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Immunoexpression of cathepsin D in rat salivary glands

Cathepsins are a group of lysosomal proteases, which play an important role in the intracellular degradation of exo-and endogenous proteins, activation of enzyme precursors, biosynthesis of peptide hormones, cell growth and maturation. They are also involved in the tumors invasion and metastasis, autoimmune diseases and neuronal degeneration (8). Based on the mechanism of enzyme activity, the structure of catalytic centre, optimum of pH and sensitivity to inhibitors cathepsins were divided by the International Union of Biochemical and Molecular Biology into four primary groups: cysteine carboxypeptidases, e.g., cathepsin X; serine endopeptidases, e.g., cathepsin G; cysteine endopeptidases, e.g., B, L and K; and aspartic endopeptidases, e.g., cathepsin D and E (2, 8).

Cathepsins are stored mainly in lysosomes. However, the enzymes intracellular capacity can be divided into bound and free fraction. The bound fraction comprises all active forms of enzymes and proenzymes located inside the endoplasmic reticulum, Golgi apparatus and lysosomes. The free fraction contains only active forms of enzymes present in the cytoplasm and is not connected with any endoplasmatic membranes (10). The tissue and cellular distribution of the enzymes varies greatly among the organs (5, 10, 11,13).

One of the most extensively studied enzyme from this group of proteases is cathepsin D (EC 3.4.23.5), which regulates intracellular proteolysis and protein turnover. It is characterized by molecular weight of 42KD and optimal activity at pH 3.8 (5).

The present study was undertaken to evaluate the localization of cathepsin D in the salivary glands in rats.

MATERIAL AND METHODS

The experiment was designed in accordance with international guidelines and the guidelines #0038/ 2000 of the Local Bioethical Committee.

Sexually mature albino rats of Wistar CRL:(WI)WUBR strain, obtained from a commercial breeder (Warsaw-Rembertów, Poland) were used. The rats were acclimated for at least 2 weeks, housed and maintained in an animal care facility, as described before (3, 4). All the examined animals came from the control group used in other studies and were not exposed to any xenobiotics through the life. The animals were sacrificed on day 84 after the quarantine that corresponds to the 22nd week of postnatal life.

The mandibular, parotid and sublingual glands were dissected during autopsy, fixed in 10% buffered formaldehyde solution, routinely processed, embedded into paraffin blocks and sectioned into 4 μm slides. The Cathepsin D Kit (NCL-Cath-Paraffin, Novocastra Laboratories Ltd.; New Castle, UK) with monoclonal mouse anti-human cathepsin D antibody (clone C5) was used according to the manufacturer directions. Epitope retrieval was applied with two cycles of heating in the microwave oven at 750 W for 5 minutes. The positive control was the sample of invasive ductal breast cancer, known to be strongly cathepsin D-positive. The negative control was the section treated in the same way as in the study group, but with the omission of the primary antibody. The evolution of immunostaining was performed in light microscope (Olympus BX45).

RESULTS

In the sublingual glands, the immunexpression of cathepsin D was found in the epithelial cells of the striated ducts and in the myoepithelial cells of the acini (Fig. 1 A-B). The staining pattern was cytoplasmic and granular. In the cells of striated ducts the staining was slightly more intense in the basal side of cells (Fig. 1B). The secretory mucous cells of acini were cathepsin D-negative.

