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Deposition and remodelling of elastic fibres in chronic hepatitis

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Summary. A longitudinal study of elastic fibres was conducted in liver biopsies from 21 patients with chronic viral hepatitis. Biopsies were taken at the start of treatment with alpha-interferon, and after 6 months or 1 year of treatment. No elastic fibres could be detected in portal tracts or fibrous septa when inflammation and necrosis were present, whatever the duration of the disease. Once the liver lesions had healed, elastic fibres were synthesized. Fibres were of two types and patterns: at first, they were thin, long and parallel to each other. Later, they were thicker, shorter and tortuous and were closely wrapped around thick bundles of collagen fibres. These findings suggest that the deposition of elastic fibres acts as a marker of healing in chronic active hepatitis and that their remodelling occurs concomitantly with the deposit of large bundles of collagen fibres.

Key words: Elastic fibre – Chronic hepatitis – Liver – Extracellular matrix

Introduction

Elastic and collagen fibres are the two fibrillar components of the extracellular matrix. Although collagen fibres have been extensively studied in the liver (Chojkier 1989; Martinez-Hernandez 1985), elastic fibres (EF) have received less attention. Two previous reports have shown that EF are absent in areas of liver necrosis and collapse (Scheuer and Maggi 1980; Thung and Gerber 1982) but that they are abundant in chronic lesions. These data suggest that, like collagen fibres (Grimaud et al. 1980), EF are synthesized during the evolution of a chronic fibrotic process. Chronic viral hepatitis and healing of the hepatic lesions after anti-viral drug treatment provides a model for the observation of this process in the liver. A prospective trial of alpha-interferon therapy in chronic hepatitis C, including sequential liver biopsies prompted us to study the chronology of EF deposition and its relationship to collagen fibres synthesis and the healing of liver lesions. Our study shows that EF deposition is concomitant with the repairing of the liver injuries and that a remodelling process of EF occurs during healing of the lesion.

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Materials and methods

Fifty-two liver biopsies from 21 patients were included in the study. Biopsies were obtained at the beginning and after 6 months of a low-dose alpha-interferon therapy for chronic active hepatitis C. In 10 cases, a third liver biopsy was obtained 1 year after the initial biopsy. The first biopsy from all patients displayed the features of chronic active hepatitis as defined by international criteria (Bianchi et al. 1977) with cirrhosis in 5 cases. Sections were routinely processed and stained with haematoxylin and eosin, Masson's trichrome or Weigert and Shikata's orcein stain (Shikata et al. 1974).

Lesions were graded according to the Knodell scoring system (Knodell et al. 1981). Fibrosis, necrosis and inflammation were also assessed semi-quantitatively using the scoring system included in the Knodell grading. After treatment, chronic active hepatitis was considered to be healed when necrosis had totally disappeared and when the score for inflammation was at least half the initial value.

In the sections stained with Shikata and Weigert's stain, EF and collagen fibres were graded as absent=0, + =thin and lightly stained, or + + =thick and deeply stained. Staining of normal fibres in the portal tract was disregarded for the purposes of grading. The Knodell score and the amount of EF were assessed blindly without knowledge of the period when the liver biopsy had been taken.

Results

The Knodell score was 7.8 ± 9.4 (mean $\pm$ SD) before interferon therapy and decreased to a mean value of 4.9 ± 3.4 after 6 months therapy. Clear decreases in the grades

