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The sinusoidal barrier in alcoholic patients without liver fibrosis

A morphometric study

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Summary. Alcohol induces morphological changes in the endothelial and perisinusoidal cells at the fibrotic stage of alcoholic liver diseases. Directly or indirectly, through hemodynamic disturbances linked to the enlargement of steatotic hepatocytes, alcohol may modify this barrier before the onset of fibrosis. Liver biopsies were obtained from control and from alcoholic patients and perfusion-fixed. Volume and surface densities of endothelial cells, perisinusoidal cells and their processes were measured. Liver histology was normal in the 2 groups except for steatosis in the alcoholics. Volume densities represented 8.2%, 4.7% and 3.2% of the sinusoid in controls for endothelial cells, perisinusoidal cells and their processes whereas surface densities represented respectively 0.5, 0.23, 0.21 m^2/cm^3 of sinusoid. Morphometric values were not significantly different in the alcoholic patients. In none of the alcoholic patients did fine morphological studies of sinusoidal cells give any indication of the possible evolution of the alcoholic disease towards fibrosis. These results indicate that in the group of patients studied, alcohol, before the fibrotic stage, did not significantly alter the sinusoidal barrier.

Key words: Alcohol – Human – Sinusoidal cells – Morphometry

Introduction

Exchanges between the blood and hepatocytes are regulated, at least in part, by the sinusoidal barrier (Wisse et al. 1985). This barrier is constituted by the fenestrated endothelial lining and by the processes of perisinusoidal cells (PSC) forming the framework of the sinusoid. Alcohol not only damages hepatocytes (steatosis, necrosis) but also has a direct effect on endothelial cells and PSC. In the baboon, alcohol (a) – decreases the number of pores. In spite of an increase in their size the fractional area occupied by

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Table 1. Clinical and laboratory data

	Age	Sex	Relevant clinical data	Drugs	Bilirubin (17 μmoles/L)	ASAT (40 UI/L)	ALAT (40 UI/L)	AP (80 UI/L)	PT (70%)
Control									
1	46	F	Gallbladder lithiasis	Chenodeoxycholic acid Acetylsalicyclic acid	17	13	12	75	70
2	32	F	Gallbladder lithiasis		8	–	–	–	95
3	65	F	Gallbladder lithiasis	Cimetidine	6	18	13	37	100
4	55	F	Gallbladder lithiasis		6	–	–	47	80
5	70	M	Colon cancer	Dihydroquinidine (chlorydrate) Paracetamol	9	–	–	–	95
6	72	F	Hiatus hernia	Aluminium, magnesium (Hydroxyde)	4	12	19	78	90
7	58	F	Stomach cancer		6	–	–	–	95
Alcoholics									
1	28	M	Alcohol dependance	Meprobamate tiapride	15	309	99	80	80
2	48	M	Alcohol dependance Oesophagitis	Cimetidine, Meprobamate Vitamin B1, B6	12	25	15	40	80
3	36	M	Familial polyposis coli	Metoprolol (tartrate)	14	–	–	–	100
4	59	M	Oesophagitis	Cimetidine, Piribedil	17				100
5	34	F	Alcohol dependance	Meprobamate Vitamin B1, B6	26	290	114	47	100
6	32	M	Chronic pancreatitis	Chlorazepate dipotassic Tiemonium, noramidopyrine	10	21	47	102	100

ASAT = Aspartate aminotransferases; ALAT = Alanine aminotransferases; AP = Alkaline phosphatase; PT = Prothrombin time; ( ) upper or lower limit of normal value; – not measured

fenestrations decreases (Mak and Lieber 1984), (b) – transforms PSC into transitional cells (Mak et al. 1984). These alterations are concomitant with the presence, under light microscopy of steatosis and perivenular and perisinusoidal fibrosis. Whereas steatosis is considered to be reversible, fibrosis is a sign of the potential evolution of the disease towards cirrhosis if the intoxication persists.