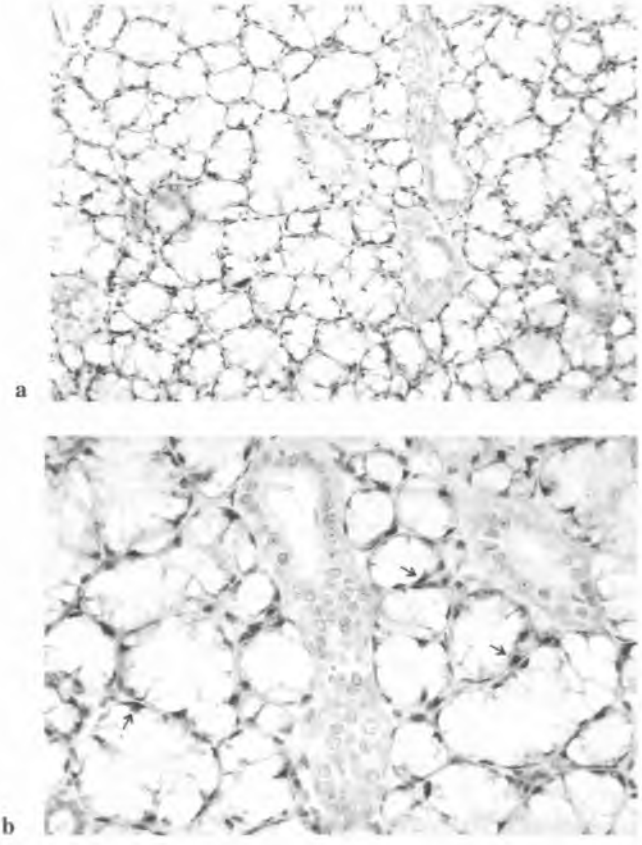

Fig. 1. Positive immunexpression of cathepsin D in the epithelial cells of the striated ducts and myoepithelial cells (arrows) whereas negative in the secretory mucinous cells of the rat sublingual gland (ABComplex/HRP; magn. A – x100; B – x200)

In the mandibular glands, the immunexpression of cathepsin D was similar as far as the striated ducts cells and myoepithelial cells is concerned, however the serous cells of acini revealed fine granular cytoplasmic reaction whereas the mucinous cells were negative (Fig. 2 A-B).

In parotid glands the secretory serous cells of acini exhibited staining pattern similar to that observed in serous component of the mandibular gland. Furthermore, positive reaction with anti-cathepsin D antibody was also found in the endothelial cells of the blood vessels and thrombocyte plugs. This reaction served as an internal positive control for evaluated slides.

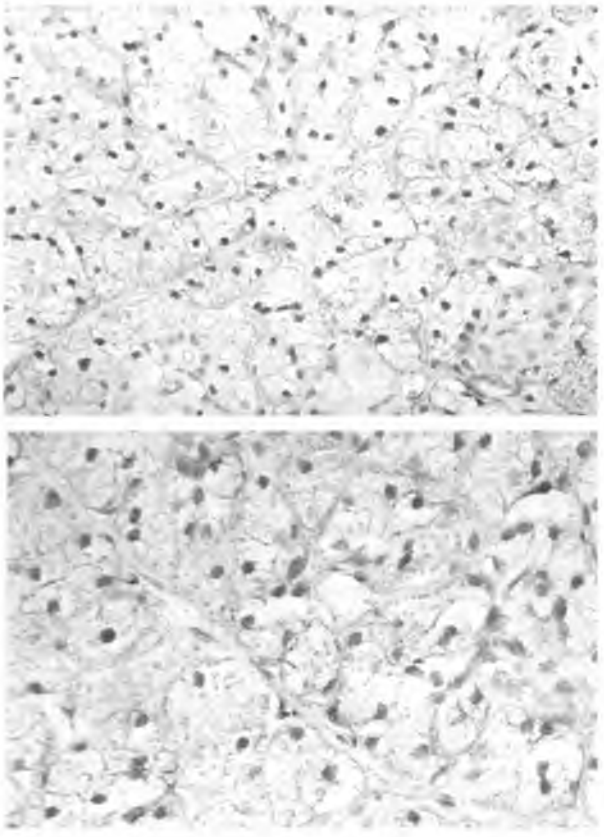

Fig. 2. Positive immunexpression of cathepsin D in the serous cells of the rat mandibular gland (ABComplex/HRP; magn. A – x200; B – x400)

