Original Article

HISTOCHEMICAL INVESTIGATION OF MAST CELLS IN THE PARANAL SINUS (SINUS PARANALIS) OF SEXUALLY IMMATURE DOGS

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Summary
A histochemical investigation of mast cells in the paranal sinus of both the external and internal anal sphincters was carried out in 8 dogs (4 male and 4 female) at the age of 2 months. Mast cells density in mm^2 was determined after staining of cross sections with toluidine blue and alcian blue-safranin. It was found out that alcian blue-safranin stained mast cells (MCsAS) were considerably more numerous as compared to toluidine blue stained mast cells (MCsTB). The lowest number of MCsAS and MCsTB was observed in musculus sphincter ani externus, and the highest - in the sinus stroma. The localization of heparin-containing mast cells and the ratio between them and MCsTB was established. Sex dimorphism was not observed throughout this study. On the basis of our results it could be assumed that mast cells in canine paranal sinus were probably involved not only in local homeostasis maintenance, but were also important for the motoric activity of smooth muscle cells of musculus sphincter ani internus and of vascular wall, as well as for the contraction of skeletal muscle cells of musculus sphincter ani externus as early as the first months of life.
Key words: mast cells, sinus paranalis, dog

Introduction
It is known that mast cells participate in allergic and inflammatory reactions, by secreting biological mediators in response of immunoglobulin E and specific antigens [1]. Mast cells are described in almost all animal and human organs and tissues, but their cytochemical traits in the paranal sinus (PS) of the dog are not elucidated. In a previous study of ours, we established the histochemical features of mast cells in this organ in sexually mature dogs by staining with toluidine blue and alcian-blue-safranin [2].

It is believed that mast cells specialization in the different tissues was probably related to their role in physiology and pathology [3]. A morphological and histochemical similarity of mast cells in the heart and kidney of guinea pigs and men was demonstrated [4], but at the same time, they were different from mast cells in rat peritoneum. In pigs, histochemical investigations of mast cells in various layers of the jejunum, tongue and skin were performed in pigs [5].

In the available literature, there are no data about histochemical features of mast cells in canine PS. Our aim therefore was to determine the histochemical properties of mast cells in this organ with regard to throw light onto their role not only in homeostasis, but for the motoric activity of muscle cells in musculus sphincter ani internus and musculus sphincter ani externus.

Material and Methods

Material for investigation was obtained from the wall of the paranal sinus of 8 mongrel dogs (4 male and 4 female) at the age of 2 months. They were euthanized with 5% Thiopental solution (Biochemie, Austria) i.v. Pieces of 1 cm^3 from all parts of the PS were fixed in Carnoy's fixative for 4 hours, dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin. Serial cross sections of 6 μm were stained with 0.1% aqueous toluidine blue solution (pH 3) [6] and with alcian blue-safranin solution, pH 1.42 [7]. For comparative purposes, 0.02% aqueous solution of berberine sulfate (pH 4) was also used, and cross sections were stained for 20 min [5]. These cross sections were mounted in glycerol, studied immediately with fluorescence microscope and photographed. Then they were demounted with distilled water and stained with 0.1% aqueous solution of toluidine blue (pH 3), dehydrated, cleared, mounted and photographed again. The number of mast cells in both staining techniques was compared on microphotographs.

In the wall of Sinus paranalis, Musculus sphincter ani internus, Musculus sphincter ani externus, a total of 240 fields were investigated (120 in male and 120 in female dogs). All visible cells in the fields were enumerated, including those in which the cross section was not through the nucleus.

The density of mast cells (number/mm^2) was determined by ocular eyepiece.

The statistical analysis of data was done by the Student`s t-test with the StatMost for Windows software.

Results

The results about the distribution (density) of alcian blue-safranin (MCsAS) and toluidine blue stained mast cells (MCsTB) in the entire sinus wall in both genders are presented in Table 1.

Comparing both staining techniques, it was found out that MCsAS were considerably more numerous vs MCsTB - 95.2±20.1 vs 56.1±22.1 in male dogs ($P<0.001$), and 94.8±23.7 MCsAS vs 55.8±20.7 MCsTB ($P<0.001$) in females, (mean values for the entire PS wall, Table 1).

Table 1. Comparison of two techniques of mast cells staining - toluidine blue (MCsTB) and alcian blue (MCsAS) with mean values of the entire paranal sinus wall

Parameter	Male dogs	Female dogs
MCsTB number	56.1±22.1	55.8±20.7
MCsAS number	95.2±20.1***	94.8±23.7***

Data for number/mm^2 are given as mean ± SD.
*** $p< 0.001$ - statistically significant difference vs mast cells stained with toluidine blue (Student's t- test).

The highest MCsAS and MCsTB density per 1 mm^2 in both genders was observed in the stroma - 113.3±21.6 and 84.4±7.8 in males and 116.8±22.4 and 81.8±8.2 in females, respectively (Table 2).