The effect of alcohol on sinusoidal cells, when the optical lesion is strictly limited to steatosis, has not been studied. The toxic effect may be either directly or indirectly linked to the hypertrophy of hepatocytes (Miyakawa et al. 1985; Vidins et al. 1985).

In this study, we measured the volume and surface densities of endothelial cells and processes of PSC, in order to assess indirectly whether alcohol impairs exchanges between blood and hepatocytes when the damage is strictly limited to steatosis. Because data in "normal" humans are not available, we also studied control patients.

Patients and methods

After submitting written informed consent, 6 alcoholics for more than 12 months who underwent either abdominal surgery (2 patients) or had a percutaneous liver biopsy (4 patients) were studied. They drank more than 60 g/day of alcohol for men and women. Clinical and laboratory data on liver functions are shown in Table 1. Wedge or needle liver biopsies were immersed in phosphate buffer (0.1 M, 4° C, 345 mosmoles). Part of the biopsy was fixed for routine liver histology (Blouin) and stained (haematoxylin eosin, Sirius red, Masson's trichrome). The hepatic vein thickness and the area % of the perisinusoidal collagen network

Table 2. Methods: quantitative analysis

I. 3 μm section (paraffin section – Sirius red staining)
– automatic image analyzer (IBAS I–II, Zeiss – FRG).
a) Hepatic vein thickness (μm)
– small veins (far away from the capsule for wedge liver biopsy
– objective: either 16,40 or 63 according to their size
– number of measurements = 6.4 (2–24) mean and extremes
b) Area % of perisinusoidal collagen network (% of hepatic parenchyma)
– field = 100 μm around the periphery of the hepatic vein
– number of measurements = 7.5 (3–17) mean and extremes
II. Ultrathin sections – 3 to 4 blocks (from zones 1 and 3) per patient
– Photographs at random over sinusoids representing more than 50% of the hepatic parenchyma – initial ×4,500 – final ×12,500 – 5 photographs per block
a) Volume density (% of sinusoid)
– endothelial cells
– perisinusoidal cells (cell body and processes)
lattice: test system with square unit (200 points)
b) Surface density (m^2/cm^3 of sinusoid)
– endothelial cells
– perisinusoidal cells (cell body and processes)
lattice: multipurpose test system (50 lines)

Table 3. Hepatic vein thickness and area of perisinusoidal collagen network

	Hepatic vein thickness (μm)	Area of perisinusoidal collagen (% of hepatic parenchyma)
Controls (7)	7.4±2.8	7.1±1.2
Alcoholics (6)	9.6±4.5	8.4±2.6

(zone 3) were determined with an automatic analyzer in sections stained with Sirius red (Junquiera et al. 1979) as shown in Table 2. The other part of the biopsy was perfusion-fixed with 1.5% glutaraldehyde (Bioulac-Sage et al. 1986). Small blocks were immersed in the same fixative for 2 h, post-fixed in 1% osmium tetroxide and processed for electron microscopy. Well perfusion-fixed areas (clean sinusoids with easy identification of sinusoidal cells) and without artifacts (dilatation of the Disse space, lateral spaces and bile canaliculi related to hyperpressure) were chosen around small portal veins (<40 nm) and small hepatic veins (<40 nm) on 1 μm sections stained with toluidine blue. Ultrathin sections were double-contrasted with uranyl acetate and lead citrate. Grids were observed in a Philips EM 301 (Centre of electron microscopy, Université de Bordeaux II). Morphometry was performed according to Weibel's method; technical details are presented in Table 2. 7 Patients without case histories of excessive alcohol intake (<20 g/day for women and <60 g/day for men) were used as controls (Table 1). Results are expressed as the mean ±1 SD. Comparisons were evaluated by student's *t*-test.

Results

Liver histology was interpreted as normal in control patients and in alcoholic patients except for steatosis. In this group steatosis was graded (0 to 3+) 1+:1 case, 2+:4 cases and 3+:1 case; there was no fibrosis, inflammation or necrosis. Hepatic vein thickness and area percents of perisinusoidal collagen network were not significantly different in the 2 groups (Table 3).

