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Role of sinusoidal endothelial cells in liver disease

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Abstract We characterized the structural changes of sinusoidal endothelial cells in chronic ethanol-fed rats and rats with cirrhosis induced by thioacetamide. The phenotypic changes of sinusoidal endothelial cells in fibrotic rats induced by thioacetamide and the reversibility of these changes were also investigated under transmission and scanning electron microscopy, regular microscopy and by immunohistochemistry with laminin and von Willebrand factor antibodies. The diameter and porosity of sinusoidal endothelial fenestrations were increased in chronic ethanol-fed rats without liver fibrosis, however, they decreased within 4 weeks of the cessation of thioacetamide treatment. A basement membrane-like structure in Disse's space was noted 6 weeks after thioacetamide treatment. Laminin was detected in Disse's space after 4 weeks and von Willebrand factor was detected in the cytoplasm as granular fluorescence after 6 weeks of thioacetamide treatment. Reversibility of the phenotypic changes of the sinusoidal endothelial cells was demonstrated in fibrotic liver of rats that received thioacetamide for 6 weeks after long-term discontinuation of thioacetamide administration. These results indicate that the structural and immunohistochemical characteristics of sinusoidal endothelial cells change in chronic ethanol-fed rats and fibrotic rats and these changes are reversible.

Key words: sinusoidal capillarization, sinusoidal endothelial cell, sinusoidal endothelial fenestration.

INTRODUCTION

Hepatic sinusoidal endothelial cells have no basement membranes but have many sinusoidal endothelial fenestrations (SEF) in their cytoplasm. These fenestrated cells play an important role in the transport of many substances from sinusoids to hepatocytes.[1] Fenestrations vary their porosity in various conditions. To determine the structural and phenotypical changes of sinusoidal endothelial cells in the capillarization of sinusoids, we studied sinusoidal endothelial cells *in vivo* and *in vitro*, in chronic ethanol-fed rats and fibrotic rats using thioacetamide (TAA).

METHODS

Male Wistar rats were fed Lieber's liquid diet for 6 weeks or they received an intraperitoneal injection of TAA for 2, 4, 6 or 12 weeks. The rats that received TAA for 6 weeks or 12 weeks were fed a standard laboratory diet for 6 to 12 months. Their tissues were used for light microscopy, electron microscopy, scanning electron microscopy and immunohistochemical study using laminin antibody and von Willebrand factor antibody. Morphometrical analysis was undertaken for the evaluation of sinusoidal endothelial fenestrae.

RESULTS

Lieber's liquid diet-fed rats showed fatty liver and ballooned hepatocytes predominantly in the pericentral area (zone 3). After 6 weeks of TAA treatment, collagen fibres and disarrangement of hepatic lobules were observed on light microscopy. TAA administration for 12 weeks produced micronodular cirrhosis (Fig. 1). In chronic ethanol-fed rats, the diameter and porosity of SEF were significantly increased compared with the controls as demonstrated by scanning electron microscopy. These changes were more prominent in zone 3. After 4 weeks of TAA treatment, the mean diameter of SEF decreased slightly and, after 6 weeks of TAA treatment, the mean diameter decreased significantly ($P < 0.001$) from 108.0 ± 3.8 nm to 98.7 ± 3.8 nm in zone 1 and 104.0 ± 3.6 nm to 89.9 ± 7.6 nm ($P < 0.001$) in zone 3 (Table 1). Sieve plates were rarely seen at this time. Twelve weeks later, a few SEF could be seen, and their intrasinusoidal surfaces were irregular. The porosity of the endothelial surface was also significantly ($P < 0.001$) decreased after 4 weeks of TAA treatment. Isolated sinusoidal endothelial cells from control rats spread, forming a monolayer in culture 6 h after seeding. Many SEF and sieve plates were noted in the cytoplasm, however, few SEF or cytoplasmic protrusions such as bullae were seen in the isolated endothelial cells isolated from rats treated with TAA for 12 weeks. TAA administration for 6 weeks

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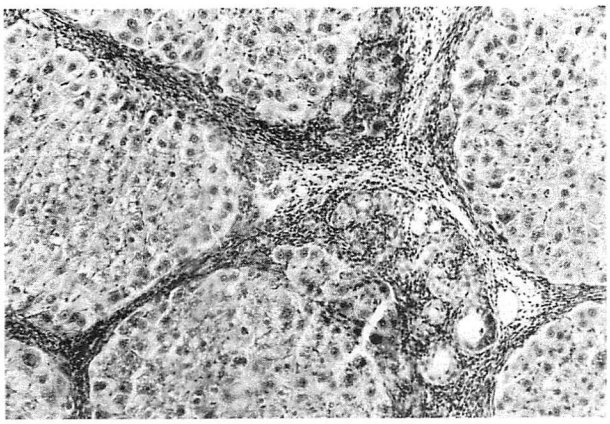

Figure 1 Light micrograph of rat liver treated for 12 weeks with thioacetamide. Fibrous septa and inflammatory cell infiltration of the portal tract are seen; (haematoxylin and eosin staining).

