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Original Contribution

EXPRESSION OF TISSUE ALKALINE AND ACID PHOSPHATASES IN THE PARANAL SINUSES AND EXTERNAL ANAL SPHINCTER OF SEXUALLY IMMATURE DOGS

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ABSTRACT

In this study, the localization of activities of the tissue enzymes alkaline and acid phosphatases has been established in the stratified squamous cornified epithelium, the sweat and sebaceous glands, the paranal sinus stroma and the external anal sphincter in sexually immature dogs from both genders. Both enzymes were detected by the Gomori's method. The results showed that both enzymes were important for the normal functioning of the organ at an early age. Most probably, these enzymes could be used as markers in the diagnosis of various pathologic states affecting the studied organ.

Key Words: alkaline and acid phosphatase activity, sinus paranalis, dog

INTRODUCTION

Alkaline phosphatase (ALP) is a membrane-bound enzyme that catalyzes the hydrolysis of organic and inorganic monophosphate esters in medium of alkaline pH from 9.8 to 10.5 It is known to be involved in membrane transport processes (1). The role of ALP in hair biology and pathology has been elucidated (2). The main isoenzymes of ALP are synthesized in the liver, bones, intestines and the placenta (3, 4, 5).

Acid phosphatase (ACF) is a lysosome marker (6,7). Its pH optimum is in acid environment (4.5). The enzyme is encountered in high concentrations in prostate gland, bones, blood cells, the spleen and reticuloendothelial cells. Several ACF isoenzymes are known, the prostatic one being of greatest importance in laboratory diagnostics (8).

The localization of both enzymes is established in a number of organs in animals and humans (9, 1, 7, 10) determined ALP localization and ACF expression in anal, apocrine and sebaceous glands in the dog. The localization of both enzymes in the canine paranal sinus (PS) has been reported (11). The alterations in tissue ACF activity in the prostate gland are utilized as important diagnostic and prognostic markers in prostate gland carcinogenesis (12, 13). The reduced alkaline phosphatase levels in disseminated prostatic cancer are a biochemical marker for good prognosis (14).

The present investigation was motivated by the lack of literature data about the localization of enzyme activities of ALP and ACF in the paranal sinus of sexually immature dogs.

MATERIALS AND METHODS

The material for the study was obtained from the paranal sinuses of 14 mongrel dogs, after euthanasia with 500 mg Thiopental (Biochemie, Austria), applied i.v. Six male and 6 female dogs aged 1–2 months were used.

Frozen tissue cross sections with a thickness of 8 µm, fixed in neutral 10% formalin (24 hours at 0-4^{o} C) were used. The alkaline phosphatase activity (ALP) was demonstrated by the Gomori's method (15): the cross sections were put in incubation medium for 4 hours at 37 ^{o} C, then washed under running water, treated with 2% cobalt (II) chloride for 3-5 min, washed in distilled

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water, treated with ammonium sulphide until it turned black. Then it was washed in distilled water, dehydrated, cleared and embedded in Entellan. The presence of the enzyme in tissue was demonstrated by the presence of black cobalt sulphide deposits.

The localization of acid phosphatase (ACF) activity was determined by the Gomori's method by putting the cross sections in incubation medium for 4 hours at 37 ^{o} C, washed in distilled water, treated with ammonium sulphide for 1 min, washed in distilled water and embedding in glycerol-gelatin. The obtained black-brownish deposit of lead nitrate was indicative of ACF activity. The light microscopy and microphotography of exhibited histochemical reactivity were carried out with a light microscope (Hund Germany) and Digital camera MDCE-5.

