MORPHOLOGY AND PATHOMORPHOLOGY

Electron Histochemical Localization of Cathepsin L in the Liver

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 137, No. 1, pp. 104-105, January, 2004
Original article submitted May 19, 2003

The location of cathepsin L in rat liver was studied by electron histochemical methods. Enzyme activity was detected in lysosomes of hepatocytes, Kupffer cells, and endotheliocytes and extracellularly on hepatocyte microvilli.

Key Words: *cathepsin L; liver; electron histochemistry*

Cathepsin L is an important lysosome proteinase working in a wide spectrum of acid pH (3.5-6.0) [6]. This cysteine proteinase exhibits *in vitro* very high specificity to protein substrates and, specifically, to collagen. The enzyme is involved in many physiological and pathological processes. About 90% intracellular intralysosomal proteolysis is believed to be realized at the expense of coordinated activities of three cysteine proteinases (cathepsins B, H, and L) [1]. Cathepsin L apart from intralysosomal cleavage of proteins and peptides is involved in the processing of bioactive peptides [3], participates in ossification processes [4], invasion of malignant tumors [7,9], induces local increase in the concentrations of matrix-degrading enzymes in inflammation [2]. The enzyme directly participates in spermatogenesis [10]. The participation of cathepsin L in proteolytic cascade in apoptosis is hypothesized [7].

The only study of the location of cathepsin L in the liver was performed at the photooptic level; the enzyme was detected in hepatocytes and Kupffer cells [5].

We investigated the location of cathepsin L at the ultrastructural level.

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MATERIALS AND METHODS

The liver of male albino rats (180-200 g body weight) was examined. The animals were decapitated under ether narcosis. The material was processed by histochemical methods for detecting activity of cathepsin L at the ultrastructural level as described previously [8] using Z-Phe-Arg-4MβNA (Bachem) as the substrate. Control samples were incubated in the absence of the substrate. Some sections were contrasted with uranylacetate, others were not contrasted.

RESULTS

Depending on the activity cathepsin L the product of reaction was seen as solitary small granules or their more or less homogeneous conglomeration of varying density.

The reaction was extremely intensive in Kupffer cell lysosomes (Fig. 1, *a*). In hepatocytes the reaction product was detected in solitary lysosomes and myelin-like bodies (Fig. 1, *b*). Slight extracellular activity of cathepsin L was also detected. Solitary granules of the reaction product were situated on the hepatocyte microvilli in the Disse space (Fig. 1, *c*). Cathepsin L activity was detected in the endothelial cell lysosomes as well (Fig. 1, *c*).

No reaction product was detected in control preparation (Fig. 1, *d*).

