ELECTRON HISTOCHEMICAL DETECTION OF INTRA- AND EXTRACELLULAR LOCALIZATION OF CATHEPSIN D IN THE LIVER

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KEY WORDS: cathepsin D; liver; localization; immunohistochemistry

Cathepsin D, the most important lysosomal proteinase, is present in various cells and tissues and functions most actively at acid pH values [3, 6, 13]. The enzyme can hydrolyze many different proteins, including the basic components of the intercellular matrix: collagen, proteoglycans, and glycoproteins (fibronectin) [2, 4, 7, 9, 11, 14]. The localization of cathepsin D in the liver has not been finally settled. Only one investigation has been devoted to this question [15], in which an immunohistochemical method was used, so that the localization of the protein could be determined. However, during processing of material for immunohistochemistry, and in particular during fixation, the antigenic determinants of the protein are modified, making its detection more difficult especially at the ultrastructural level, whereas fixation has a much weaker effect on its activity [1, 15].

Accordingly, in the present investigation the localization of cathepsin D in the liver was determined by a basically different approach to that used in the study mentioned above, namely by an electron-histochemical method, in which the enzyme was demonstrated with reference to its activity.

EXPERIMENTAL METHOD

The liver of male albino rats weighing 180-200 g was studied. The animals were killed by decapitation under ether anesthesia. Material was treated histochemically for detection of cathepsin D at the ultrastructural level by the method described in [12]. Pieces of tissue measuring 0.5 × 0.5 × 1 mm were fixed for 3 h with 1.5% glutaraldehyde in cacodylate buffer, pH 7.2. For the next few days the material was washed with cacodylate buffer, pH 7.2, containing 7% sucrose. Incubation continued for 30 min at 37°C in medium containing as the substrate 24 ml of BL-Arg-Gly-Phe-Phe-Pro-MBNA, dissolved in 1 ml of dimethylformamide with the addition of 25 ml of glycine buffer, pH 3.1. The reaction was stopped by the addition of 10% KOH to the medium followed by washing with HEPES buffer, pH 7.0. Incubation followed in medium containing 10 μg of dipeptidylaminopeptidase II in cacodylate buffer, pH 5.4, with hexazotized pararosaniline (1 ml to 20 ml medium) at 37° for 15 min. After fixation in OsO_4 the material was dehydrated and embedded in Epon. As the control reaction, incubation was carried out in medium with substrate.

Some sections were stained with uranyl acetate and elad citrate, others with uranyl acetate alone. The preparations were studied in the JEM-100S electron microscope.

EXPERIMENTAL RESULTS

Determining on the degree of activity of the enzyme, the product of the reaction for cathepsin D was found in the form either of single small granules or of a more or less homogeneous conglomerate of them, of varied density. The reaction product was found in hepatocytes in individual lysosomes, located most frequently at the biliary pole (Fig. 1a), and also extracellularly — on microvilli of the hepatocytes in Disse's space (Fig. 1b). An intensive reaction for cathepsin D was observed in individual lysosomes of certain endothelial cells (Fig. 1b).

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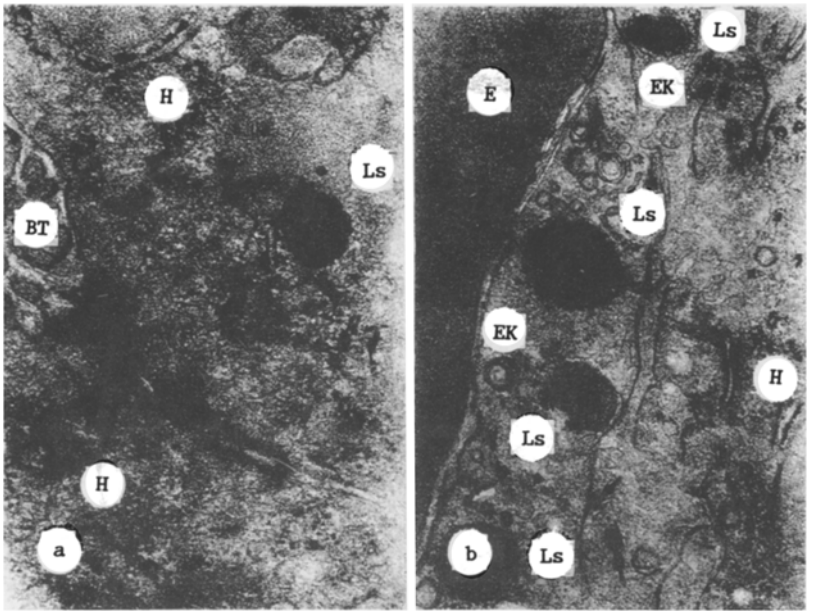

Fig. 1. Macrophage after 10 days of involution of cirrhosis. Product of reaction for cathepsin D in lysosomes (Ls), and also extracellularly (arrow) on collagen fibers (C). 10,000×.