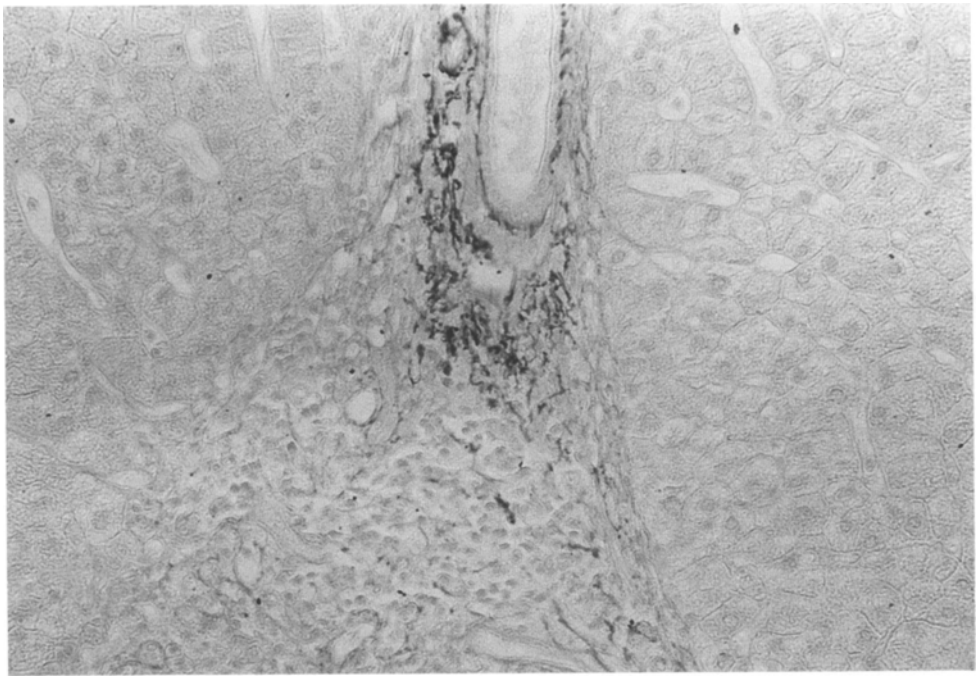

Fig. 1. Chronic active hepatitis. Portal tract with a lymphocytic infiltrate and piecemeal necrosis. No elastic fibres are present from the periportal area. Shikata, ×60

of necrosis (2.7±1.9 to 1.1±1.8) and of inflammation (3.4±0.7 to 2.1±1.2) were obvious after interferon treatment, but the score for fibrosis did not change (1.9±1.6 before and after treatment). According to the previously described criteria, the features of chronic active hepatitis were no longer present in 15 of the 21 patients after 6 months of alpha-interferon treatment.

Analysis of the liver biopsies taken at the beginning of treatment and 6 months or 1 year later clearly showed that EF deposition increased with the duration of disease but was only apparent once the inflammation had subsided and the lesions had healed. Newly synthesized EF were absent or rare in all the liver biopsies taken at the beginning of interferon treatment. In the 5 cases with cirrhosis, some EF were centrally located around vessels and bile ducts in the portal tracts or fibrous septa but were absent from the periportal area, where lymphocytic infiltrates and piecemeal necrosis were present (Fig. 1). Newly synthesized collagen fibres were also rare or absent.

EF were still absent from 6 of the biopsies performed after 6 months of treatment, and 4 of those taken 1 year after the beginning of treatment. These cases were resistant to treatment, according to both the biochemical data and the histological lesions. In the patients concerned, liver biopsies continued to exhibit piecemeal necrosis and features of inflammation but no EF synthesis. Correlations between the amount of EF and the Knodell scores are shown in Table 1. In the biopsies which did display EF synthesis, two different patterns could be distinguished:

1. In the second biopsies taken when inflammation and piecemeal necrosis had disappeared, EF were clearly apparent. They were arranged in an ordered pattern consisting of straight and thin elastic fibres oriented in parallel. In cases with cirrhosis, they stained lightly with orcein and were located at the periphery of the portal tracts or of the fibrous septa (Fig. 2). These EF were generally associated with parallel and elongated collagen fibres. Such EF were not seen in the patients who still exhibited the features of chronic active hepatitis at the second biopsy.

2. In the 10 cases with longstanding fibrosis, a different pattern was observed in the biopsies taken after 1 year of treatment, when inflammation had disappeared totally. EF were more abundant and had grown thick, short and tortuous. They were distorted and closely wrapped

Table 1. Elastic fibres in sequential liver biopsies according to the score of Knodell

	Knodell score (mean±SD)	Amount of elastic fibres[a]		
		0	+	++
Initial biopsy	7.8±9.4	21	–	–
After 6 months of alpha-interferon therapy	4.9±3.4	6	13	2
After 1 year of therapy	2.5±2.2	4	3	3

[a] Semi-quantitatively assessed on sections treated with Shikata's stain: 0, no elastic fibres; +, thin, lightly stained, long, newly synthesized fibres; ++, thick, deeply stained tortuous fibres

In the 5 cases of cirrhosis, the fibres present in the first biopsy at the centre of the portal tract and septa were disregarded for grading purposes