In control patients, biopsies were well perfusion-fixed; on 1 μm sections, sinusoids were clean and sinusoidal cells well visible. In alcoholic patients, during the perfusion, lipid droplets were seen in the effluent and in general the quality of the perfusion was more variable. It was not always possible, in areas with hepatocytes loaded with lipids, to recognize easily PSC on 1 μm section even in well fixed zones.

Under electron microscopy the sinusoidal barrier was not qualitatively different in the two groups (Figs. 1, 2). It was mainly constituted by the thin fenestrated endothelium and the processes of PSC. Endothelial cells overlap over a small area but without visible organized junctions. In rare cases a small or large portion of a Kupffer cell intercalated in the endothelial wall forming the barrier. Occasionally filopodes of Kupffer cells were in contact with the Disse space through the endothelial pores. Processes of PSC were easily identified and differentiated from the endothelial lining, filopodes of Kupffer cells and hepatocyte microvilli: these processes were discontinuous, they contained numerous pinocytic vesicles, parallel microfilaments and occasionally lipid droplets. In general they ran parallel to the endothelial wall and were thicker near the cell body and thinner far away.

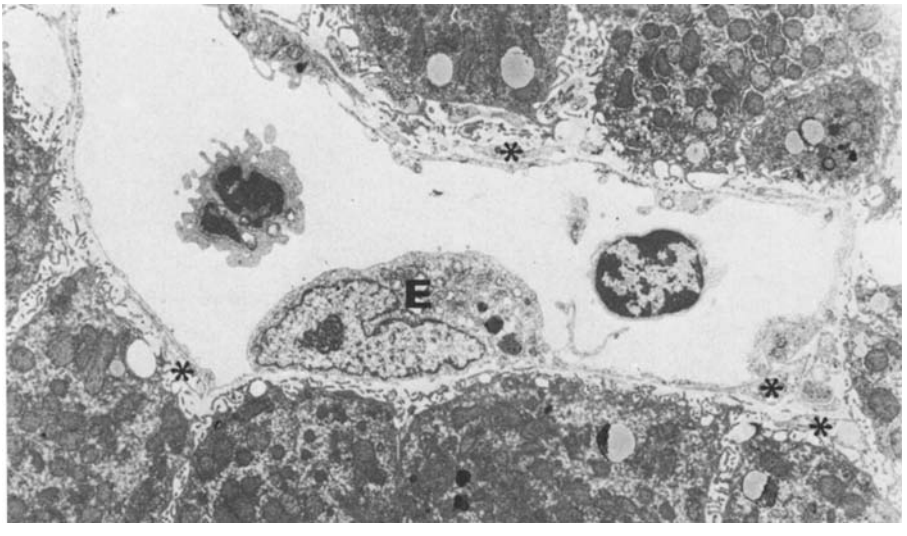

Fig. 1. Control patient, surgical liver biopsy. The sinusoidal barrier is well visible: the endothelial lining (*E*) and some processes (*asterisk*) of perisinusoidal cells in the Disse space. × 3,250

Occasional fragments of more or less clearly defined basement membrane-like material were found between the endothelial wall and the PSC. Often short digitations sprouted from processes, and sometimes, near the cell body, long digitations formed a second process layer occasionally covering the cell body in part or totally. The PSC body was often encased in hepatocyte recesses and therefore played a very small part in the constitution of the barrier. Bundles of collagen were often in close association but not always with PSC. In the Disse space, numerous cut sections of hepatocyte microvilli, and fibrillar or granular material were observed; pit cells and Schwann cells surrounding unmyelinated axons were encountered exceptionally.

Morphometric results are presented in Fig. 3. In control patients, the volume density (V_v) of the Disse space, endothelial cells and PSC processes represented respectively $20.4 \pm 3.3\%$, $8.2 \pm 1.2\%$ and $3.2 \pm 0.7\%$ of sinusoids. Processes accounted for 7/10 of PSC V_v. Surface densities (S_v) were 0.5 ± 0.1 m^2/cm^3 and 0.21 ± 0.04 m^2/cm^3 for endothelial cells and processes respectively; processes accounted for 9/10 of PSC S_v. Expression of the results per zone indicate that V_v and S_v of endothelial and PS cells were higher in the periportal zone than in the centrolobular zone, and that none value but one (S_v of PSC processes) was significantly different (Table 4). In alcoholic patients morphometric results were not significantly different (Fig. 3, Table 4).