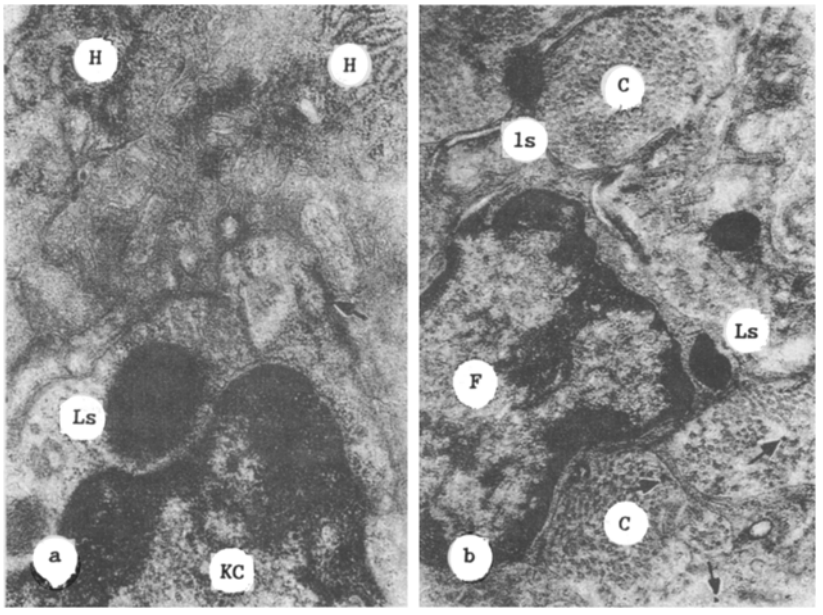

Fig. 2. Product of reaction for cathepsin D (arrows) located extracellularly on collagen fibers (C) and on microvilli of a hepatocyte (H). Release of enzyme from hepatocyte into extracellular space can be clearly seen (double arrow). 20 days of involution of cirrhosis. 20,000×.

An extraordinarily intensive reaction was found in lysosomes and various vacuoles, containing material undergoing digestion, in the Kupffer cells (macrophages). The reaction product was found extracellularly also, directly on the cytolemma of the macrophages (Fig. 2a). The presence and intensity of the reaction varied both between different lysosomes and within the same lysosome, i.e., considerable heterogeneity in the distribution of the reaction product was observed. This was a characteristic feature of all types of cells studied.

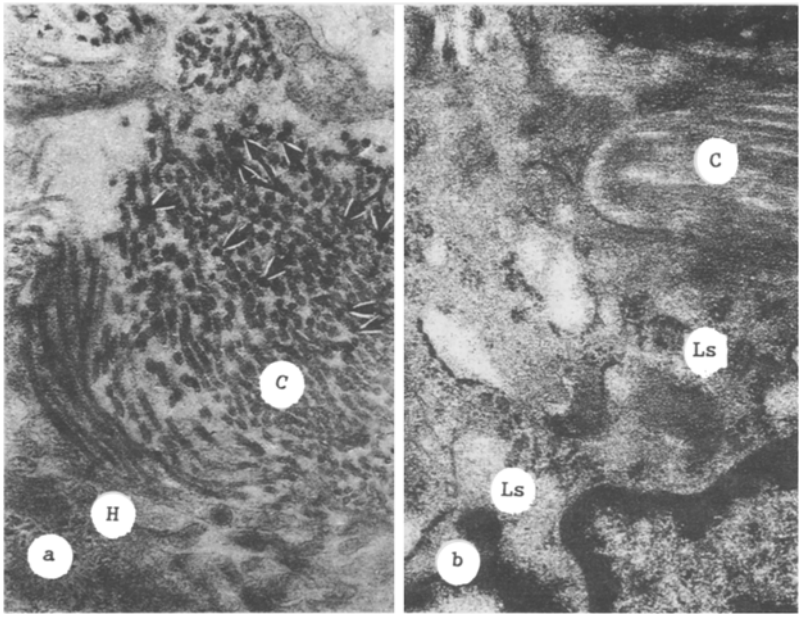

Fig. 3. Product of reaction for cathepsin D (arrows) located extracellularly on collagen fibers (C) in Disse's space (a) and control preparation (b) after 20 days of involution of cirrhosis. a) Magnification 40,000; b) reaction product absent both in lysosomes (Ls) of connective tissue and also extracellularly, on collagen (C). 20,000×.

A very intensive reaction for cathepsin D was observed in lysosomes of fibroblasts from the portal tracts. We observed granules of the reaction product extracellularly on collagen fibers located immediately next to fibroblasts (Fig. 2b), and also in Disse's space (Fig. 3a). The control preparations did not contain the reaction product (Fig. 3b).

