ELECTRON-HISTOCHEMICAL DETECTION OF THIOL PROTEINASE ACTIVITY IN THE NORMAL AND CIRRHOTIC LIVER

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Thiol (cysteine) proteinases, which are found in many different cells and tissues of animals, play an important role in intracellular protein degradation [4, 5]. Activity of these lysosomal proteinases with different substrates is optimal at pH of about 6.0. It is suggested that thiol proteinases take part in various physiological and pathological processes [3, 8, 10, 14]. Thiol proteinases are known to take part in degradation of the main components of the intercellular matrix, including insoluble collagen in vitro [6, 7, 9, 12].

The aim of this investigation was to discover the part played by thiol proteinases in collagen degradation. For this purpose, the combined activity of two thiol proteinases, cathepsin B and cathepsin H, was determined in the normal and cirrhotic liver.

EXPERIMENTAL METHOD

Noninbred male albino rats weighing initially 150 g were used in the experiments, Cirrhosis of the liver was induced by subcutaneous injection of 50% solution of CCl_4 in olive oil, in a dose of 0.3 ml/100 g body weight twice a week for 15 weeks. Samples of liver for investigation were taken 3 weeks after the last injection of CCl_4. According to our previous data [1], this period of regression of cirrhosis is characterized by a sharp increase in activity of lysosomal hydrolytic enzymes in the liver and intensive lysis of collagen. The liver of intact rats also was studied, The material was subjected to histochemical treatment to detect the combined activity of the two thiol proteinases, cathepsin B and cathepsin H, at the ultrastructural level, according to the principle described in [13] in our modification, using BZ-Arg-2NNUP ("Serva") as the substrate [2]. Other known thiol proteinases (cathepsin L and cathepsin N) do not hydrolyze this substrate [11]. As the control reactions, incubation in medium without substrate and incubation with the addition of the enzyme inhibitor para-chloromercuribenzoate, were used as control reactions.

EXPERIMENTAL RESULTS

Depending on the degree of activity of the enzymes (cathepsin B and cathepsin H) the reaction product appeared either in the form of single granules or in the form of a more or less homogeneous conglomerate of the granules, with varied electron density.

In both the normal and cirrhotic liver an intensive reaction was discovered in the Kupffer cells. The reaction product was found in the lysosomes and also extracellularly, directly on the cytolemma of the macrophages (Fig. 1a). Considerable heterogeneity was observed in the distribution of the reaction product between the lysosomes. A rather intensive reaction for thiol proteinases was observed in lysosomes of endothelial cells (Fig. 1b). The reaction product was found in the hepatocytes in individual lysosomes and in various membrane-derived particles (Fig. 1c).

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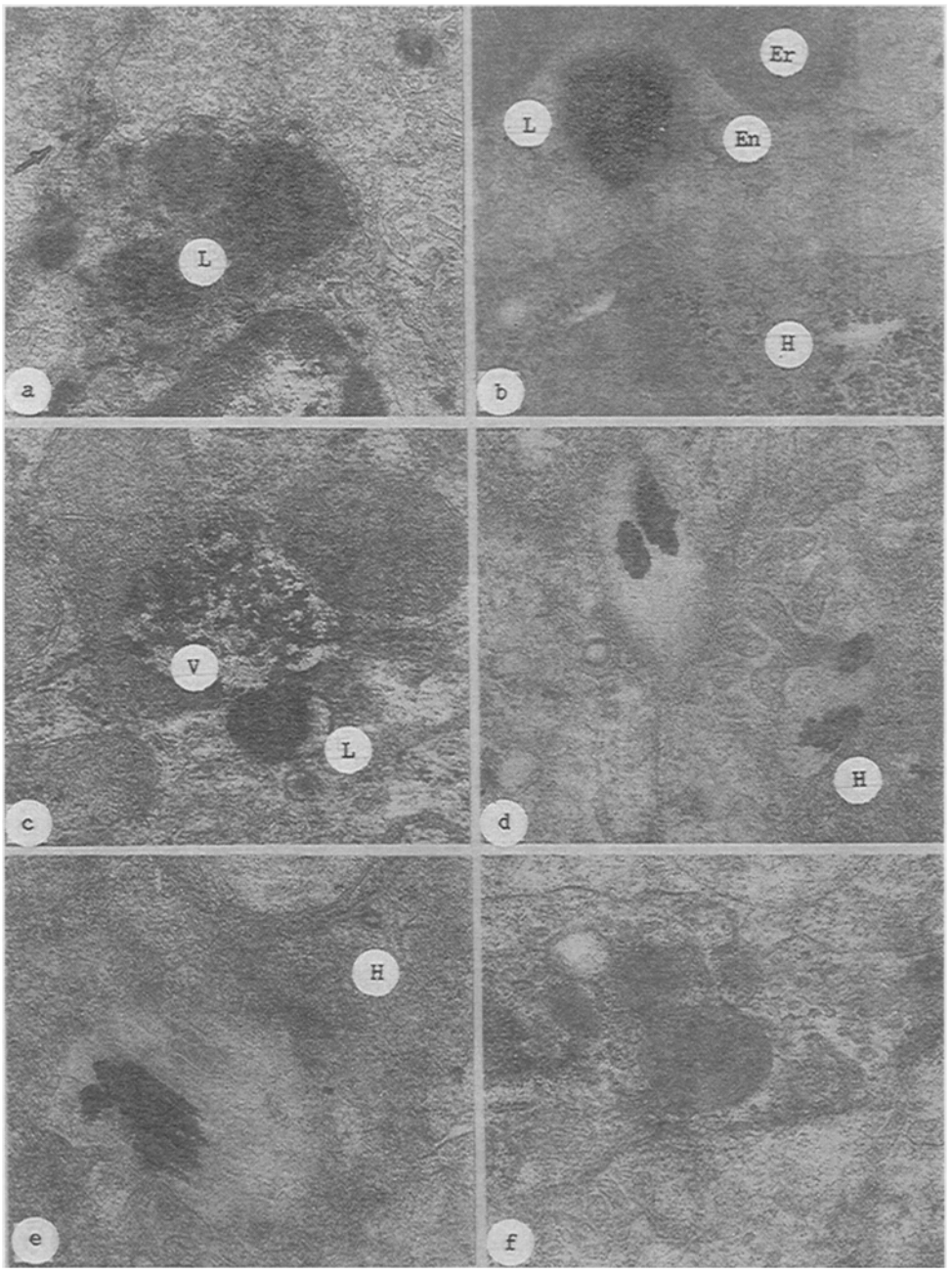

