Original Article

LOCALIZATION OF THE ENZYME EXPRESSION OF TISSUE ALKALINE AND ACID PHOSPHATASES IN THE BULBOURETHRAL GLANDS OF THE TOMCAT

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Summary

The alkaline and acid phosphatases are hydrolytic enzymes, whose tissue localization is a probable indicator about malignity, benignity or intactness in the bulbourethral glands of the tomcat. Bulbourethral glands of six clinically healthy, sexually matured tomcats were studied. The animals were euthanized. Proving of the acid phosphatase by Gomori: we used frozen slices, with thickness 10 μm, fixed in 10% neutral formalin. They were put on objective slides. The slices were translocated in an incubating medium, treated with Ammonium sulfide, who stained them dark brown. They were included in glycerine-gelatine. Proving of alkaline phosphatase by Gomori: the same frozen slices were transferred in an incubating medium, they were treated with Cobaltous chlorate afterwards and translocated in Amonium sulfide till they get black colour.The most considerable localization of the tissue acid phosphatase activity was found lightmycroscopically in the apical parts of the glandular epithelial cells, in the lumen and the basal parts of the glandular tubules in the feline bulbourethral glands. Higher enzyme activity was found closely the basal membranes and lower one-in the perilobular interstitium. A remarkable expression of tissue alkaline phosphatase in the bulbourethral glands of the tomcat was hystochemically remarked. A high activity of tissue alkaline phosphatase was found in the basal parts of the epithelial cells, while this one was missing in their apical parts.

***Key words*:** phosphatases, bulbourethral glands, tomcat

Introduction

The alkaline phosphatase is a hydrolytic enzyme, who is synthesized in the liver, bones and placenta. It is presented normally in high concentrations in the growing bones and the bile. Its level increases in case of bone metastatic prostatic cancer.

According to [1] the quality of the bone alkaline phosphatase is indicator for bone metastases and development of prosatic cancer.

The acid phosphatase is also a hydrolytic enzyme, who is catalyzed in an acid medium and is synthesized in the liver, spleen, bone marrow and prostate. The high levels of this enzyme are indicatior for glandular pathology [2].

The expression of the both enzymes is investigated in the tomcat`s prostate gland, where enzymes` localization is found in the glandular parenchyma and stroma [3].

In the dromedary camel the acid phosphatase activity

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is mostly expressed in the prostate gland, moderately-in the urethra, there are traces of acid phosphatase activity in the bulbourethral glands and of alkaline phosphatase activity-in the apical cytoplasm of the glandular epithelium in the bulbourethral and prostate glands [4].

In the dog`s male sex organs is found, that the activity of the alkaline phosphatase is highest in the epididymis, and it is considerably lower in the testis and the prostate. The results prove that the biggest fraction of this enzyme is secreted by the epididymis, and not by the prostate [5].

The epithelial cells from the transitional epithelium demonstrate a high alkaline phosphatase activity, while these ones from the other kinds of epithelium-a low expression of this enzyme. There aren't such enzyme histochemical differences in the acid phosphatase activity in these two types of cells [6].

In the man the tissue acid phosphatase activity exists in four isoenzyme forms. The lysosomal and the erythrocyte ones are expressed in more cells, while the macrophagic and the prostate ones are with limited expression. The variations in the activity of the tissue prostate acid phosphatase are used as diagnostic and prognostic marker about the prostatic cancerogenesis [7].

There isn't correlation between the acid phosphatase activity, the individual age, the degree of the disease and the prostatic cancer [8].

The acid phosphatase isoenzymes in the prostate of the man vary in its carcinomatosis lesions, compared with the serum acid phosphatase, where it is stabile.These isoenzymes are found in malignant and fetal prostate, and they are absent in the intact gland [9].

The tissue acid phosphatase, in benignant prostate glands is distributed in the cylindrical epithelial secretory cells, compared with the basal ones, where it is absent [10].

There is a decreased tissue capacity for producing of acid phosphatase in big part of the malignant prostatic lesions, compared with the intact tissues [11].

The prostatic acid phosphatase is a tissue specific enzyme, whose localization is in the epithelium of the tubular and alveolar parenchyma in the ventral prostate of the rat [12].

