

Original Article

LOCALIZATION OF THE ENZYME EXPRESSION OF TISSUE ALKALINE AND ACID PHOSPHATASES IN THE FELINE PELVIC URETHRA

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Summary

The acid and alkaline phosphatases are hydrolases, whose tissue expression is a probable marker about the proceeding of normal and pathologic processes in feline the pelvic urethra. Pelvic urethras of six clinically healthy and sexually matured tomcats were studied. The animals were euthanized. Proving of acid phosphatase by Gomori: we used frozen slices with thickness 10 µm, fixed in 10% neutral formaline. Afterwards they were put on objective slides. The slices were translocated in an incubating medium and 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 translocated in an incubating medium, treated with 2% Cobaltous chlorate afterwards and translocated in Ammonium sulfide, till they get black colour. Via enzyme histochemical investigation of the pelvic urethra was found that the most considerable tissue acidphosphatase activity is found in the propria and the adventitia of the pelvic urethra, following by the epithelial cells' apical parts of the disseminated prostate and in the lumen of the glandular tubules. A remarkable tissue alkaline phosphatase expression is marked in the basal parts of the epithelial cells and in the lobules' peripheral zones of the disseminated prostate. The perivascular regions and the urethral adventitia demonstrated also an increased enzyme activity. Alkaline phosphatase expression wasn't observed in the lumen of the disseminated prostatic alveolus.

Key words: alkalinephosphatase, acid phosphatase, urethra, tomcat.

Introduction

The alkaline phosphatase expression in the seminal liquid is a marker about the presence of epididymal fluid in the ejaculate of the dog and about its quality in case of asospermia and it is used as indicator about the degree of the tubular lesions in the epididymis. Compared to the alkaline one, the acid phosphatase is a contradictive indicator about the diagnosis of carcinoma in the accessory sex glands [1].

The tissue activity of the both enzymes is investigated in the prostate gland of the tomcat, where their localization is found in the parenchyma and the stroma of the gland [2].

A lot of authors investigate the expression of the tissue alkaline and acid phosphatases in swine endometrium, before and after fecundation, for early provement of pregnancy. They observe a high alkaline phosphatase

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activity in the basal parts of the glandular uterine epithelium, and a high acid phosphatase one-in the apical parts [3].

The enzyme activity of the tissue alkaline and acid phosphatases activities is studied by [4] in the renal primordiums of the rat. The alkaline phosphatase expression is found in the luminal part of the differentiating proximal tubules, whereas the acid phosphatase one-in the cytoplasm of all morphologically determined cells of the nephron.

The ratio between the prostatic specific antigen and the prostatic acid phosphatase is a remarkable indicator about the prostatic cancerogenesis in the man [5].

The acid phosphatase in the Golgi complex, in the rat's prostate is different from this one in the lysosomes [6].

In the rodents the vesiculosus and bulbourethral glands demonstrate a high alkaline and acid phosphatase expression in the epithelium, but in the bulbourethral gland the alkaline phosphatase is expressed only in the stroma. There isn't any phosphatase activity in the prostatic epithelium and secretion [7].

The decreasing alkaline phosphatase level in the monitoring of the disseminated prostate cancer is a biochemical marker about positive prognosis [8].

Via enzyme histochemical investigation of the dog's male sex organs is found that the activity of the alkaline phosphatase is highest in the epididymis, considerably lower in the testis and the prostate. The results prove, that the biggest fraction of this enzyme in the seminal plasma is secreted by the epididymis, and not by the prostate [9].

In the male sex organs (rat, guinea pig, rabbit, dog and cat) a different localization of acid and alkaline phosphatase activity is found. The alkaline one is presented as secretory, stromal, nuclear, and vessel, and the acid one as secretory and nuclear [10].

According to the other authors, the decreased levels of alkaline and acid phosphatase activity are observed in benignant and malignant prostates [11].

Via enzyme histochemical investigation of the rat's prostate, the lysosomal acid phosphatase activity increases after castration, compared to the activity of the secretory one, who decreases after androgenous deprivation [12].

The scarce data about the localization of the alkaline and acid phosphatase activity in the tomcat's pelvic urethra, motivated us to make this enzyme histochemical investigation.

Our aim was founding the different parts of the normal feline pelvic urethra, in which there is activity of the hydrolases, investigated by us.

Materials and Methods

Proving of acid phosphatase by Gomori

Pelvic urethras 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 formalin 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 thermostat at 37° C for 3 h. They were washed with distilled water, treated with Ammonium sulfide for 1 min., who stained them dark brown. They were included in glycerine-gelatine afterwards [13].

Proving of alkaline phosphatase by Gomori

The same frozen slices were translocated in an incubating medium and put in a 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 was determined lightmicroscopically.

The studies are made, following strictly keeping of ethic principles and law-making requirements about animal welfare, according to article 58, paragraph 1 and article 60, paragraph 1, 2, 3 from the Law of Biological Diversity (Government News, number 77/09.08.2002.

Results

Via enzyme histochemical study was found, that the most considerable localization of tissue acid phosphatase activity was observed in the pelvic urethra's propria and adventitia, following by the epithelial cells' apical parts in the disseminated prostate and in the lumen of the glandular tubules. Enzyme expression was seen in the basal membranes and in the subcapsular zone of the gland (Figures 1 and 2).

