MORPHOLOGY AND PATHOMORPHOLOGY

Electron-Histochemical Localization of Liver Collagenase

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Localization of collagenase in normal rat liver is studied by the electron-histochemical method. Enzyme activity is detected in Kupffer and endothelial cells and on hepatocyte microvilli.

Key Words: *collagenase; liver, electron histochemistry*

Collagenase is a key enzyme in collagen metabolism. Tissue collagenases are zinc-dependent metalloenzymes that cleave various types of interstitial collagens across the three chains into 1/4 and 3/4 length fragments [6]. The investigation of collagenases has been hampered by a number of difficulties, one of them being that the enzymes are not accumulated in the cell but are synthesized as necessary. Therefore, only small amounts of enzyme have been detected in cells and tissues, and the majority of collagenase studies have been carried out *in vitro*. Morphological identification of the enzyme is very difficult. In the liver, two forms of collagenase are present: active and latent [7]. Proenzyme can be activated by trypsin, plasmin, kallikrein [1,11], and cathepsins B [2] and G [14]. Since collagenase normally occurs in the latent state, it can be visualized only by immunomorphological methods. Collagenase activity in the liver was first described by Japanese scientists [10] and then confirmed by other researchers [4,12]. It is generally accepted that Kupffer cells are the major producers of collagenase [3,5]. They contain the enzyme in the active form. In addition, two types of collagenase have been demonstrated in the liver; one type is produced by hepatocytes and the other by sinusoidal cells [9]. However, none of these biochemical investigations provide information regarding the intracellular localization of the enzyme in the liver. The only morphological study of collagenase at the light microscopy level with the use of immunological methods has shown the enzyme to be localized extracellularly, being associated with collagen fibrils and basal membranes [8].

This study was designed to identify the cells producing collagenase and to reveal the structural localization of active collagenase in normal rat liver.

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MATERIALS AND METHODS

Livers of male albino rats weighing 180-200 g were studied. Electron-histochemical identification of collagenase was performed using the techniques developed in our laboratory, based on the reaction of successive nitrogen coupling [3]. Tissue pieces (0.5 mm^3) were fixed for 3 h in 1.5% glutaraldehyde on 0.05 M cacodylate buffer (pH 7.4) containing 2% sucrose. The material was then washed with the same buffer containing 7% sucrose. The incubation medium contained 10 ml 0.05 M Tris-HCl buffer (pH 7.5), 10 mM $CaCl_2$ [14], and 10 mg Z-Pro-Ala-Gly-Pro-4-MBNA substrate (Serva) dissolved in dimethylformamide (DMF, 10 μl/10 ml final volume). After 1-h incubation at 37°C, the material was washed with cacodylate buffer (0.1 M, pH 6.5) and incubated in the same buffer containing hexazotated pararosaniline (50 μl/ml) at 37°C for 30 min. After washing with cacodylate buffer (0.05 M, pH 7.4) containing 7% sucrose, the material was treated with 2% OsO_4 for 90 min and embedded in Epon. Control reactions were carried out in medium containing EDTA, an inhibitor of collagenase, and in medium containing no substrate. Ultrathin sections were not contrast-stained.

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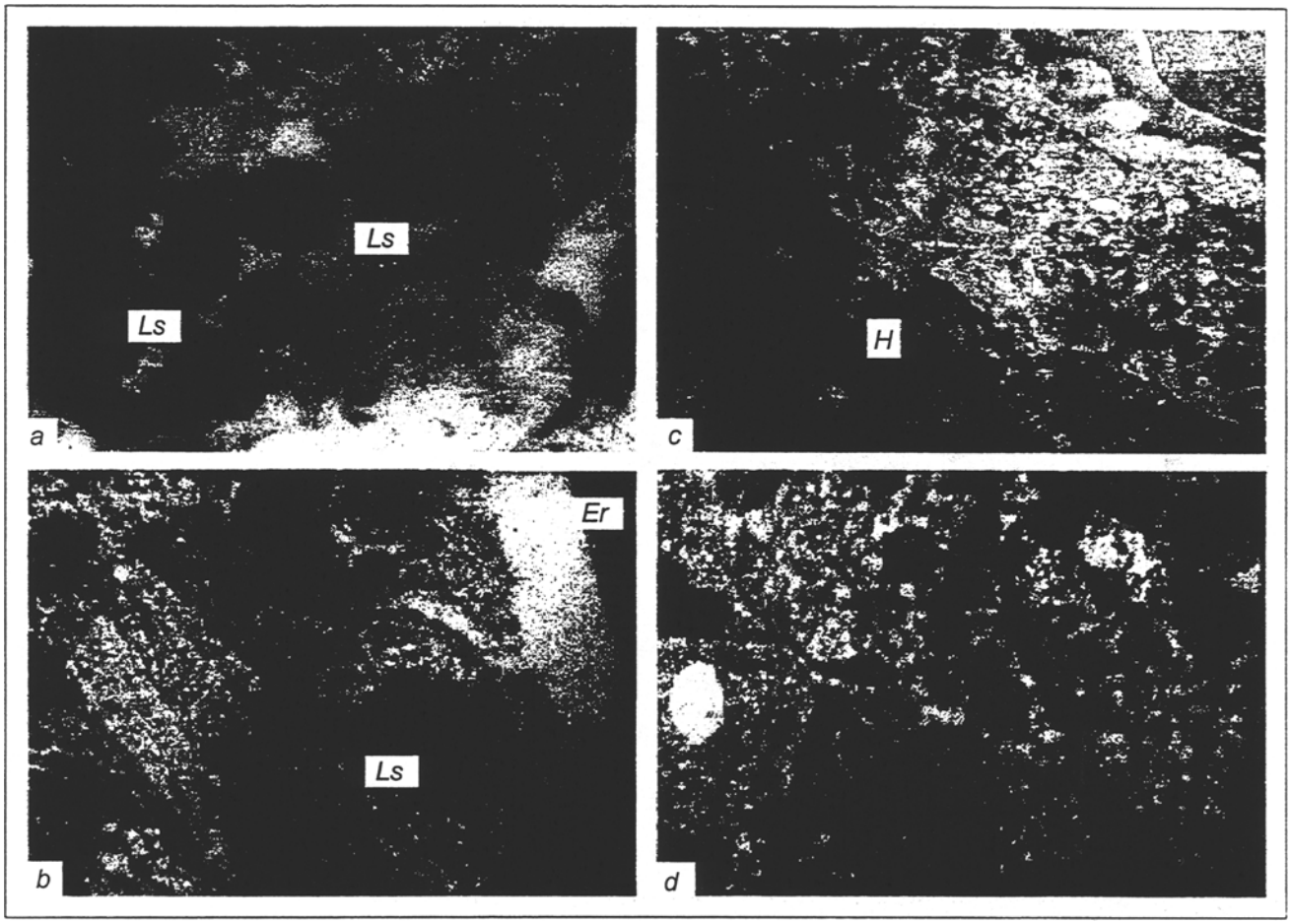