The scarce data about the localization of the alkaline and acid phosphatase activity in the tomcat`s bulbourethral gland, and the probable importance of these enzymes in the malignant and benignant diseases of this gland, motivated us to make this investigation.

Materials and Methods

Proving of acid phosphatase by Gomori

Prostate glands of six clinically healthy and sexually matured European shorthair tomcats (aged between one to two years) and with weight 2,8 to 4 kg were investigated. The animals were euthanized with 200mg Thiopental (Biochemie, Austria) iv-in V. cephalica. We used frozen slices with thickness 10 m, fixed in 10% neutral foprmalin for 24, at temperature 0°-4° C. They were put on objective slides afterwards. The slices were translocated in an incubating medium and put in a termostat at 37° C for 3 h. They were washed with distilled water, treated with Ammonium sulfide for 1 min., who satined them dark brown. They were included in glycerine-gelatine afterwards [13].

Proving of alkaline phosphatase by Gomoi

The same frozen slices were translocated in an incubating medium and put in thermostat at 37° C for 2 h, they were treated with Cobaltous chlorate for 3 min and translocated in Ammonium sulfide, till they get black colour [13].

The localization of the tissue alkaline and acid phosphatase enzyme expression were determined lightmicroscopically.

The studies are made, following strictly keeping of ethic principles and law-making requirements about animal wellcare, according to article 58, paragraph 1 and article 60, paragraph 1, 2, 3 from the Low of Biological Diversity (Government News, number 77/09. 08. 2002).

Results

The enzyme histochemical investigation demonstrated that the most considerable tissue acid phosphatase activity is found in the apical parts of the glandular epithelial cells, in the lumen and the basal parts of the glandular tubules in the tomcat`s bulbourethral gland. Increased enzyme activity was found closely the basal membranes, and lower expression-in the perilobular interstitium. In the perivascular parts of the glandular vessels and in the periphery of the bulbourethral ductules, the enzyme activity was remarkable too. The lowest acid phosphatase activity was remarked in the glandular interstitium (Figure 1).

Moderate enzyme expression was seen in the loose fibrous connective tissue, between the skeletal muscle cells, and in the dense connective tissue of the glandular adventitia, whereas in the muscle cells the glandular activity was lowly expressed (Figure 2).

A high tissue alkaline phosphatase activity, in the tomcat`s bulbourethral gland was found in the basal parts of the epithelial cells, this one was absent in their apical parts. It was remarked, that in the lumen of the glandular tubules, alkaline phosphatase expression was observed only in the cases when they were filled with secretory-like unnuclear matter (Figure 3).

The glandular epithelial cells demonstrated a low alkaline phosphatase activity. A high enzyme expression was found in the loose fibrous connective tissue, located between the glandular tubules and the skeleton muscles layers. The adventitia demonstrated a low enzyme activity (Figure 4).

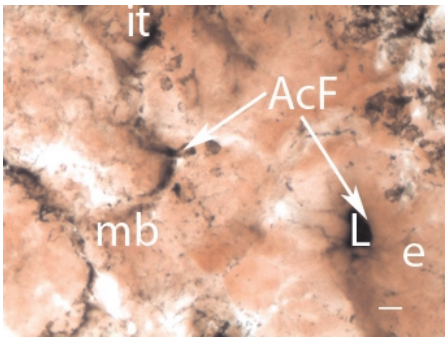

Figure 1. Region from the bulbourethral gland, indicating the localization of the acid phosphatase activity (AcF) in the epithelial cells (e) and the tubular lumen (L); interstitium (it). By Gomori. Bar = 10μm.

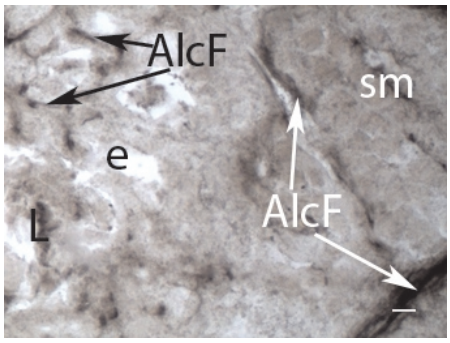

Figure 3. Region from the bulbourethral gland, indicating alkaline phosphatase activity (AlcF) in the epithelium (e) and around the skeleton muscle cells (sm); lumen (L). By Gomori. Bar= 20μm.