Table 2. Localization of number of mast cells (MC), stained with toluidine blue (MCsTB) and alcian blue (MCsAS) in sinus paranalis, musculus sphincter ani internus, musculus sphincter ani externus in male and female dogs

MCs localization	MCsTB number/mm^2 male dogs	MCsTB number/mm^2 female dogs	MCsAS number/mm^2 male dogs	MCsAS number/mm^2 female dogs
Stroma	84.4±7.8 63-99	81.8±8.2 68-101	113.3±21.6*** 27-135	116.8±22.4*** 31-136
Apocrine glands	37.2±6.8 23-52	37.4±7.4 25-55	87.0±14.1*** 33-104	83.9±17.9*** 36- 105
Sebaceous glands	47.8±7.8 33-61	49.1±7.9 31-62	88.5±11.9*** 49-108	86.6±14.5*** 47-109
Internal anal sphincter	31.0±6.2 19- 43	33.5±6.1 23- 49	59.6±8.2*** 46-81	63.2±7.4*** 53-83
External anal sphincter	51.7±10.7 21- 68	55.1±6.6 42- 66	71.5±8.9*** 59-91	73.8±8.1*** 61-92

Data for number/mm^2 are given as mean ± SD.
*** $p< 0.001$- statistically significant difference vs mast cells stained with toluidine blue (Student's t-test).

The difference between MCsAS and MCsTB counts in this layer was statistically significant in the Student`s t-test (P<0.001). In the interstitial connective tissue among the glandular tubules, MCsAS were significantly more abundant compared to MCsTB in both males (87.0±14 vs 37.2±6.8) and females (83.9±17.9 vs 37.4±7.4) (P<0.001, Student`s t-test). Around the mast acini, MCsAS were also found out to prevail in male dogs (88.5±11.9) as compared to MCsTB (47.8±7.8) (P< 0.001, Student`s t-test). In female dogs, the almost same ratio was present 86.6±14.5 MCsAS vs 49.1±7. MCsTB (P<0.001, Student`s t-test). Similar distribution was observed in both external and internal anal sphincters (Table 2).

Two types of granules were differentiated in mast cells cytoplasm after alcian blue-safranin staining: red and blue. It should be emphasized that both types of granules were not found out in all cells. Some mast cells contained only large red granules. These cells were situated mainly near the blood vessels of the microcirculatory bed. Mast cells with red granules were also located in the vicinity of apocrine glands, around the glandular acini, as well as in the sinus propria. The berberine sulfate staining for detection of heparin by florescence (Fig. 1) and subsequent staining of the same sections with 0.1% toluidine blue (Fig. 2) showed that the following proportions of MCsTB were heparin-positive: those located in the connective tissue layer between the epithelial coating of the sinus and the apocrine glands - 64%, in the interstitium among apocrine glands tubules - 64%, around the sebaceous glands acini - 62%, in the external anal sphincter - 31% and in the internal anal sphincter - 100%.

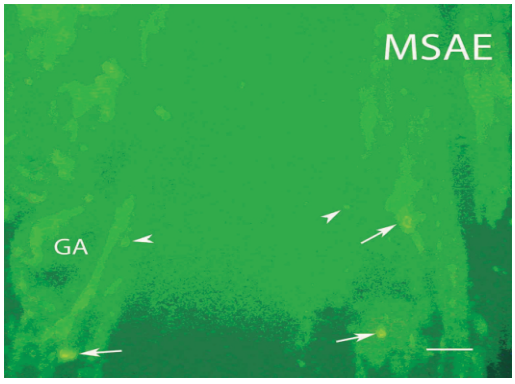

Fig. 1. Heparin-containing mast cells (arrows). Heparin-negative mast cells (arrowheads). Berberine sulfate. MSAE- external anal sphincter. GA- apocrine glands. Male dog at the age of 2 months. Bar = 70 μm.

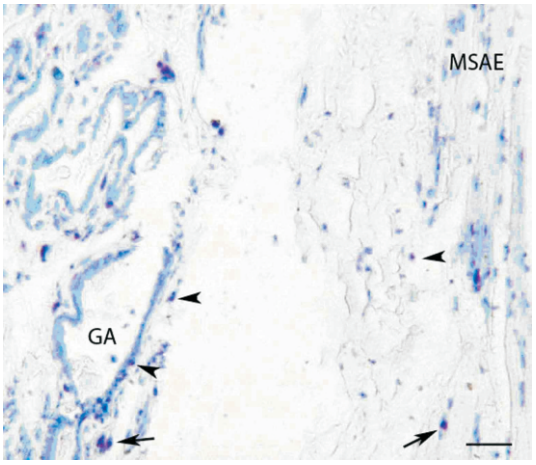

Fig. 2. Mast cells stained with toluidine blue (arrows), heparin-positive.
Mast cells stained with toluidine blue (arrowheads), heparin-negative.
MSAE- external anal sphincter. GA- apocrine glands. Male dog at the age of 2 months.
Toluidine blue, bar = 50 μm.