RESULTS

High alkaline phosphatase (ALP) activity was established in Stratum basale, Stratum spinosum and Stratum granulosum of the stratified squamous cornified epithelium of PS. In the stroma of the sinus, moderate enzymatic reaction was detected in single cells. In the secretory cells of apocrine glands, a strong histochemical reaction was observed in the basal part of cells, adjacent to the basement membrane as well as a moderate reaction among adjacent secretory cells (**Figure 1**). The blood vessels of the microcirculation bed around the secretory tubules manifested a moderate to strong expression of the enzyme. In the germinative cells of sebaceous glands in the excretory PS duct, a strong reaction was observed (**Figure 2**). Moderate to weak histochemical reaction was observed in centrally located cells. A strong enzymatic activity was present in the endomysium and perimysium of the external anal sphincter.

A strong to moderate acid phosphatase (ACF) activity was observed in all layers of the stratified squamous cornified epithelium (**Figure 3**). Moderate to weak enzyme activity was exhibited by some of the cells in the sinus stroma. A similar moderate to weak ACF activity was detected in the cytoplasm of apocrine glands cells, and a strong one – in the apical part of secretory cells, the lumen of glandular tubules and the basal membranes (**Figure 4**). In the mature cells of sebaceous glands, a weak enzyme reaction was observed, whereas in the peripheral cells – a strong reaction. Strong enzymatic reaction was exhibited by muscle cells, in the peri- and the endomysium.

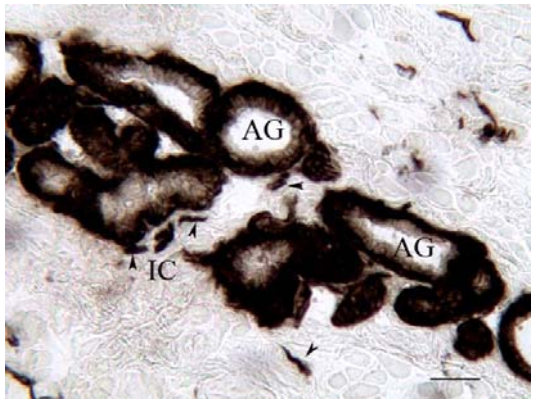

Figure 1. *Strong ALP activity in the basal part of cells of apocrine glands (AG), in blood vessels (arrowheads) of the interstitium (IC). Magnification ×100, Bar = 50µm*

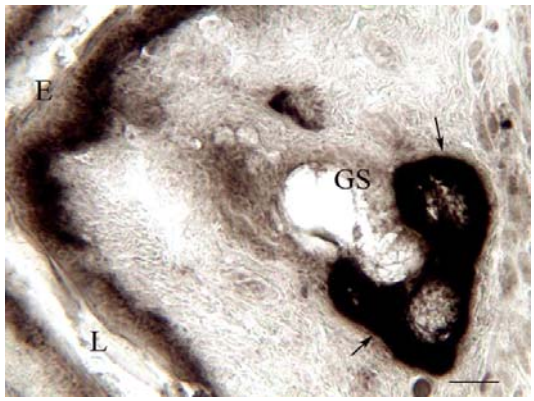

Figure 2. *Strong enzyme reaction in the germinative cells (arrow) of sebaceous glands (GS) in the excretory duct of the sinus, and moderate to weak reaction 0 in centrally located sebocytes. Strong ALP activity in the stratified squamous cornified epithelium (E). L- lumen of the excretory duct. Magnification ×100. Bar = 50µm*

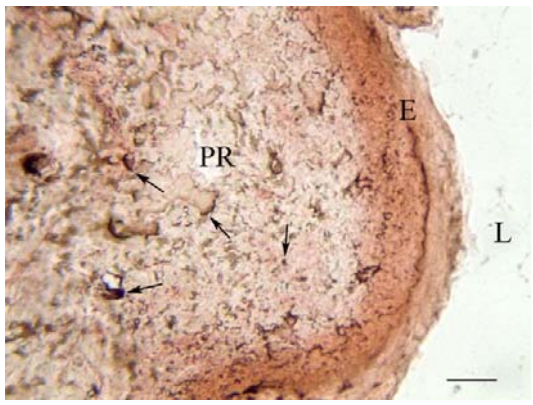

Figure 3. *Moderate to strong activity of acid phosphatase in all layers of the stratified squamous cornified epithelium (Å). Moderate to weak enzyme reaction in some cells in the lamina propria (PR) of the sinus (arrows). Magnification ×100. Bar = 50µm*

In this study, no sex dimorphism in the expression of both enzymes was found.