Discussion

Exchanges between blood and hepatocytes can be assessed in many ways of which morphological techniques are one. So far they have not been

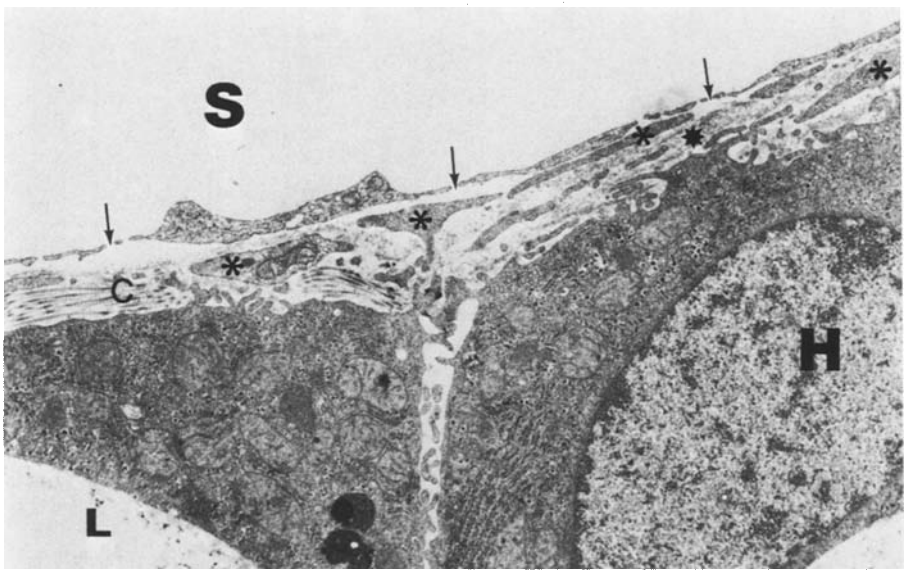

Fig. 2. Alcoholic patient, surgical liver biopsy. The sinusoidal barrier is constituted by the fenestrated (*arrows*) endothelium and the processes (asterisk) of a perisinusoidal cell neighbouring with some collagen fibers (*C*) and fibrillar material (*star*) in the Disse space. *S*, sinusoidal lumen; *H*, hepatocytes containing lipids (*L*). ×7,650

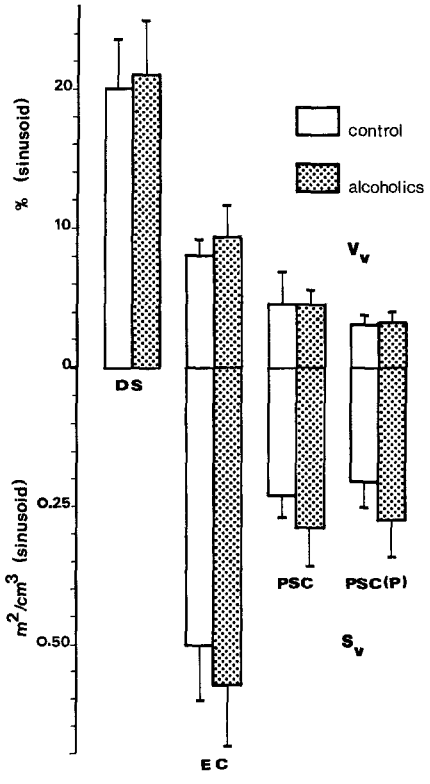

Fig. 3. Morphometric results in control and alcoholic patients. *DS*, Disse space; *EC*, endothelial cells. *PSC*, perisinusoidal cells (cell body plus processes); (*P*) processes of PSC; V_v, volume density; S_v, surface density

Table 4. Morphometric results in control and alcoholic patients – Zonal differences