Discussion

The present results showed that mast cells counts in the paranal sinus in sexually immature animals (MCsAS - 96.0±21.9, MCsTB 56.0±21.3) were more numerous as respective counts reported in sexually mature dogs (MCsAS - 86.2±25.2, MCsTB- 40.6±20.7) [2]. In both investigations, there was a statistically significant difference. Our results corresponded to [8], claiming that mast cells density in the ventral prostate in rats was the highest during the puberty and then decreased with age. On the contrary however, it was reported that mast cells prevailed in the intestinal mucosa and submucosa of adult pigs [5]. The considerable amount of mast cells in the paranal sinus wall in sexually immature dogs presumes the active participation of these cells in the normal functioning and development of the organ as early as the first months of life. It is known that normally, a secretion with high microbicide activity is secreted and temporarily stored in the sinus lumen, serving for spraying as well as a pheromone [9]. Thus, the secretion of the sinus probably stimulates the increase in mast cells numbers mainly in the subepithelial connective tissue, where their density was found to be the highest.

It should be stated that despite mast cells counts were more in female as compared to male dogs, the difference was not statistically significant. A sex dimorphism in mast cells counts was described by some authors in various tissues in rodents, with higher counts in females than in males [10]. As recognized, oestrogens and androgens have a different influence on mast cells in rodents: oestrogens stimulate cell proliferation [11], whereas testosterone has a suppressive effect [10,12]. The absence of sex dimorphism in canine paranal sinus wall was probably related to tissue specificity.

The higher MCsAS counts as compared to MCsTB in the studied organ confirmed data reported for bovine trachea [13, 14] and domestic swine ureter [15]. The various mast cells counts could be attributed to the effect of surrounding tissue [16] and the occurrence of various proteoglycans in mast cells granules [3,1]. It is also known that the maturation of mast cells in rats is related to a higher degree of proteoglycans sulfation, resulting in increased affinity to safranin [17].

The localization of mast cells in the interstitial connective tissue among the glandular tubules and around sebaceous glands acini could be attributed to the role of these cells for the normal functioning of these glands from one part, and for their pathological changes, on the other.

Mast cells found out in Musculus sphincter ani internus and Musculus sphincter ani externus (with statistically significant difference between MCsAS and MCsTB counts, $P<0.01$, Mann-Whitney test) are probably involved in the retention and especially in the discharge of the secretion synthesized in the organ, by release of substances influencing the motoric activity of muscle cells.

Some MCsAB in the PS contained only large red granules, whereas others - small red and blue granules, this confirming the results from investigations carried out in bovine tracheal mast cells [14]. Red granules are believed to contain heparin [18] whereas blue ones-chondroitin sulfate. As already mentioned, the presence of heparin-containing mast cells in the PS was demonstrated by berberine sulfate staining. The obtained results are supported by studies [5], showing the presence of heparin in mast cell granules in the connective tissue of the skin, tongue and intestinal submucosa in pigs.

Activated mast cells inhibit the proliferation of smooth muscle cells in Tunica media of the human arterial wall and histamine stimulates it [19]. An inhibiting effect of heparin was also shown [20] with regard to proliferation of human myometrium and it was supposed to induce the differentiation of uterine smooth muscle cells and to influence tissue remodeling and reconstruction in a number of physiological and pathological events. In t his connection, it is essential to affirm that mast cells observed in the vicinity of capillaries and arterioles of the PS, as well as in the internal anal sphincter, were heparin-positive. This allowed us to hypothesize a possible role of these mast cells in the regulation of smooth muscle cells growth in the vascular wall and the internal anal sphincter. On the other side, heparin in mast cells located near the vessels from the PS microcirculatory bed participates in binding of the enzyme lipoprotein lipase and hence, in lipid metabolism regulation [21].

Alcian-positivity of mast cells is considered an evidence for the presence of biogenic amines in their granules [7]. This study showed for the first time mast cells containing biogenic amines in the paranal sinus. Their presence was the most noticeable near the wall of vessels from the microcirculatory bed of the sinus stroma as well as around the sebaceous and apocrine glands of the organ. IN our opinion, the vicinity of mast cells to smooth muscle cells was most probably due to their biogenic amine content that, after release, has an effect mainly on smooth muscles.

Conclusions

In conclusion, it could be assumed that mast cells in canine paranal sinus were probably involved not only in local homeostasis maintenance, but were also important for the motoric activity of muscle cells of musculus sphincter ani internus and of vascular wall, as well as for the contraction of skeletal muscle cells of Musculus sphincter ani externus as early as the first months of life.

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