Scanning Electron Microscopic Study on the Three-Dimensional Structure of the Collagen Fibrillar Framework in the Chronic Active Hepatitis and Liver Cirrhosis

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The three-dimensional collagen framework of human liver parenchyma in surgical specimens from patients with chronic active hepatitis (CAH), viral or alcoholic cirrhoses (LC) was observed by scanning electron microscopy after cell-maceration by the method of Ohtani (1988). In control livers, the tubular sheaths of collagen fibers housing the sinusoid were shown to form a labyrinthine network, which we called the perisinusoidal collagen sheaths (PSCSs), and a loose band-like collagen sheath was seen embracing each hepatocyte, which we called the perihepatocellular collagen sheath (PHCS). In CAH, thin septa partially surrounded the lobules, but the PHCS and PSCS appeared almost unchanged. In viral LC, thick collagen septa surrounded pseudolobules of various sizes. In these conditions, the collagen density was slight in PSCSs, while in PHCSs, it was prominent and associated with an enlargement of sheaths themselves. In alcoholic LC of mixed type, the basic structure of the collagen framework closely imitated that of viral LC. ——— liver cirrhosis; chronic active hepatitis; collagen fiber; scanning electron microscopy

The increase in collagen fibers in chronic liver diseases has already been extensively studied by light microscopy (Gerber and Popper 1972; Popper 1977; Scheuer 1979a, b) and transmission electron microscopy (TEM) (Schaffner and Popper 1963; Phillips and Steiner 1965; Popper and Udenfriend 1970; Henriksson et al. 1984; Takahashi et al. 1987). However, the methods of observation used in these studies did not clearly visualize how the collagen fibrillar framework changes in these diseases. Recently, the three-dimensional structure of the collagen fibrillar framework of the normal rat and human livers was observed by scanning electron microscopy (SEM) after digestion of the parenchymal tissue.
with 2.5 N NaOH solution (Ohtani 1988). In the present study, the same method was applied to liver specimens taken from patients with chronic active hepatitis (CAH) and viral and alcoholic liver cirrhoses (LC). All of them were obtained at hepatectomy for liver tumors. The three-dimensional structure of the collagen fibrillar framework in these lesions was observed under SEM and compared with that of control liver specimens.

**Materials and Methods**

Liver specimens from 3 patients with CAH, 8 patients with viral LC and 10 patients with alcoholic LC, obtained at hepatectomy for liver tumor, were used. Examined as control were liver specimens from four patients without histological or biochemical abnormalities, which were all obtained at hepatectomy for metastatic liver tumors. The patients with CAH and viral LC have the past histories of HB viral infections with HBsAg and HbcAb, but without alcohol habit. The patients with alcoholic LC have habits of daily ethanol consumption of more than 70 g for more than 20 years and have no histories of blood transfusion and of HB viral infections.

Light microscopic specimens were fixed in 20% formaldehyde or 2% phosphate-buffered glutaraldehyde (pH 7.4) for 1 week. Three µm sections were cut and stained with hematoxylin-eosin (H-E).

NaOH-macerated specimens were prepared according to the method of Ohtani (1988). The resected liver tissues were immediately fixed in 20% formaldehyde or 2% glutaraldehyde solution in 0.2 M phosphate buffer (pH 7.4) for 1 week, and further cut into small pieces, each about 5×5×3 mm. These specimens, after being washed in distilled water, were subjected to parenchymal maceration by immersing in 10% (2.5 N) NaOH solution at 20°C for about 1 week. Then they were washed in running water for 1 to 2 days to remove cellular debris. The specimens thus macerated were further fixed in 1.5% tannic acid solution for 6 hr and washed in running water for 3 hr. They were then post-fixed in 1% osmium solution at 4°C for 2 hr (Murakami 1974), washed in running water for 2 hr, and dehydrated in graded ethanol. All specimens, were dried in critical point drier (HCP-2, Hitachi, Tokyo), and mounted on metal stubs. Subsequently they were coated with gold in an ion coater (IB-5, Eiko, Ibaraki) and observed under SEM (S-430, Hitachi, Tokyo) at an accelerating voltage of 15 KV.

**Results**

*Light microscopy*

Light micrographs of the liver specimens are shown in Fig. 1 so as to represent the control group, CAH, viral LC and alcoholic LC. In the CAH, an inflammatory infiltrate rich in lymphocytes extended from the portal tracts into the adjacent parenchyma. The fibrous septa extended into the parenchyma in the so-called incomplete septal pattern (Fig. 1b). Almost all cases of viral LC were mixed micronodular and macronodular type (Fig. 1c). Portal tracts and septa were infiltrated by inflammatory cells. The parenchyma became surrounded by complete septa, forming pseudolobules. In the early stage of alcoholic LC, the micronodular pattern was predominant. However, almost all alcoholic cirrhoses were of mixed type and showed almost the same features as the viral LC (Fig. 1d).
Scanning electron microscopy

After NaOH-maceration, nothing but collagen fibers remained in the specimens in each group as Ohtani already reported (1988).