In the perivascular regions of the vessels, in the propria and the adventitia, the enzyme expression was remarkable. The lowest acid phosphatase reaction was observed in the basal and middle zones of the epithelial cells in the disseminated prostate (Figure 1).

Well expressed acid phosphatase activity was found in the loose fibrous connective tissue, located between the skeletal muscle fibers (Figure 2).

A remarkable tissue alkaline phosphatase expression in the urethra, was marked in the epithelial cells' basal parts and in the peripheral zones of disseminated prostate gland's lobules. The perivascular regions and the urethral adventitia demonstrated also a high enzyme activity. There wasn't enzyme reaction in the tubules' lumen of the disseminated prostate. A low alkaline phosphatase reactivity was seen in their epithelial cells (Figure 3 and 4).

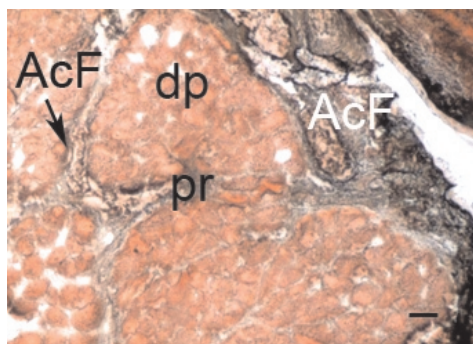


Figure 1. Localization of acid phosphatase activity (AcF) in the wall of the pelvic urethra-disseminated (dp), propria (pr). By Gomori. Bar = 50 μ m.

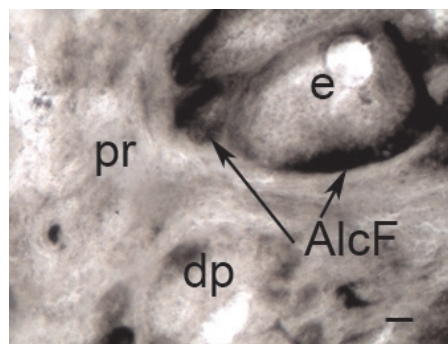


Figure 3. Alkaline phosphatase expression (AlcF) in the wall of the pelvic urethra-prostatic epithelium (e), propria (pr), disseminated prostate (dp). By Gomori.

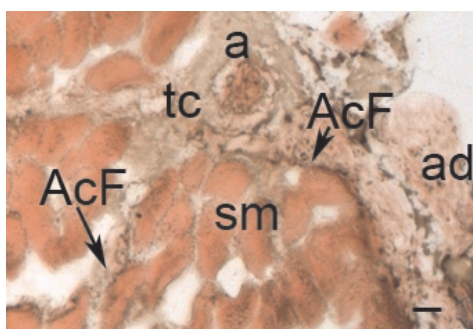


Figure 2. Acid phosphatase expression (AcF) in the wall of the pelvic urethra-connective tissue (tc), skeletal muscle cells (sm), adventitia (ad). By Gomori. Bar = 50 μ m.

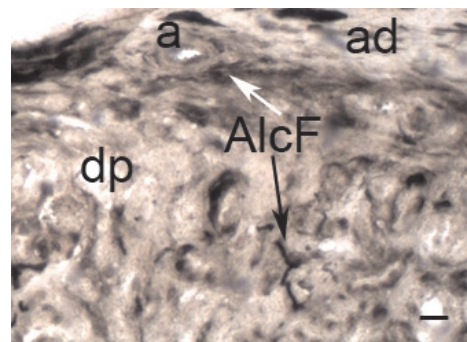


Figure 4. Alkaline phosphatase expression (AlcF) in the wall of the pelvic urethra-disseminated prostate (dp), arteriola (a), adventitia (ad). By Gomori. Bar = 25 μ m.

Discussion

The results, obtained via enzyme histochemical study, demonstrated, that the most considerable tissue acid phosphatase activity was observed in the propria and adventitia of the pelvic urethra, compared with the interstitium of the disseminated prostate. The enzyme activity, found in the epithelial cells' apical parts of the disseminated prostate and in the glandular alveolus' lumen, coincides with the results about the prostate in the other animal species [2, 3, 7, 10].

The enzyme expression in the basal membranes and in the zone under the adventitia is similar to the obtained results about the prostate in the rat and the man [4, 6, 12].

A distinct enzyme expression in the perivascular regions of the vessels, in the propria and adventia, corresponds with the observations in the human prostate, made by [5, 8].

The lowest acid phosphatase reaction was found in the basal and middle zones of disseminated prostate's epithelial cells, which corresponds with the enzyme expression in the epithelial cells from the glandular body [2].

The good acid phosphatase activity, observed in

the loose fibrous connective tissue, located between the skeletal muscle fibers, as it is concluded, similar to the enzyme activity in the propria of the pelvic urethra and the stroma of the feline prostate [2].

The obtained data indicate, that a distinct tissue alkaline phosphatase expression in the feline pelvic urethra, is marked histochemically in the epithelial cells' basal parts and in the peripheral zones of the prostatic lobules in the disseminated prostate. They coincide with our investigations about feline prostate gland [2].

The high enzyme activity, observed in the perivascular regions and the urethral adventitia, corresponds with the activity in the prostate gland of the tomcat [2], of the man [11], of the dog [1, 9], and the rodents [7, 10].

Conclusion

Alkaline phosphatase expression wasn't found in the lumen of the disseminated prostatic alveolus, whereas their epithelial cells demonstrated a low alkaline phosphatase activity, which coincides with our investigations about the feline prostate [2].

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