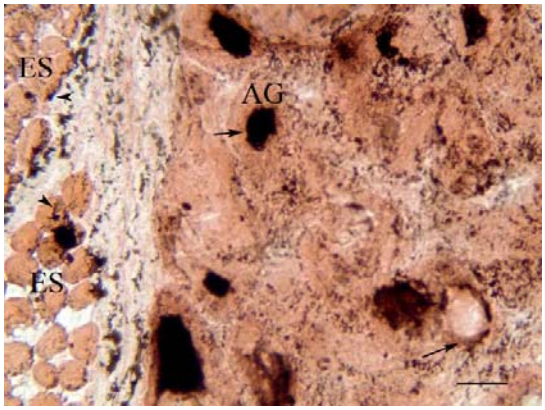

Figure 4. *Moderate to weak ACF activity in the cytoplasm of cells of apocrine glands (AG), and strong reaction – in the apical part of secretory cells, in the lumen of glandular tubules (arrows) and in basal membranes. ES – external anal sphincter. Magnification ×100. Bar = 50μm*

DISCUSSION

The ALP localization was mainly established in myoepithelial cells and the basal membrane of glandular tubules in canine paranal sinus (11). In the apical part of cylindrical apocrine cells, the authors found a weak expression of the enzyme but a strong one in the basal part. In sebaceous glands, including the sebum, enzyme activity was also present. The localization of enzyme in the stratified squamous cornified epithelium and in PS stroma was not described. The ALP localization in the stratified squamous cornified epithelium of canine paranal sinus, confirmed the findings of other investigators about the expression of this enzyme in the epidermis of skin (16, 17). According to them, ALP was primarily located in the basal layer and in stratum granulosum. The cells that reacted positively for ALP in the skin epidermis were described as cells of the Langerhans type, whose dendrites penetrated among the cells of stratum spinosum, whereas keratinocytes have not reacted. In the view of some authors (17) the activity of the enzyme was related to plasmatic membrane of these cells and sometimes, with the typical granules.

ALP expression has been also observed in the plasmatic membrane of secretory cells of apocrine sweat glands and of adjacent myoepithelial cells (17, 18)**.** These authors reported that the histochemical expression of ALP was related to the function of sweat glands, but it could also assist in the diagnosis and in determining the differentiation course of sweat gland tumours. In our study, we have found a similar localization of the enzyme in PS apocrine glands. In our opinion this enzyme expression was related with apocrine glands and could be probably used for diagnosis of neoplastic growth in them. According to (19), ALP is associated with the transport of substances through capillaries. An androgenic control of membrane-bound enzyme, manifested in increased amounts of it, was reported (20).

In the PS stroma, an intensive enzymatic reaction was detected. Thus, we confirm the enzyme activity in the endothelial cells of capillaries in rats as well as in skin capillaries in primates, reported by others (16, 18, 21). The localization of the enzyme in human fibroblasts is described by (22). ALP also participates in calcium secretion and transport (23). This enzyme is probably involved in the pathogenesis of tumours, connected in calcium metabolism, described in canine PS (24). The anticancer effect of vitamin A in pancreatic cancer was established (25). This suppressive effect is explained by authors via inhibition of the cell cycle of tumour cells and apoptosis induction. They also observed increase in ALP – an indicator for cell differentiation.

The cells of PS sebaceous glands reacted positively for ALP activity, similarly to cells in the multiacinar glands of the skin in primates and human skin sebaceous glands (2, 16). The role of ALP in hair biology and pathology is elucidated (25). According to others (19), ALP participates in the synthesis of sebum.