Control liver. A closely knit meshwork of collagen proved to extend over the parenchymal area, uniformly among the capsule, portal tracts, hepatic lobules and central veins (Fig. 2a). The diameter of bundles in the portal tracts varied from 0.8 to 3.4 \( \mu \text{m} \) (Fig. 5a).

Many fibers were extending from the portal tracts into the lobular parenchymal tissues. The sinusoids were housed in sheaths made of fibers which we called the perisinusoidal collagen sheaths (PSCSs). The PSCSs were abundantly interconnected with each other, forming a labyrinthine system corresponding to the spatial arrangement of sinusoids (Fig. 2b, c). The fibers of the PSCS extended in the lateral direction, forming septa at an interval of about 10–20 \( \mu \text{m} \) in diameter and separating hepatocytes, which we called the perihapatocellular collagen sheath (PHCS) (Fig. 2b, d). Both PSCS and PHCS were composed of a mixture of coarse fibers, about 0.1–1.0 \( \mu \text{m} \) in diameter, and fine fibers, about 0.05–0.1 \( \mu \text{m} \). The coarse fibers formed rough networks whose meshes were filled with fine fibers (Fig. 2c, d).

Chronic active hepatitis. The portal tracts were slightly enlarged due to periportal fibrosis. Light microscopically, the hepatic lobule was partially surrounded by thin septa 10–15 \( \mu \text{m} \) in thickness. Some of these septa extended so as to link adjacent portal tracts in the form of porto-portal septa, while there were also septa linking portal tracts and central veins, creating the porto-central septa (Christoffersen and Poulsen 1979) (Fig. 3a). The diameter of bundles at the central portion of the portal tract was similar to that of the control liver, ranging 0.6–3.1 \( \mu \text{m} \) (Fig. 5b). At a similar magnification, the septa were composed of bundles ranging from 0.8 to 3.5 \( \mu \text{m} \) in diameter with many branches of fine fibers, whose bundles were of almost the same size as those in the portal tract of the control livers (Fig. 3b). In the lobules, the PSCS and PHCS appeared similar to those of the control livers (Fig. 3c) without showing evident increase of fibers in the PSCS and PHCS. But in the peripheral areas of the lobules, the density of fibers was undoubtedly elevated.

Viral liver cirrhosis. At a low magnification, pseudolobules were surrounded by thick collagen septa of 50–200 \( \mu \text{m} \) in width (Fig. 4a). The fibrotic areas including portal tracts and septa were markedly enlarged. The diameter of bundles at the central portion of the portal tract ranged from 0.8 to 4.8 \( \mu \text{m} \) (Fig. 5c), i.e., their average diameter was larger than in the control (Fig. 5a) or the CAH (Fig. 5b) liver specimens.

Most of the pseudolobules did not contain clearly discernible central veins (Fig. 4a). The PSCSs decreased in density, running irregularly in pseudolobules, and interhepatocellular spaces were seen at some places (Fig. 4b). The diameter
of the PSCS was a half or two thirds smaller than in control livers. However, the density of fibers in the PSCS seemed almost unchanged or slightly increased (Fig. 6a). At a low magnification, the PHCSs varied in size (Fig. 4a, b). While in the control liver there was usually only one hepatocyte in each PHCS, in the LC, a PHCS was frequently seen housing two or more hepatocytes (Fig. 4b). Higher magnification showed that the PHCSs were markedly increased in width and density, especially around portal tracts and septa. The PHCSs around portal tracts and septa were composed mainly of densely packed fine fibers (Fig. 6b). In the areas with more advanced fibrosis the PHCSs were bowl-like, with their bottom often nearly or perfectly closed (Fig. 6c). But the fiber density gradually decreased toward the center of pseudolobules and the ratio of coarse to fine fibers increased apparently (Fig. 6d).

Alcoholic liver cirrhosis. In the alcoholic LC, small pseudolobules were surrounded by thin septa about 50 µm in thickness (Fig. 7a). Some of these linked two adjacent portal tracts, others a central vein to a portal tract. However, in some cases, pseudolobules were surrounded by thick septa of 50 to 200 µm (Fig. 7b), leaving no difference from the septa in viral LC (Fig. 4a). Thus, the basic three-dimensional structure of the collagen framework of the alcoholic LC of mixed type closely imitated that of viral LC. At a higher magnification, however, the ratio of the coarse to fine fibers in the PHCS and in the PSCS in the alcoholic LC was higher than in the viral LC (Fig. 7c, d). This tendency was more prominent around the portal tracts and the septa than at the central area of pseudolobules.

