Electron Microscopy of Fat-Storing Cells in Liver Diseases with Special Reference to Cilia and Cytoplasmic Cholesterol Crystals

Kazuo Tobe, Takahiro Tsuchiya, Tatsuya Itoshima, Hideo Nagashima¹ and Toshinari Kobayashi²

The First Department of Internal Medicine (Prof. H. Nagashima)¹, Okayama University Medical School and Department of Internal Medicine,² Kawasaki Hospital, Kawasaki Medical College, Okayama, Japan

Received June 19, 1985

Summary. Two hundred and ten fat-storing cells in the liver of patients with liver diseases were examined electron microscopically, and 5 of them were found to project a single cilium from the perinuclear region to the space of Disse. Three cilia were in the space between a hepatocyte and their own cell body, and 2 were between a sinusoidal endothelial cell and their own cell body. In cross sections of the cilium, the axoneme consisted of 9 pairs of microtubules arranged in a cylinder and lacked a central pair of microtubules ("9 + 0" pattern). Arms and spokes were also absent in the axoneme. Therefore, the single cilium of fat-storing cells was considered to be immotile. Some fat-storing cells from a patient with alcoholic liver cirrhosis contained an angular electron lucid inclusion, which was identical to cholesterol ester crystals, suggesting the presence of disturbed cholesterol metabolism.

The single cilium of fat-storing cells in the human liver was first reported by Ito and Shibasaki (1968) and later by many authors in other vertebrates (Wake, 1971; Yamamoto and Enzan, 1975; Tanuma and Ito, 1978; Tanuma et al., 1981; Ohata et al., 1982a, 1984; De Leeuw et al., 1984). Its axonemal structure, however, has not been demonstrated because it is rarely encountered under the electron microscope. In this paper, we report the ultrastructure of the cilium and unexpected cytoplasmic cholesterol crystals in the fat-storing cells of human patients.

MATERIALS AND METHODS

Needle liver biopsy specimens were taken under a laparoscope from five patients with liver cirrhosis, including one with alcoholic liver cirrhosis, and two patients each with idiopathic portal hypertension and the Budd-Chiari syndrome. A part of each liver specimen was sliced into small pieces and fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After postfixation in 1% osmic acid in 0.05 M phosphate buffer, the tissues were dehydrated through a graded ethanol series, embedded in Epon 812, and sectioned with a Porter-Blum MT2–B ultramicrotome. The ultrathin sections were
stained with uranyl acetate and lead citrate for observation under a Hitachi H-300 electron microscope. The rest of the biopsy specimens were used for routine paraffin sections for histological examination under a light microscope.

RESULTS

Two hundred and ten fat-storing cells with a nucleus were observed in the ultrathin

![Fig. 1. A. An empty fat-storing cell (F) located in the space of Disse (D) contains a cross-sectioned cilium (arrow). H hepatocyte, S sinusoid. \( \times 10,000 \). B. The cilium is located close to the Golgi apparatus (G) and surrounded by a tunnel (T), a space formed by invagination of the cytoplasmic membrane. \( \times 29,000 \). C. The axoneme of the cilium is composed of 9 pairs of microtubules and lacks a central pair of microtubules, arms and spokes. \( \times 105,000 \) ]
sections (Table 1), and five of these cells were found to project a single cilium from the perinuclear cytoplasm into the space of Disse. Three of the five cilia stretched into Disse's space between a hepatocyte and their own cell body, and two in the space between a sinusoidal endothelial cell and their own cell body.

In cross sections of the cilium (Fig. 1), nine pairs of microtubules were arranged in a cylinder and surrounded with a unit membrane. The diameter of the cilium was 0.25 $\mu$m. No central pair of microtubules, arms or spokes were observed.