Table 1 Morphometric analysis of the diameter of SEF in TAA-treated rats

	Diameter (nm)	
	Zone 1	Zone 3
Control ($n=6$)	108.0 ± 3.8	104.0 ± 3.6
TAA treatment		
2 weeks ($n=5$)	110.2 ± 1.4	99.6 ± 1.5
4 weeks ($n=5$)	102.3 ± 5.6*	91.1 ± 5.1**
6 weeks ($n=5$)	98.7 ± 3.8**	89.9 ± 7.6**

Values are expressed as mean ± s.d. * $P<0.05$, ** $P<0.001$.

resulted in the formation of a discontinuous basement membrane-like structure in Disse's space. Laminin was not observed in the hepatic sinusoids in rats treated for 2 weeks with TAA, however, it was detected in the space of Disse in rats treated with TAA for 4 weeks. Isolated endothelial cells obtained from rats treated for 2 to 4 weeks with TAA were negative for von Willebrand factor on indirect immunofluorescence study. However, isolated endothelial cells from rats treated for 6 weeks with TAA were positive for von Willebrand factor (Fig. 2).

For the study of the reversibility of phenotypic changes of sinusoidal endothelial cells in TAA-treated rats, we used the rats that received TAA for 6 weeks and 12 weeks. These rats were fed standard diet for 6 months and 12 months. The rats that received TAA for 6 weeks followed by discontinuation of TAA for 6 months showed no basement membrane formation in Disse's space and 6 months later the ultrastructure of SEF was almost the same as the controls (Fig. 3). At this time, von Willebrand factor was not stained in the cytoplasm of sinusoidal endothelial cells. However, the reversibility of phenotypic changes of sinusoidal endothelial cells in the cirrhotic rats was not demonstrated after 12 months discontinuation of TAA. At this time, defenestration and von Willebrand factor were noted in the sinusoidal endothelial cells, however, liver cirrhosis was not seen at this time.

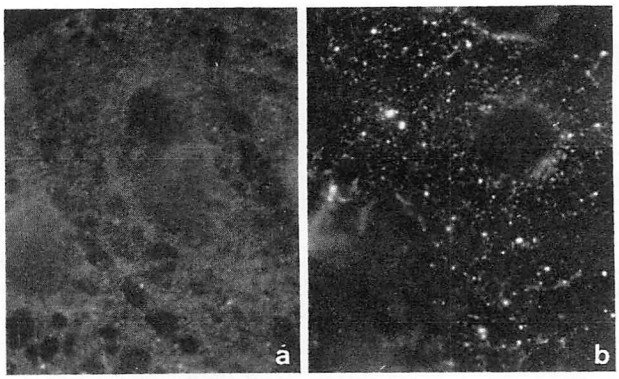

Figure 2 Immunofluorescence staining reveals the distribution of von Willebrand factor in cultured endothelial cells. After (a) 4 and (b) 6 weeks of thioacetamide treatment, von Willebrand factor was seen as granular fluorescence in the perinuclear and in the cytoplasm of cultured endothelial cells isolated from rats treated for 6 weeks (original magnification × 1000).

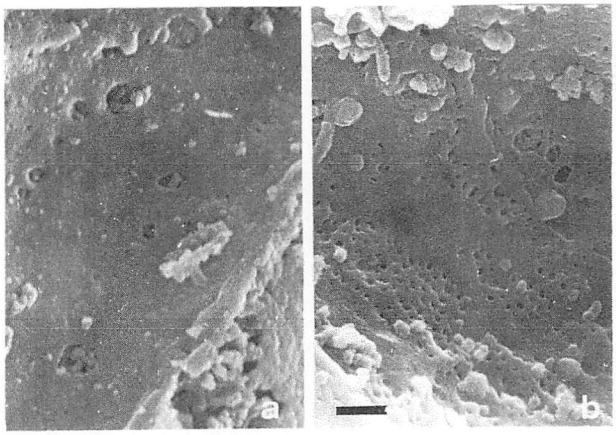

Figure 3 Scanning electron micrograph of endothelial cells cultured on type I collagen for 6 h after isolation. (a) Thioacetamide (TAA) for 6 weeks; (b) TAA for 6 weeks and discontinuation of TAA for 6 months. Sieve plates are not visible in the rat that received TAA for 6 weeks (a), however, sieve plates are seen after 6 months discontinuation (b). Bar, 1 μm.

DISCUSSION

We demonstrated a significant increase in the diameter and the porosity of SEF in chronic ethanol-fed rats without liver fibrosis. These changes were more prominent at the sinusoidal endothelial cells in zone 3, resulting in the compensation of hypoxia of hepatocytes in zone 3 in ethanol metabolism.[2] Hypoxia in zone 3 has been considered to be one of the mechanisms of hepatocyte damage in alcoholic liver disease, however,

hypoxia in zone 3 might be compensated in part by the increased porosity of SEF and increased portal blood flow in an early stage of alcoholic liver disease.[3]

In an advanced stage of alcoholic liver disease and chronic hepatitis, defenestration of SEF associated with basement membrane formation in Disse's space was noted. This is called sinusoidal capillarization. Sinusoidal capillarization results in the disturbance of the transport of many substances from sinusoids to hepatocytes. It is important to clarify whether the phenotypic changes[3] of sinusoidal endothelial cells seen in the sinusoidal capillarization are reversible. Our study using the TAA-induced fibrotic rat model demonstrated the reversibility of sinusoidal capillarization associated with phenotypic changes of sinusoidal endothelial cells. However, phenotypic changes of sinusoidal endothelial cells in cirrhotic rats were not demonstrated 12 months after discontinuation of TAA administration. Long-term follow-up study is needed to clarify the reversibility of phenotyic changes of sinusoidal endothelial cells in cirrhosis.

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