	Periportal zone		Centrolobular zone	
	E	PSC	E	PSC
Control				
Volume density (% of sinusoid)	9.0 ±1.1	4.9 ±2.3 (3.5 ±0.7)	7.3 ±2.4	3.6 ±1.2 (2.9 ±1.0)
Surface density (m^2/cm^3 of sinusoid)	0.56±0.13	0.26±0.04 (0.24±0.03)[a]	0.45±0.12	0.19±0.06 (0.18±0.05)
Alcoholics				
Volume density (% of sinusoid)	10.1 ±3.3	4.8 ±0.9 (3.4 ±0.7)	9.7 ±2.2	4.2 ±1.1 3.1 ±1.1
Surface density (m^2/cm^3 of sinusoid)	0.60±0.17	0.30±0.10 (0.29±0.10)	0.51±0.10	0.24±0.07 (0.22±0.07)

[a] Significantly different form the opposite zone ($P<0.05$) ( ) values for PSC processes

used in humans on a quantitative basis because identification of sinusoidal cells was poor on biopsies fixed by immersion. Although perfusion-fixed biopsies represent suitable material some problems remain linked, (a) – to the size of the sample, (b) – to the difficulty of obtaining a homogeneous distribution of flow through the sinusoids (as is the rule when the liver is perfused through the vein) and therefore of controlling the perfusion pressure in the sinusoids. For these reasons morphometric results were expressed per sinusoid and not per parenchymal tissue.

In this study we checked to see whether alcohol ingestion directly, or indirectly (Mak and Lieber 1984) through hemodynamic disturbances (Fraser et al. 1980) related in part to hepatocyte enlargement (Vidins et al. 1985, Miyakawa et al. 1985), could modify the sinusoidal barrier and therefore exchanges. Impaired exchanges might represent one obligatory step in the chain of events leading to fibrosis. All patients were chronic alcoholics. The absolute criterion for inclusion in the work was not the amount of alcohol consumption, the clinical manifestations nor the values of liver function tests but the normality, (except for steatosis) of the liver histology, in particular with regard to perivenular and perisinusoidal fibrosis.

In the control group, data were similar to those obtained in the rat by Blouin (Blouin et al. 1977). This confirms that endothelial cells represent a large surface density for a small volume density. In addition this study has clearly shown that processes of PSC represent 7/10 and 9/10 of the volume and surface densities of PSC respectively and approximately half the value of the surface densities of endothelial cells. These results underline the important role played by processes in the constitution of the sinusoidal barrier. V_v and S_v of endothelial and PS cells were higher in periportal zone than in centrolobular zone. These results support the hypothesis that the barrier is more porous in zone 3 than in zone 1 as shown in the rat for the endothelial cells (Wisse et al. 1985).

In this study we did not find differences in the V_v and S_v of endothelial and PS cells between alcoholics and unmatched controls suggesting that at this stage of the disease the primary target of alcohol is represented by hepatocytes and not sinusoidal cells.

Amongst alcoholics, only 10 to 20% will develop cirrhosis. It could be possible that in this group, selected from patients without fibrosis, none will develop cirrhosis. It could also be that individual qualitative or quantitative changes, as yet unknown, may be indicative of the future progression of the disease towards fibrosis and from thence towards cirrhosis. These may have been missed through statistical analysis. We looked therefore at individual morphometric values and at cell morphology with a particular attention to perisinusoidal cells. There was no evidence in any of the patients of such changes. In PSC the amount of lipids was not decreased, and was in fact increased in half the cases. The RER was apparently not increased and microfilaments in the processes were not more numerous. We need to follow up patients who have undergone repeated liver biopsies as well as taking a larger group of patients in order to have a better understanding of the onset of fibrosis. In addition, other techniques such as scanning electron microscopy should be used. This approach is more suitable for assessing the number and size of endothelial pores (Wisse et al. 1985) although difficult technical problems often arise linked to the perfusion pressure (Miyakawa et al. 1985, Fraser et al. 1980) or to the preparative procedures (Wisse et al. 1985).

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