Discussion

In the present investigation, the cell maceration method (Ohtani 1988) has proved to be very useful in demonstrating the three-dimensional structure of perisinusoidal and pericellular collagen fibers in the control livers and pathological conditions. With this method, the configuration and location of collagen fibers of different sizes can be clearly demonstrated without minimum artifact.

Our observations on the three-dimensional structure of collagen fibrillar sheaths for housing the sinusoid (PSCS) in the control liver were almost the same as those reported by Ohtani (1988), but he did not mention the existence of the PHCS. From SEM observations, Ohtani (1988) reported that the collagen fibers extended from one PSCS to another intercellularly in normal human livers. According to the present observations, these fibers formed a thin band-like structure about 10–20 µm in width around each hepatocyte. They consisted of a loosely arranged mixture of thick and thin fibers, a finding we called the pericellular collagen sheath (PHCS). The presence of collagen fibers between the sinusoidal lining cells and hepatocellular microvilli have been previously observed by light microscopy and TEM (Wassermann 1958; Schaffner and Popper
Collagen Framework in Chronic Liver Diseases

1963; Phillips and Steiner 1965; Ito and Shibasaki 1968; Popper and Udenfriend 1970; Henriksen et al. 1984; Ohtani 1988). In the subendothelial spaces of Disse, tube-like collagen fibrillar sheaths around the sinusoids were shown forming a labyrinthine network system, as described by Ohtani (1988), which we have called the perisinusoidal collagen sheaths (PSCSs). Three-dimensionally, the concept of the PSCS and the PHCS has been never reported.

Light microscopic observation of silver impregnated specimens and TEM studies revealed an increased fiber density around hepatocytes in livers with chronic hepatitis and those with LC. This increase was called perihepatic or pericellular fibrosis (PCF) (Popper et al. 1960). In viral hepatitis, liver cells around portal tracts are involved in piecemeal necrosis in small groups (Popper et al. 1965; Boyer and Klatskin 1970; Bianchi et al. 1977). As a result, the pre-existing fibrillar framework collapses and new collagen fibers appear near the portal tract (Gerber and Popper 1972). Consequently the fibrosis near the portal tract increases and forms septa between the adjacent portal tracts (Popper and Udenfriend 1970). In the CAH, Scheuer (1979a) showed that collagen fibers in the space of Disse increased. However, in the SEM observations, the size and density of the PSCS were almost the same as in the control hepatic parenchyma. And the width of the PHCS and its density of fibers only slightly increased if any in the CAH.

The PHCSs of the LC specimens, however, were markedly enlarged with their collagen density apparently increased, especially around the portal tracts and septa. It has been suggested that the increase in collagen fibers in the spaces of Disse, together with the formation of basement membrane around the endothelial cells and the decrease of hepatocellular microvilli, works as a barrier against the supply of metabolic substances and oxygen to the underlying hepatocytes (Schaffner and Popper 1963; Popper and Udenfriend 1970; Popper 1977; Takahashi et al. 1987). The results of the present study makes us assume that in the LC, the marked enlargement of PHCSs with their increased density causes and aggravates the hepatocellular insufficiency. Our observations showed that the fibrosis in the PHCS in the LC tends to be more prominent around portal tracts and septa than in other regions of the pseudolobule, as already described by Popper (1977). The difference in the PHCS between the viral and alcoholic LC is not so clear, with the cases showing a mixed type, at a lower magnification in the present SEM observation. However, at a higher magnification, there was a difference between the two types of LC. In the viral LC, the PHCSs around the portal tract and the septa were mainly formed by fine fibers, while they comprised an increased ratio of coarse fibers in the alcoholic LC. These findings have not been reported in the previous papers three-dimensionally.

The number in a unit volume of PSCSs decreased in the LC as reported by Phillips and Steiner (1965). In addition, the diameter of PSCS was two thirds or a half smaller than in the control livers. However, their density was not so
markedly increased as Aihara reported (1984). The decrease in the number and size of PSCSs probably contribute to a certain extent to the reduced blood flow in cirrhotic livers and hinder the metabolism of hepatocytes, as suggested in previous studies (Schaffner and Popper 1963; Scheuer 1979a, b; Uchikoshi et al. 1987). In the control livers, the confronting surfaces of the PHCSs between neighbouring hepatocytes are closely apposed. But in the cirrhotic livers, narrow gaps between opposed PHCSs (Fig. 5b) were seen as already reported by Phillips and Steiner (1965) under TEM. It is considered that these gaps, communicating to the spaces of Disse, compensate the disturbance of the blood supply. In the PSCS, the difference between viral and alcoholic LC is not so clear. However, in the alcoholic LC the ratio of coarse to fine fibers was elevated around the portal tract, compared with that of the viral cirrhotic livers. This difference has not been reported previously.