Fig. 1. Intracellular and extracellular thiol proteinase activity in normal and cirrhotic liver: a) intensive reaction in lysosomes (L) of Kupffer cell and also extracellularly, on cytolemma (arrow), 15,000×; b) Intensive reaction in L of endothelial cell (En), H) Hepatocyte, Er) erythrocyte. 15,000×; c) Fusion of L with vacuole (V) in hepatocyte (H) after 20 days of regression of cirrhosis. Due to distribution of reaction products, release of lysosomal enzymes from L into V is clearly visible, 25,000×; d) Intensive reaction extracellularly alongside H and connective-tissue cell. Reaction product lies directly on collagen fibrils. 30,000×; e) Intensive reaction connected with collagen fibrils in collagen bundle "ingested" by H after 3 weeks of regression of cirrhosis. 25,000×; f) Control reaction with enzyme inhibitor para-chloromercuribenzoate. No reaction present. 7000×.

An intensive reaction also was observed in lysosomes of fibroblasts from the portal tracts.

Extracellular thiol proteinase activity, which we found in the normal and cirrhotic liver, deserves particular mention. The reaction product, in the form of long conglomerates, was located directly on the collagen fibrils,

alongside hepatocytes, and also alongside connective-tissue cells (Fig. 1d). During regression of the cirrhosis a much larger quantity of reaction product than normally was observed in the extracellular space (Fig. 1e). The control preparations contained no reaction product (Fig. 1f).

The extracellular activity of the two proteinases, cathepsin B and cathepsin H, which we discovered in vivo in normal and cirrhotic liver tissue is evidence that besides the role in the intracellular degradation of various proteins, thiol proteinases also are secreted by the hepatocytes and connective-tissue cells of the liver into the intercellular space and they can participate in extracellular collagen resoration.

REFERENCES

1. V. V. Ryvnyak, Byull. Éksp. Biol. Med., No. 7, 37 (1988).
2. A. J. Barrett, Analyt. Biochem., **47**, 280 (1972).
3. A. J. Barrett, Acta Biol. Med. Germ., **36**, 1959 (1977).
4. A. J. Barrett, Proteinases in Mammalian Cells and Tissues, Amsterdam (1977), p. 181.
5. A. J. Barrett, Protein Degradation in Health and Disease, Amsterdam (1980), p. 1.
6. M. C. Burleigh, A. J. Barrett, and G. S. Lazarus, Biochem. J., **137**, 387 (1974).
7. M. C. Burleigh, Proteinases in Mammalian Cells and Tissues, Amsterdam (1977), p. 285.
8. Y. Eeckhout and G. Vaes, Biochem, J., **166**, 21 (1977).
9. D. J. Etherington, Biochem. J., **153**, 199 (1976).
10. H. Hanewinkel, J. Glossl, and H, Kresse, J. Biol. Chem., **262**, 12351 (1987).
11. C. Hirayama and Y. Murawaki, Collagen Degradation and Mammalian Collagenase, Amsterdam (1985), p. 166,
12. R. I. G. Morrison, A. J. Barrett, J. T. Dingle, et al., Biochim. Biophys. Acta, **302**, 411 (1973).
13. R. E. Smith and R. M. van Frank, Lysosomes in Biology and Pathology, New York (1975), p. 193.
14. Y. Uchiyama, M. Watanabe, T. Watanabe, et al., Cell Tissue Res., **256**, 355 (1989).

BIOCHEMICAL AND MORPHOLOGICAL CHANGES IN THE MYOCARDIUM OF ACUTE VASCULAR SURGICAL PATIENTS (EARLY AUTOPSY RESULTS)

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Acute surgical states in angiology constitute extremal situations in which the risk of complications and a lethal outcome increases with age. A most suitable method which allows objective evaluation of the state of the myocardium from the morphological and functional standpoints is an enzyme-histochemical investigation [6]. In this connection a combined biochemical and enzyme-histochemical study of enzyme activity and its comparison with the results of histologic investigation, conducted on material from patients dying in a vascular surgical department is particularly interesting.

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