volume (V) of the fluid and to the length of the cylinder (L), and inversely to the fourth power of the radius (r) and to time (t). The K coefficient depends on the viscosity of the fluid.

K should be similar in cirrhotic and in non-cirrhotic livers. In our experience the formula (c) could not be used to derive any of the variables because more than one of them were unsettled by our data. However, an increase in r for the cylinder, as supported by the (b) for our cirrhotic specimens, without substantial changes in the Lv would militate against the sinusoidal system being responsible for an increase in the differential pressure, i.e. portal hypertension. In addition, other experimental works (Mitra 1966) support the concept that the sinusoidal flow (F; $F=V/t$) is decreased in cirrhosis. Finally, collagen fiber deposition around the sinusoids in cirrhosis could hardly increase the tension (T) in the wall, because collagen fibers resist extension only after notable stretching (Burton 1969). Accordingly, the transmural pressure (TMP), which is expressed by the Lamé approximation of Laplace's law,

$$TMP=T/r$$

should decrease, hence it is unlikely to affect the $P[2]-P[1]$ in cirrhosis.

Ryoo and co-workers (1983) reported an increased Vv of the vessels in the livers of rats with experimental hypertrophic cirrhosis. Although the Vv of the sinusoids is not only related to their radii or lengths but also to the general arrangement of the liver tissue, they inferred that blood flow in the sinusoids does not cause portal hypertension in cirrhosis.

Rappaport and co-workers (1983) studied the pathways of microcirculation in cirrhosis according to the acinar concept, and indirectly confirmed the view that the sinusoids are not responsible for portal hypertension. Through tridimensional reconstruction of the liver architecture, they identified three phases in the changes of the vascular network of the cirrhotic nodule. The "triadal" nodule receives blood from the portal venules, the hepatic arterioles and the perinodal plexus; but it may be segregated from the hepatic veins. The "para-triadal nodule" is an aggregate of nodules derived from adjacent acini; it receives blood mainly from the perinodal plexus. Finally, the "A-triadal nodule" receives blood only from its perinodular plexus, for it is separated from the other nodules by thick bundles of collagen fibers.

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