The portal tracts were enlarged slightly in the CAH and markedly in the LC. In the LC, the collagen bundles were more dense and thicker in the central areas of portal tracts than those in the peripheral portal areas and in the septa. In addition, the bundles were thick in the central areas of the portal tracts in the LC, while the bundles in the CAH remained almost the same as in the control livers. These findings have not been reported previously and indicate that the more thickened bundles in the central area may be older than those in the peripheral area.

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References


Abbreviations used in Fig. 1 to Fig. 7: C, central vein; Cp, capsule; H, perihepatocellular collagen sheath (PHCS); H1, hepatic lobule; P, portal vein; Ps, pseudolobule; Pt, portal tract; S, perisinusoidal collagen sheath (PSCS).

Fig. 1a-d. Light micrographs of the HE stained liver specimens. ×10. a. Control liver of a 60-year-old woman. b. CAH of a 71-year-old man. Portal tracts are inflamed and the infiltrate extends into the lobules and the fibrous septa incompletely surround the parenchyma. c. Viral LC of a 60-year-old woman. d. Alcoholic LC of a 49-year-old man. Both viral and alcoholic LC show almost the same pattern as follows. Complete septa surround pseudolobules, and inflammatory cells are seen in portal tracts and periportal areas. The portal tracts are enlarged.
Fig. 2a-d. SEM pictures of the control liver after cell maceration: a-d correspond to the case shown in Fig. 1a. a. Low magnification. All cells are removed and only collagen fibrillar frameworks remain. ×70. b. Moderate magnification. Collagen fibers extend from portal tracts into hepatic lobules and form PSCSs and PHCSs. ×550. c. High magnification. The framework of the PSCS consists of coarse and fine fibers. ×1,760. d. High magnification. The collagen fibers of the PHCS are continuous to those of the PSCS. The PHCS appears as a band-like structure formed by collagen fibers of various diameters. ×1,900.
Fig. 3a-c. SEM pictures of the CAH: a-c correspond to the case shown in Fig. 1b.

a. Low magnification. The portal tract is slightly enlarged and thin septa partially surround the pseudolobules. The arrow indicates the porto-portal septa and the arrowhead indicates porto-central septa. ×80. b. High magnification of the porto-portal septa. Each bundle has many branches. ×2,200. c. The periportal area. Note that the PSCS and the PHCS are almost the same as those of the control liver, corresponding to the case shown in Fig. 2b. ×800.
Fig. 4a, b. SEM pictures of the advanced viral LC, corresponding to the case shown in Fig. 1c. a. The pseudolobules are surrounded by thick septa. Note the various sizes of the PHCSs. The central vein cannot be discerned in the pseudolobules. ×100. b. The periportal area. The PHCS has a band-like appearance. Note the narrow gap between two PHCS (arrow). The number of PSCSs (arrowhead) decreases. ×550.

Fig. 5a-c. Comparison of the bundles in the portal tract. a. Control liver, corresponding to the case shown in Fig. 1a. b. CAH, corresponding to the case shown in Fig. 1b. c. Viral LC, corresponding to the case shown in Fig. 1c. Note that the average size of the bundles in the LC is larger than in the control liver and the CAH. ×1,850.
Fig. 6a-d. SEM pictures of viral LC.  a. The PSCS. The PSCS decreases in size but its fiber density remains almost similarly to the control liver. ×4,100.  b. The PHCS. Fine fibers, 0.05–0.1 μm in diameter, are densely packed. ×3,100.  c. The PHCSs in the further advanced fibrosis around the portal tract. Their bottom parts are almost (arrow) or completely (arrowhead) closed, showing bowl-like appearances. ×470.  d. The central area of the pseudolobules. The density of the PHCS decrease as compared with that around the portal tract, and coarse fibers prominent. The PSCSs show almost the same as those of the control liver. ×960.
Fig. 7a-d. SEM pictures of the alcoholic LC. b-d correspond to the case shown in Fig. 1d. a. The alcoholic LC of micronodular type. The pseudolobules are surrounded by thin septa. \( \times 45 \). b. The alcoholic LC of mixed type. The pseudolobules are surrounded by thick septa. \( \times 50 \). c. The PHCS of the periportal area in the LC of mixed type. Note the marked increase of coarse fibers with 0.5–1.0 \( \mu m \) in diameter. \( \times 1,300 \). d. The PSCS of the periportal area. The density of fibers is not so prominently increased but the ratio of the coarse to fine fibers increased slightly. \( \times 1,890 \).