DISCUSSION

Activity of cathepsin D in salivary glands was previously detected using various biochemical and immunoprecipitation methods in rat (9), rabbit (9) and human (1, 10, 13). In most of the studies it was seen that the cathepsin activity is relatively low when compared with other lysosomal enzymes like acid phosphatase, beta-galactosidase, beta-N-acetyl-glucosaminidase and lipase (3, 4). However, the activity of cathepsin D depends on the organ, or even the type of cells. According to Sakai (11), it is ubiquitously distributed in all of the tissues and cells, except for erythrocytes. Based on those observations, the highest activity of cathepsin D characterizes lacrimal gland and stomach mucosa,

while skeletal muscles, thrombocytes and lymphocytes have very low enzyme concentration. Gastrointestinal tract (e.g. stomach, colon) and lymphatic system (e.g. lymph node, thymus) present lower levels of cathepsin D than the E one. Similar to endocrine organs, cathepsin D was well detected in epithelial cells of exocrine glands, like salivary, lacrimal and pancreatic glands (11).

Immunoelectron microscopic techniques confirmed mostly lysosomal localization of cathepsin D (5, 12, 14). Such observations were partially confirmed in biochemical studies. The free cytoplasmatic fraction was higher than the bound one in hepatocytes (2, 3) but higher bound fraction activity was measured in neutrophils (11). The lysosomal localization of cathepsin D in the parotid gland was recently proved. The average number of gold particles with anti-cathepsin D in lysosomes reached 68.25 per μm^2, while in cytoplasmatic granules and nucleus was 0.89 and 0.55 per μm^2, respectively (12). The cisternal stock in Golgi apparatus and in lysosomes subcellular localization in parotid gland was confirmed in another paper (14).

The enzyme activity and immunostaining increased in various pathological processes of salivary glands. Kawasaki et al. (6) reported high activation of cathepsin D in parotid glands after the exposure to polychlorinated biphenyl. Overexpression was also reported in salivary gland tumors (13). The enzyme activity was significantly higher in carcinomas when compared with mixed tumors. Immunoreactivity in cancer cell of the primary tumor also correlated with an increased risk of metastasis. The positive correlation between expression of cathepsin D in salivary adenoid cystic carcinoma and distant metastases, but not with the histological type, local recurrence and survival rate was also reported (10). However, according to Barnes et al. (1) expression of cathepsin D is not a prognostic factor in salivary duct carcinoma.

The present study confirmed the cross-reactivity of the used human antibody for the rat tissues. It is secondary to the high homology of the cathepsin D amino acid sequence among various species, including rats and humans (5).

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SUMMARY

Cathepsin D is an aspartic proteinase that regulates intracellular proteolysis and protein turnover. The aim of the study was to evaluate the enzyme imunoexpression in salivary glands in untreated mature Wistar rats. Positive immunexpression of cathepsin D was found in the epithelial cells of the striated ducts and in the myoepithelial cells of the sublingual, parotid and mandibular glands. The immunoreactivity was revealed in the serous cells of the mandibular and parotid glands whereas the mucous cells of the sublingual and mandibular glands were negative.

Immunoekspresja katepsyny D w śliniankach szczura

Katepsyna D jest jedną z proteaz aspartylowych regulujących proteolizę wewnątrzkomórkową i obrót białkowy. Celem pracy była ocena immunoekspresji enzymu w śliniankach u dorosłych nieleczonych szczurów szczepu Wistar. Dodatni odczyn immunohistochemiczny z przeciwciałem przeciwko katepsynie D wykazano w komórkach nabłonka przewodów prążkowanych oraz w komórkach mioepitelialnych ślinianki podjęzykowej, żuchwowej i przyusznicy. Dodatni odczyn obserwowano także w komórkach surowiczych ślinianki żuchwowej i przyusznicy, natomiast ujemny w komórkach śluzowych ślinianki podjęzykowej i żuchwowej.