The results are evidence that cathepsin D is localized in the liver not only in lysosomes of hepatocytes and Kupffer cells [15], but also in lysosomes of endothelial cells, and also of fibroblasts from the portal tracts. The smallest number of lysosomes containing reaction product, and also the weakest reaction were observed in the hepatocytes. According to the biochemical data [13], activity of cathepsin D in the nonparenchymatous cells of the liver is 6 times higher than in parenchymatous cells. The heterogeneity which we observed in the distribution and intensity of the reaction product is evidence of differences in the functional state of the lysosomal system in the types of cells studied.

Involvement of cathepsin D in extracellular catabolism has not been proved [3]. Some workers suggest that, besides in intracellular catabolism, cathepsin D can also participate in extracellular protein degradation [2, 5, 9]. Other workers [8, 15] consider that the enzyme acts purely intracellularly, for an acid pH is essential for its activity, whereas the normal pH of the matrix is neutral, namely 7.2 [10]. However, the investigation cited, conducted in vitro, showed that considerable degradation of proteoglycans (one of the chief components of the intercellular matrix) by cathepsin D is observed at pH 7.2 also [10]. Consequently, activity of cathepsin D is maintained at a high level at neutral pH.

The extracellular activity of cathepsin D which was found in vivo is evidence that, besides its role in intracellular degradation of various proteins, cathepsin D is secreted by the liver cells into the extracellular space where it can take part in catabolism of the intercellular matrix.

LITERATURE CITED

1. G. Geyer, Electron Histochemistry [Russian translation], Moscow (1974).
2. A. C. Allison and P. Davies, Mononuclear Phagocytes in Immunity, Infection, and Pathology, R. Van Furth (ed.), Oxford (1975), p. 487.
3. A. J. Barrett, Fed. Proc., 39, 9 (1980).
4. M. C. Burleigh, A. J. Barrett, and G. S. Lazarus, Biochem. J., 137, 387 (1974).
5. J. T. Dingle, Philos. Trans. R. Soc. B, 271, 315 (1975).

6. D. J. Etherington, Biochem. J., **127**, 685 (1972).
7. R. Hynes, Ann. Rev. Cell. Biol., **1**, 67 (1985).
8. G. B. Kolski, J. Levy, S. K. Ackerman, et al., J. Lab. Clin. Med., **110**, 41 (1987).
9. R. J. G. Morrison, A. J. Barrett, J. T. Dingle, and D. Prior, Biochim. Biophys. Acta, **302**, 411 (1973).
10. A. I. Sapolsky, R. D. Altman, J. F. Woessner, and D. S. Howell, J. Clin. Invest., **52**, 524 (1973).
11. P. G. Scott and C. H. Pearson, FEBS Lett., **88**, 41 (1978).
12. R. E. Smith and R. M. Van Frank, Lysosomes in Biology and Pathology, ed. by J. Dingle et al., New York (1975), p. 193.
13. J. C. Van Berkel, J. K. Kruijt, and J. F. Koster, Eur. J. Biochem., **58**, 145 (1975).
14. J. F. Woessner, Tissue Proteinases, ed. by A. J. Barrett et al., Amsterdam (1971), p. 291.
15. S. Yokota, H. Tsuji, and K. Kato, Histochemistry, **82**, 141 (1985).

CORRELATION BETWEEN INTRACELLULAR AND CELLULAR REGENERATION OF THE RENAL TUBULAR EPITHELIUM OF THE KIDNEY IN NECROTIZING NEPHROSIS

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Mercuric chloride (corrosive sublimate) has nephrotoxic properties, and on entering the animal or human body it induces necrosis of the tubular epithelium, accompanied by a varied degree of acute renal failure. This model of toxic necrotizing nephrosis has attracted close attention in connection with the study of the mechanism of action of mercury salts and the principles governing restoration of the structure and function of the renal tubular epithelium since an article was published in 1860 by Pavy [10] describing the effect of mercury on animals, and the place of damage to the renal epithelium was established in rats, rabbits, and dogs [12].

Investigations using transmission and scanning microscopy have revealed the early ultrastructural changes of destructive and regenerative character in the tubular epithelium of the proximal part of the nephron [3-9, 11] and showed that undamaged epitheliocytes may be the source of the proliferating tissue which covers the denuded areas of the basement membrane. Since damaged epithelial cells may also be the source of regeneration of the tubular epithelium [2], the question arises: does intracellular regeneration of the damaged cells take place in mercuric chloride necrotizing nephrosis (MCNN) and, if it does, at what stage of the life cycle does this occur, and what is its connection with the cellular form of regeneration.

In the present investigation, by using information obtained by light and electron autoradiography, an attempt was made to find the answer to the above questions.

EXPERIMENTAL METHOD

Experiments were carried out on male rats weighing 170-210 g. The experimental animals were given a subcutaneous injection of mercuric chloride in a dose of 0.4 mg/100 g body weight, followed 72 h later by injection of ^{3}H-thymidine with specific

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