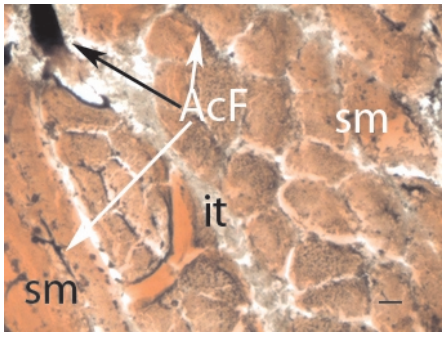

Figure 2. Region from the bulbourethral gland, indicating the acid phosphatase expression (AcF) around the skeleton muscle cells (sm); interstitium (it). By Gomori. Bar = 30μm

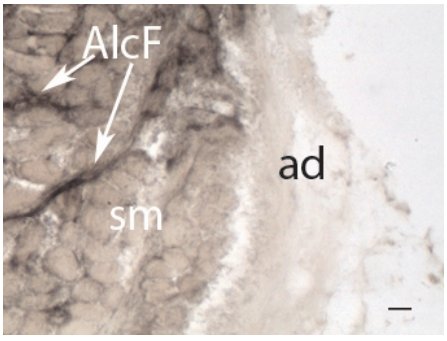

Figure 4. Region from the bulbourethral gland, indicating alkaline phosphatase activity (AlcF) between the skeleton muscle cells (sm); adventitia (ad). By Gomori. Bar = 40μm.

Discussion

Via our results, it could be make the conclusion that in the feline bulbourethral glands, the most considerable expression of tissue alkaline phosphatase activity is marked in the apical parts of the epithelial cells, and in the lumen and basal parts of the glandular tubules. That confirms the studies of [9, 10, 12] about the human prostate.

The high enzyme activity, observed by us in the basal membranes and in lower degree in the perilobular interstitium, support the confirmation of [11], about the human prostate gland. These facts are identical with our results about the expression of this enzyme in the tomcat [3]. Therefore, the bulbourethral glands in this animal species are enzyme histochemically similar to the prostate and its disseminated part, and that proves the high secretory activity of these three glandular parts.

The enzyme expression was considerable in the perivascular parts of the glandular vessels and in the periphery of the bulbourethral glands, and that according to [7, 8] is probably connected with the communications between the vessel wall and the glandular parenchyma, and between the draining ductules and the stroma.

The lowest acid phosphtase activity was found in the glandular interstitium, which is probably connected with its lower contribution about the secretory activity.

The moderate acid phosphatase expression, observed by us in the loose connective tissue, between the skeleton muscle cells, and in the dense connective tissue of the glandular stroma, was corresponding to the enzyme expression in the same structures of the pelvic urethra.

The high tissue alkaline phosphatase activity in the tomcat`s bulbourethral gland, expressed in the basal parts of the epithelial cells, was missing in their apical parts. Our results about the prostate gland and the pelvic urethra are similar, and they correspond with these ones of [6] about the man.

Alkaline phosphatase activity, in the lumen of the glandular tubules, was observed only when they were filled with secretory-like matter, compared to the same ones in the prostate and the pelvic urethra [3]. That motivated us to make the supposition about its lower expression in the bulbourethral epithelium and its lower secretory activity.

The glandular epithelial cells demonstrated a comparatively low alkaline phosphatase activity in the epithelium and in the pelvic urethra, which corresponds with the investigations in these organs of the camel [4].

A high alkaline phosphatase activity was observed in the loose fibrous connective tissue, located between the skeleton muscle cells, whereas the adventitia demonstrated a low one. These results are similar to our data about the feline prostate and urethra, and to these ones about the dog [5].

Conclusion

According to us, the enzyme histochemical parameters of the bulbourethral gland are aggregate from the same ones in the prostate gland and the pelvic urethra, which motivates us to make a conclusion about the intermediate character of the enzyme phosphatase features of these glands.

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