In the cytoplasm of cells of PS sebaceous glands, we observed histochemically ACF expression, thus confirming the data about the enzyme activity in sebaceous glands in rat skin (7). This is evidence that this enzyme is closely related to the function of these glands, being responsible for the holocrine secretion (26).

The strong enzymatic reaction in the endo- and the perimysium of the external anal sphincter confirmed the results of others (27), reporting that ALP was connected with the sarcolemma of cells in the skeletal muscles and the plasmatic membrane of endothelial cells in adjacent capillaries.

The observed ACF localization in the canine paranal sinus is similar to other results (14). Unlike these authors, we described the enzyme localization in the cells of the stratified squamous cornified epithelium as well. A good ACF activity was observed in the cytoplasm of cells of the epidermis. The acid phosphatase is primarily included in organelles with lysosomic traits and is related to cornification (28). Besides that this enzyme is encountered in membrane-limited

organelles, it was described and diffusely distributed in the cytoplasm (9). It is also present in the intracellular space of stratum granulosum and stratum corneum. It is presumed that ACF participates in the degradation of nucleotides and phospholipids (29).

The localization of ACF in the stratified PS epithelium and its hydrolytic effect allowed us to assume a role of this enzyme in the cornification of cells of the underlying layers. The increased number of lysosomes enhanced the regression of tumours. There are data about a considerable regression of keratoacanthoma after local application of vitamin A (30). The release of ACF from the lysosomes in the epidermis is positively influenced by retinol (28). As known, the transportation of vitamin A in the blood plasma in dogs and other carnivores, is performed not only as retinol, coupled to retinol-binding protein but predominantly as retinyl stearate and retinyl palmitate associated with all lipoprotein fractions (31, 32). Other animal species are less susceptible to intoxication, provoked by excessive vitamin A intake with food. Probably, the supplementation of dog food with retinol would result in control of neoplastic growth in the paranal sinuses. According to some authors, the lipids in the cornified layer of the epidermis are supplied via exocytosis of lamellar granules in the intercellular space. The lamellar granules contain ACF. Both lipids and ACF participate in the functioning and desquamation of stratum corneum (28). ACF is involved in the degradation of phospholipids and the necrobiosis of keratinocytes (18). The connection between ACF and lipids is revealed by other authors too. For instance, increased ACF activity was observed in altered smooth muscle cells with massive lipid deposits in rabbit atheromatous aortas that, is thought to be related to increased number and size of lysosomes (34). A moderate to weak enzymatic reaction was detected in the stroma of the sinus. This could be explained by the localization of enzyme activity in human fibroblasts, established by (35). According to them, the activity of this enzyme could be used as a marker of lysosome enzyme activity in the different functional stages of fibroblasts, namely the collagen-secreting and the collagen-resorbing fibroblasts. In the human adrenal cortex, ACF activity was found in lysosomes and secretory vacuoles of cells in the reticular zone that is the most active with regard to steroidogenesis (1). The observed enzymatic expression in the cytoplasm of cells of apocrine glands with a good activity in the apical part of secretory cells and the lumen of glandular tubules could be related both to their secretion and steroidogenesis. Some authors have confirmed the essential role of the enzyme in the catabolism of glomerular basal membrane in rats, as well as for the performance of ultrafiltration (36). This ACF localization is probably related to the transport of substances through the basal membranes that are necessary for the function of glands. Furthermore, we hypothesize that ACF in the apocrine glands of this organ is related to tumour genesis, similarly to prostatic ACF (13).

ACF and ALP are enzymes associated with various biological functions. On the basis of the investigation for the expression of these enzymes in the skin (18) and the data obtained in this study, we believe that the principal role of ALP in PS is associated with the transport of chemical substances needed for the normal functioning of the various structures of this organ as early as the first months of life. ACF is included in the degradation of phospholipids, the necrobiosis of keratinocytes, and the secretion of sebaceous and apocrine glands in the paranal sinus. Probably, these two enzymes could serve as markers in the diagnosis and prognosis of pathological conditions in PS.

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