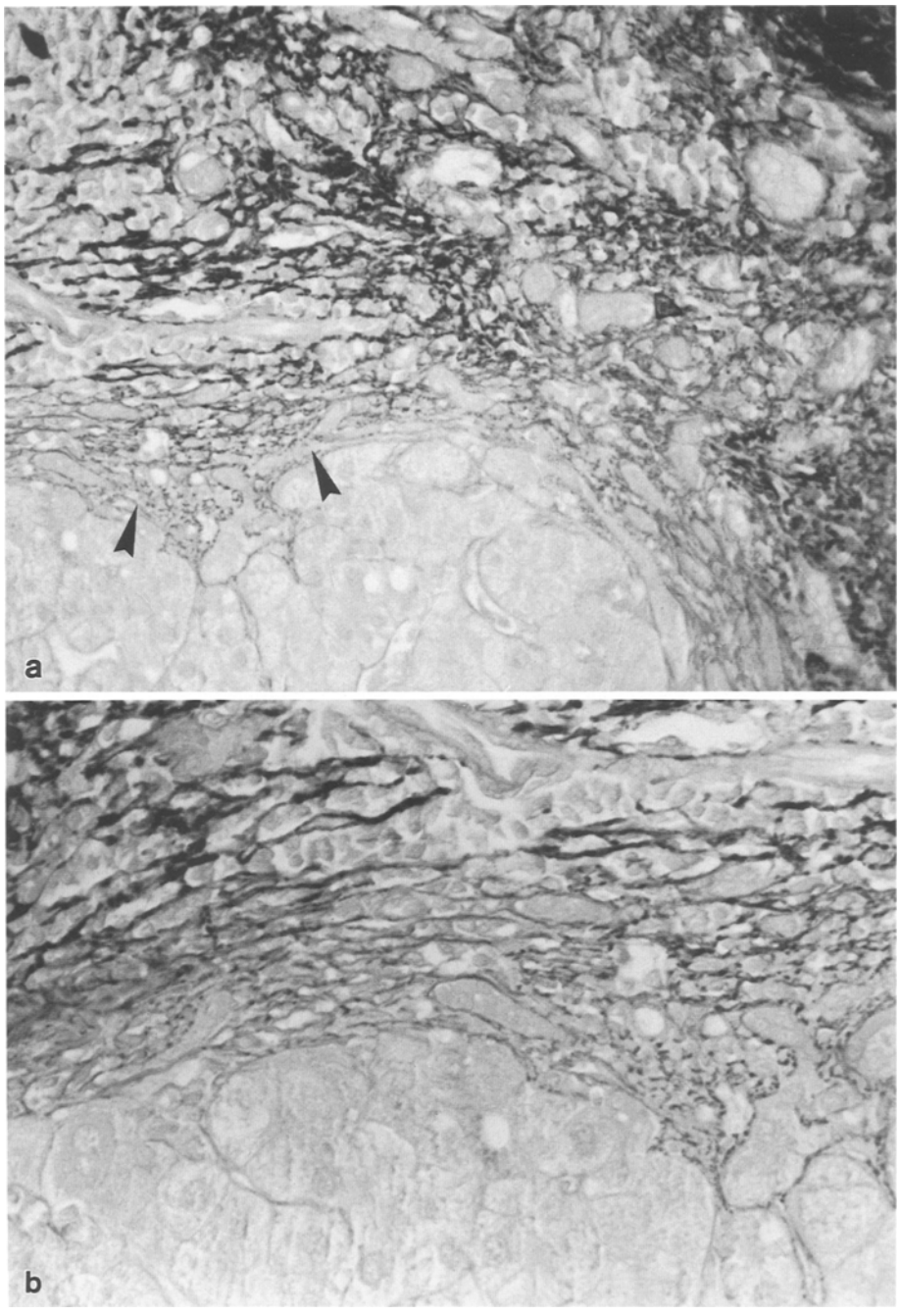

Fig. 2. **a** A fibrous septum in a case with cirrhosis, after 6 months of interferon therapy. Note the presence of newly synthesized elastic fibres (*arrow*). Shikata, ×120.
b Higher magnification showing that newly synthesized fibres are thin and long, whereas older fibres are thick and short. Shikata, ×250

around the thick collagen bundles, and stained deeply with Shikata's stain (Fig. 3).