0007-4888/04/1371-0090

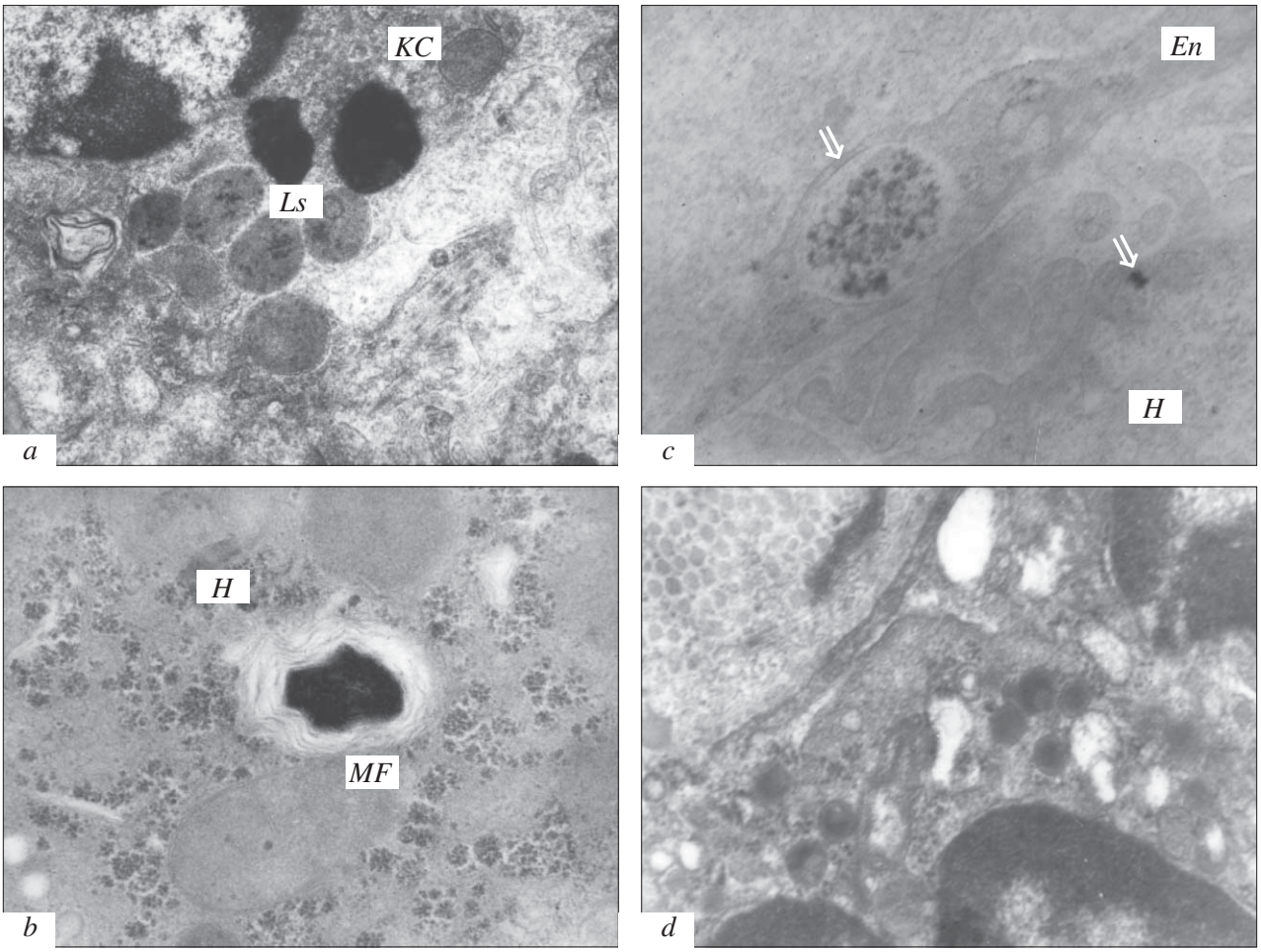

Fig. 1. Localization of cathepsin L in the liver. *a*) intensive reaction for cathepsin L in Kupffer cell (*KC*) lysosomes (*Ls*), ×20,000; *b*) cathepsin L reaction in myelin-like figures (*MF*) of hepatocyte (*H*), ×20,000; *c*) cathepsin L reaction product (arrow) in endotheliocyte (*En*) lysosomes and on hepatocyte (*H*) microvillus, ×30,000; *d*) control (no reaction product), ×15,000.

Hence, our findings suggest that cathepsin L in the liver is located in lysosomes of hepatocytes, Kupffer cells, and endotheliocytes and extracellularly on hepatocyte microvilli.

REFERENCES

1. P. Bohley, H. Kirschke, J. Langner, *et al.*, *Acta Biol. Med. Germ.*, **35**, 301-307 (1976).
2. E. Fiebiger, R. Maehr, J. Villadangos, *et al.*, *J. Exp. Med.*, **169**, No. 9, 1263-1269 (2002).
3. M. Furuhashi, A. Nakahara, H. Fukutomi, *et al.*, *Histochemistry*, **95**, No. 3, 231-239 (1991).
4. K. E. Glaser, M. E. Davies, and L. B. Jeffcott, *Equine Vet. J.*, **35**, No. 1, 42-47 (2003).
5. K. Ii, K. Hizawa, E. Kominami, *et al.*, *J. Histochem. Cytochem.*, **33**, 1173-1175 (1985).
6. H. Kirschke, *Acta Biol. Med. Germ.*, **40**, 1427-1432 (1981).
7. N. Levicar, R. A. Dewey, E. Daley, *et al.*, *Cancer Gene Ther.*, **10**, No. 2, 141-151 (2003).
8. R. E. Smith and R. M. Van Frank, *Lysosomes in Biology and Pathology*, New York (1975), pp. 123-249.
9. A. Wille, A. Heimburg, A. Gerber, *et al.*, *Biol. Germ.*, **383**, Nos. 7-8, 1291-1296 (2002).
10. W. W. Wright, L. Smith, C. Kerr, and M. Charron, *Biol. Reprod.*, **68**, No. 2, 680-687 (2003).