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Light microscopical demonstration and zonal distribution of parasinusoidal cells (Ito cells) in normal human liver

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Summary. The parasinusoidal cells of the liver (Ito cells) were demonstrated light microscopically in autopsy specimens fixed in formalin and stained with Oil red O after dichromate treatment. The method allows examination of large samples containing numerous acini.

Quantitative assessment showed a zonal gradient with 6.3 and 7.7 parasinusoidal cells per 62.5×10^3 μm^2 in zone 1 and 3, respectively.

Key words: Dichromate fixation – Liver – Ito cells – Oil red O staining – Parasinusoidal cells

Introduction

Our understanding of the function of the hepatic parasinusoidal cells (PS) (synonyms: Ito cells, fat-storing cells, lipocytes, perisinusoidal cells – see Wake 1980 and Atermann 1986 for historical review) has seen considerable advances in recent decades. Although the functions of these cells are still a matter of debate, several have been suggested. PS cells contain and store vitamin A (Wake 1980), they are responsible for parenchymal collagen production (McGee and Patrick 1972; Minato et al. 1983; Mak et al. 1984; De Leeuw et al. 1984; Horn et al. 1986b) and may have a regulatory function on the hepatic microcirculation (Horn et al. 1986a).

The cytoplasm of the PS cells contains numerous lipid droplets which are poorly demonstrable after formalin fixation and conventional paraffin embedding. Various other techniques have been used for their light microscopical identification. Vitamin A fluorescence and gold or silver reduction (Wake 1980) allow the observation of PS cells on frozen sections. A better preservation is obtained by osmium tetroxide fixation and embedding in Epon (Bronfenmajer et al. 1966; Okanoue et al. 1982; Giampieri et al. 1981; Horn et al. 1986a, b), however, this method allows the examination of small tissue samples only.

We have recently demonstrated PS cells in specimens stained with Oil red O after post-fixation in dichromate (Horn et al. 1986a). The aim of the present study was to examine the occurrence and topographical distribution of PS cells in normal adult livers using large autopsy specimens and Oil red staining after dichromate treatment.

Materials and methods

Autopsy liver tissue specimens from 12 adults (7 females, median age 79, range 46–92; 5 males, median age 78, range 48–79) were examined. They were selected from patients without a previous history of liver disease or daily alcohol consumption. The livers were all normal on gross inspection, and conventionally fixed and stained specimens showed normal morphology except for acute congestion. Autolysis was minimal.

For Oil red O staining liver specimens measuring about $15 \times 15 \times 1$ mm were fixed in 4% neutral formalin pH 7.4 for 8 to 24 h at 4° C. After washing in phosphate buffered saline (pH 7.4) specimens were treated with 2.5% $K_2Cr_2O_7$, buffered to pH 3.5 for 18 h (overnight) at 56° C, washed in running water for 6 h, dehydrated and embedded in paraffin. Five μm thick sections were deparaffinized to 70% ethanol and stained with Oil red O for 15 min followed by hematein for 2–5 min for background staining.

The number of parasinusoidal cells was counted by two of the authors (Horn and Junge) by consensus. When individual counting was compared (in 5 specimens), an interobserver variation of less than 5% was noted. Counting was performed under a magnification of ×400 using an ocular with a square grid representing an area of 62.5×10^3 μm^2 in the biopsy. All PS cells within the square and in contact with the upper and right limits were counted. For each specimen 3 to 6 areas next to portal tracts (zone 1), and 2 to 4 areas next to terminal hepatic veins (zone 3) were analyzed. Counting was done in unselected and different areas and from the border of portal tracts

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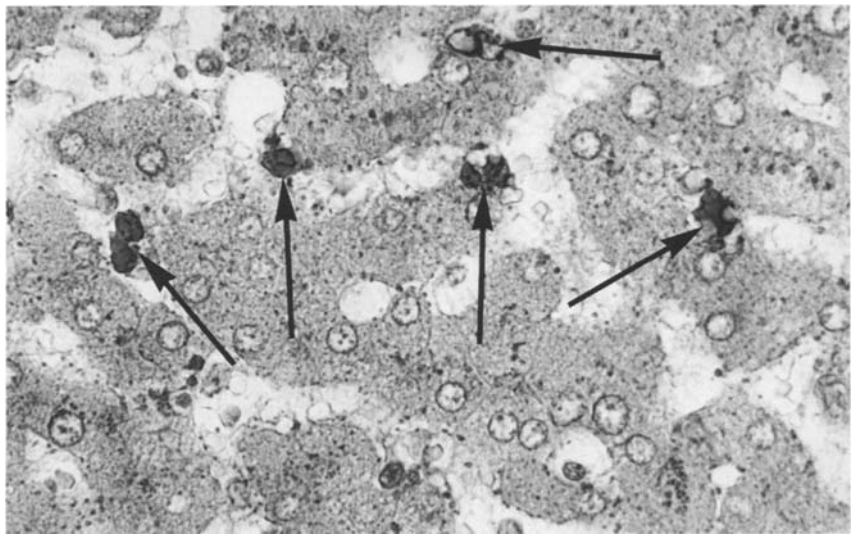

Fig. 1. Adult liver stained with Oil red O after dichromate treatment showing accumulation of large lipid droplets in parasinusoidal cells (*arrows*). Original magnification ×400

and terminal hepatic veins, respectively, to a distance of up to 250 μm from these structures.