Fig. 1. Collagenase localization in the liver. *a*) intensive collagenase reaction in lysosomes (*Ls*) of Kupffer cells. ×30,000; *b*) collagenase reaction in a lysosome of an endotheliocyte. *Er* = erythrocyte, ×40,000; *c*) reaction product (shown with arrows) on hepatocyte (*H*) microvilli, ×25,000; *d*) control, incubation in the presence of EDTA, no reaction product seen, ×30,000.

RESULTS

The reaction product was seen as small granules. The reaction was particularly intensive in the lysosomes of Kupffer cells (Fig. 1, *a*). Reaction product was also present in the lysosomes of endotheliocytes; however, enzyme activity in these cells was considerably lower (Fig. 1, *b*). The hepatocytes contained no reaction product, although a slight extracellular collagenase activity was detected. Granular reaction product was seen on the microvilli of some hepatocytes (Fig. 1, *c*).

In control preparations, no reaction product was detected either in cells or in the extracellular space (Fig. 1, *d*).

These findings indicate that in the liver collagenase is localized in the lysosomes of Kupffer cells and endotheliocytes and extracellularly on hepatocyte microvilli. The possibility that the high collagenase activity detected in Kupffer cells is associated with phagocytosis, the major function of these cells, cannot be ruled out. The absence of reaction product in hepatocytes and its presence on their microvilli probably indicates that hepatocytes contain latent collagenase which is activated after being secreted into the extracellular space.

REFERENCES

1. C. T. Brinckerhoff, M. C. Benoit, and W. J. Culp, *Proc. Nat. Acad. Sci. USA*, **82**, 1916-1921 (1985).
2. Y. Eeckhout and G. Vaes, *Biochem. J.*, **166**, 21-31 (1977).
3. K. Fujiwara, T. Sakai, T. Oda, and S. Igarashi, *Biochem. Biophys. Res. Commun.*, **54**, № 2, 531-537 (1973).
4. K. Fujiwara, T. Sakai, T. Oda, and S. Igarashi, in: *Diseases of the Liver and Biliary Tract,* Basel (1976), pp. 73-81.
5. E. Harris and E. Cartwright, in: *Proteinases in Mammalian Cells and Tissues,* Amsterdam (1977), pp. 249-283.
6. E. Harris, et. al., *N. Engl. J. Med.*, **291**, № 1-2, 557-563 (1974).
7. K. Maruyama, I. Okazaki, K. Kashihwazaki, *et al., Biochem. Exp. Biol.*, **14**, № 3, 191-201 (1978).
8. I. Montfort and R. Perez-Tamayo, *J. Histochem. Cytochem.*, **23**, № 12, 910-920 (1975).
9. I. Montfort, R. Perez-Tamayo, A.-M. Alvizouri, and E. Tello, *Virchows Arch. [B]*, **59**, № 5, 281-289 (1990).
10. I. Okazaki and K. Maruyama, *Nature,* **252**, № 202, 49-50 (1974).
11. A. Oronsky, R. Perper, and H. Schroder, *Ibid.*, **246**, 417-419 (1973).
12. R. Perez-Tamayo, I. Montfort, and E. Gonzalez, *Exp. Mol. Pathol.*, **47**, 300-308 (1987).
13. R. Smith and R. Van Frank, *Lysosomes in Biology and Pathology,* New York (1975), pp. 193-249.
14. J. Woessher, *Biochem. J.*, **161**, № 3, 535-542 (1977).