Longitudinal sections (Fig. 2) demonstrated that the cilia extended from a basal body about 0.25 $\mu$m in diameter. On the outer surface of the osmiophilic wall of the

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**Fig. 2.** A. Two fat storing cells (F) with fat droplets. One of them projects a single cilium (arrow) into the space of Disse. H hepatocyte, R red blood cell in the sinusoid. $\times 4,500$. B. Closer view of the cilium. Triangular small projections (arrowheads) are seen on the wall of the basal body. D the space of Disse, E rough endoplasmic reticulum, G Golgi apparatus. $\times 25,000$
basal body, similar osmiophilic projections were observed. In a cross section of a probable basal body (Fig. 3), nine triplets of microtubules were arranged in a cylinder about 0.18 μm in diameter and were surrounded by an osmiophilic matrix. From the outer surface of the triplets, nine osmiophilic linear thorn-like structures about 0.13 μm in length projected into the circumferential cytoplasm with a regular angle and a constant interval and showed the feature of a pinwheel.

Centrioles were more frequently observed in the perinuclear area close to the Golgi apparatus, as shown in Table 1. The fat-storing cells in each patient tended to have well-developed rough endoplasmic reticula and showed variety in the number of fat
Cilia and Cholesterol Crystals in FSC

The ultrastructure of the cells was not obviously different among the three diseases, although many collagen fibers were present in Disse's space in the tissue from the patients with the Budd-Chiari syndrome.

Some fat-storing cells in the patient with alcoholic liver cirrhosis had an angular electron lucid inclusion surrounded by an electron dense margin (Fig. 4). Similar inclusions were rarely observed in hepatocytes and Kupffer cells of the same patient.

**DISCUSSION**

This study clarified that fat-storing cells in the human liver have a single cilium with a “9+0” microtubule pattern. Cilia with a “9+2” microtubule pattern, as in the case of bronchial epithelial cell cilia, have been referred to as being motile. The central two microtubules in the axoneme have been considered to be essential for motility and for determining the direction of the movement of the cilium. In contrast to the “9+2” cilia, the “9+0” cilia in which the central pair of microtubules is absent, as is the case

**Table 1.** Total number of fat-storing cells (FSCs), the single cilium and the centriole observed in the present study

|         | FSC | Cilium | Centriole |
|---------|-----|--------|-----------|
| 210     | 5   | 33     |
| (Frequency) | (2.4%) | (15.7%) |

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with the cilia of retinal rod cells, have been referred to as being immotile. Although some "9+0" cilia are observed to move, their movements are sluggish compared with the "9+2" cilia (GHADIALY, 1982). Arms consisting of ATP-splitting enzymes and spokes are also necessary for the movement of cilia (GIBBONS and ROWE, 1965). General characteristics of cilia have been reviewed in detail (FAWCETT, 1981; GHADIALY, 1982). The present electron microscopic study revealed the absence of the central microtubules, arms and spokes in the cilia of fat-storing cells. Therefore, the cilia of these cells are probably immotile, and likely play a role as a sensory or chemical receptor as such cilia are assumed to do in other types of cells (CURRIE and WHEATLEY, 1966; OHATA et al., 1982b). Other authors have made the same assumption about the cilia of fat-storing cells (TANUMA et al., 1981; OHATA et al., 1984), although they could not demonstrate the axonemal ultrastructure.

Nine thorns radiated from the wall of the basal body into the cytoplasm with a regular angle and interval. Although similar structures have not been found in the literature, they may act as an anchor, such as the rootlets of the basal body. The basal body is morphologically identical to centrioles except for rootlets, and a single cilium develops from one of the two centrioles constituting a diplosome. In the present observation, the frequency of the cilium was 2.4% for all fat-storing cells in the three diseases, and that of centrioles was 15.7%. The difference between these frequencies was remarkable, but it was considered reasonable because the single cilium extends from one of the two centrioles arranged perpendicularly to each other (FAWCETT, 1981).

Angular electron lucid inclusions were observed in some fat-storing cells of the patient with alcoholic liver cirrhosis. Similar inclusions have been found in Kupffer cells and hepatocytes in cholesterol ester storage disease (TANIKAWA, 1979), and in Kupffer cells in Wolman's disease (LOUGH et al., 1970), which is a familial lipidosis manifested in early infancy and in which the principal stored lipid is cholesterol ester. Therefore, the inclusions were considered to be cholesterol ester crystals, although such inclusions have not been reported in fat-storing cells before. The mechanism of the present cholesterol depositions is unknown, but the deposition may indicate a disturbance of cholesterol metabolism in fat-storing cells in alcoholic liver disease.

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