Discussion

It has been suggested previously that EF are markers of chronic disease and not of acute lesions (Thung and Gerber 1982). Our study confirms this report in part, since we observed more EF in the liver biopsies performed 6 months or 1 year after the beginning of treatment than in the initial biopsies. However, a stronger correlation was found between the amount of newly synthesized EF and the healing of liver lesions than between new EF and the duration of the disease. No EF were synthesized when the features of chronic active hepatitis persisted, whatever the duration of the disease.

Once the inflammatory lesions had disappeared, two types of EF could be distinguished. They differed in shape and histochemical staining intensity. The first type, consisting of straight thin fibres, was present in cases of recent fibrosis in which collagen fibres were few or absent, whereas the second type of EF was thick, short and tortuous. These EF were detected in older fibrous tissue in which they were wrapped around the thick collagen fibres. This close relationship between EF

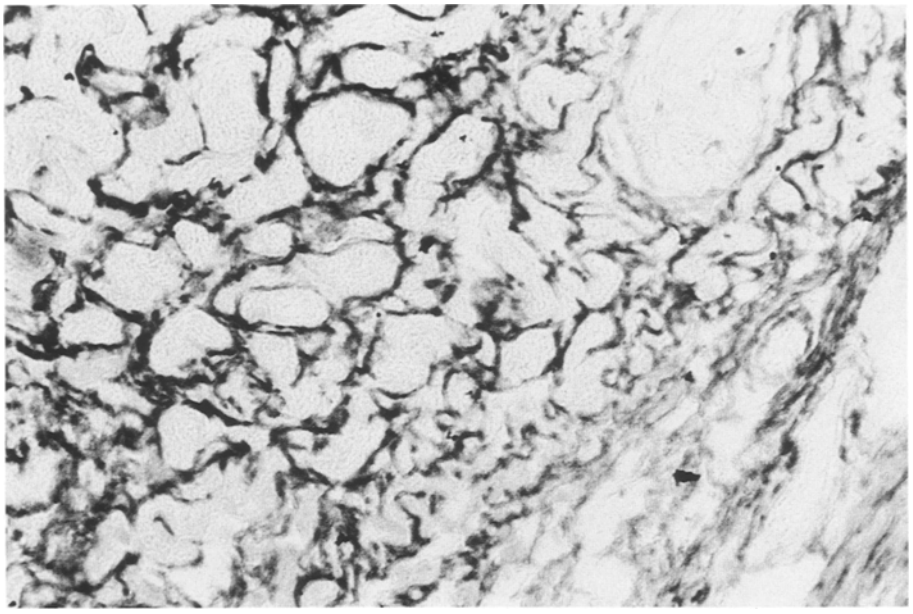

Fig. 3. Liver biopsy, 1 year after interferon therapy. Dark thick elastic fibres are wrapped around bundles of collagen fibres. Shikata, ×250

and collagen fibres suggests that in the liver, as in other tissues, EF are co-synthesized with collagen fibres (Kao et al. 1980). However, the types of cell and regulation mechanisms involved in elastin synthesis in the liver are not yet known.

It is highly probable that the two types of EF observed here reflect the degree of fibre maturation reached. In cases with cirrhosis, the two types were present together. Thus, thick tortuous EF were observed in the central part of the fibrous septa, and long thin EF, in the outer area of these septa, where synthesis of new fibres occurred.

The changes with time in EF histochemical staining intensity might reflect biochemical maturation, but as the mechanisms by which these stains work are unclear (Uito 1979), no conclusion can be drawn on this point. Changes in the shape of EF were concomitant with the deposit of large collagen bundles. It has been suggested in a recent study, that re-arrangement of the three-dimensional organization of the fibres occurs in the extracellular matrix during wound healing or the aging process. Thus, in the skin, the synthesis of collagen fibres induces stretching of the elastic fibres, which break up and wrap around the collagen fibre bundles (Imayama and Braverman 1989). This rearrangement might also occur for EF in the extracellular matrix of the liver. Whether the presence of EF in fibrous tissue is a factor of unfavourable prognosis in the potential reversibility of fibrosis is not known. Since no decrease in the score for fibrosis was noted here 6 months or even 1 year after the beginning of treatment, no conclusion can be drawn on this point.

Our study shows that deposition and remodelling of EF occur during the healing of chronic active hepatitis, in which EF seems to act as a marker of healing. Furthermore, the shape and histochemical staining intensity of EF are features that indicate whether the healing is a recent or a longstanding process.

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