PS cells were defined as cells localized in the perisinusoidal space and characterized by an irregular nucleus surrounded by a conglomerate of bright red fat vacuoles. Small isolated fat vacuoles without any relationship to a nucleus were not registered.

Data are expressed as median values with a range. A Mann-Whitney rank sum test was used to compare values in single specimens and between specimens. $P<0.05$ was considered significant.

Results

In the Oil red O stained sections orientation was easy and portal tracts and terminal hepatic veins were readily identified. Liver cells appeared with a weakly stained cytoplasm containing varying amounts of brownish red lipofuscin.

Whereas occasional lipid droplets in liver cells were unstained they appeared bright red in the PS cells (Fig. 1). The lipid occurred as clusters of smaller droplets or as a single droplet measuring up to 8 μm in diameter.

Counting of PS cells was done in 31 to 57 areas and in 20 to 55 areas in zone 1 and 3, respectively, in each specimen and each area represented 62.5×10^3 μm^2. A total area of 32.83×10^6 μm^2 ($= 525 \times 62.5 \times 10^3$ μm^2) and 28.82×10^6 μm^2 was analyzed in zone 1 and 3, respectively. The numbers of PS cells in zone 1 and 3 are presented in Table 1.

Except for two cases (case number 8, a 78-year-old male with a carcinoma of the prostate and case number 10, a 48-year-old male with an astrocytoma grade IV) a significantly higher number of PS cells was found in zone 3 when compared with zone 1 ($P<0.05$). For all cases the median values were 6.3 and 7.7 in zone 1 and 3, respectively ($P<0.05$). The number varied greatly, not only from case to case but also within single specimens and within a single field of vision; the cells were not evenly and/or symmetrically distributed in these areas. Despite this variation, it was a general impression that the number of PS cells gradually increased towards the terminal hepatic vein.

Table 1. Number of parasinusoidal cells per 62.5×10^3 μm^2 in zone 1 and 3 areas in 12 adults

Biopsy number	Zone 1		Zone 3		Difference between zone 1 and 3
	median	range	median	range	
1	5	1–11	6	1–16	*
2	7	1–19	12	2–19	*
3	6	2–13	9	5–15	*
4	4	1–9	7.5	2–14	*
5	6	2–12	7	3–13	*
6	7	2–14	10.5	4–17	*
7	8	2–14	12	6–18	*
8	7	3–15	5.5	3–12	ns
9	6.5	2–12	8	3–16	*
10	8	5–12	8	5–17	ns
11	4	2–9	6	2–12	*
12	5	1–11	6	3–12	*
All 12 biopsies	6.3	1–19	7.7	1–19	*

* $P<0.05$
ns = non significant

Discussion

PS cells are recognized light microscopically by their content of lipid droplets (Wake 1980; Horn

et al. 1986a, b). In normal tissue sections the majority of non membrane-bound lipid is dissolved during dehydration and mounting, and therefore identification of PS cells in conventionally treated liver tissue is difficult or impossible.

Fixation prior to dehydration with osmium tetroxide or chromic acid will, however, render the lipids insoluble and prevents outwashing during following procedures. Dichromate, which is also included in the gold reactions described by Wake (1980, 1986), is, however, preferable for the demonstration of PS cells since large specimens can be examined. Whereas gold and silver reactions are complicated and results often unpredictable (Lillie and Fullmer 1976) staining with Oil red O is simple and consistent and differences in colours, with lipofuscin appearing brownish red and PS cells bright red, makes identification of the PS cells easy. As for the initial fixation of specimens for Oil red O staining, Baker's formolcalcium (Horn et al. 1986a) or, as shown in the present investigation, a neutral formalin solution can be used; no differences in stainability of PC cells are seen when comparing the two fixations.

Whereas Wake (1980) using the gold reaction has shown the PS cells to be evenly distributed throughout the normal liver, Giampieri et al. (1981) demonstrated a zonal gradient with a higher number of PS cells in centrilobular areas. The results of Giampieri et al. (1981) were based on an osmium tetroxide fixation method; however, due to the slow penetration rate of osmium tetroxide only small parts of liver biopsy specimens can be processed. By the method used in the present study it is possible to process large paraffin sections containing numerous acini. The existence of a zonal gradient as described by Giampieri (1981) was confirmed, however, a wide variation was observed in both zones (Table 1).

The physiological significance of a zonal difference in the normal liver is uncertain. The PS cells possess cytoplasmic extensions encircling the sinusoids (Horn et al. 1986a) and they contain contractile elements such as tubulin and actin (De Leeuw et al. 1982) and desmin (Yokoi et al. 1984) and may therefore have a regulatory function on the hepatic microcirculation (Horn et al. 1986a). Hypothetically, the considerable number of cells around the terminal hepatic veins could function as a post-sinusoidal sphincter.

We observed two cases (number 8 and 10) without significant more PS cells in zone 3 as compared to zone 1. The reason for the reduction in number of cells in zone 3 in these two cases (Table 1) is uncertain but could be attributed to an unknown alcohol abuse. A reduction of PS cells in zone 3 areas in alcoholics has been demonstrated ultrastructurally even in biopsies showing normal morphology by light microscopy (Horn et al. 1986b).

In summary, this paper describes an easy light microscopical method for identification of normal PS cells in large formalin fixed liver tissue specimens. The PS cells are unevenly distributed in the liver but with a numerical dominance in zone 3 areas.

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