

DENSITY, SHAPE AND DIMENSIONS OF MAST CELLS IN CANINE ANAL CANAL

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Summary

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The aim of the present study was to determine the density (number), shape and dimensions of mast cells in the wall of the anal canal (AC) and wall of perianal sinuses (PS) in six healthy mongrel male dogs at the age of 3–4 years. The density of mast cells was determined in 1 mm² fields. In AC wall, 276 fields were studied and 8317 mast cells were detected, whereas in the PS wall – 9126 mast cells in 198 fields. The biggest mast cell density was observed in propria of the AC wall (53.3 ± 12.0 /mm²). Relatively less mast cells were observed in the internal anal sphincter (31.7 ± 5.0 /mm²), while their number in the external anal sphincter was considerably lower (7.3 ± 1.5 /mm²). The shape of mast cells was the most elongated in the muscle layer, whereas those in the propria were mostly oval. In the PS wall, the highest mast cell number was observed in the connective tissue, situated between the cover epithelium and the apocrine glands (67.2 ± 12.4 /mm²). Less mast cells were present in the layer with sebaceous glands (43.7 ± 7.0 /mm²), and the fewest amount – in the layer with apocrine glands (18.5 ± 4.0 /mm²). The number of mast cells in the external anal sphincter was relatively high (59.9 ± 12.1 /mm²). In the last two structures, the shape of mast cells was elongated whereas in the other – predominantly oval.

Key words: anal canal, dog, mast cells

INTRODUCTION

The specific traits of the anal canal structure and the related perianal sinus have a significant clinical importance (Emerson & Cross, 1965; Donovan, 1969; Budsberg & Spurgeon, 1983; Isitor, 1983; Budsberg *et al.*, 1985). In the anal sinuses of the anal canal and their dilatations – crypts, are retained foecal masses and that results in development of infection, onset of abscesses and fistulations (Budsberg *et al.*, 1985).

The function of perianal sinuses is mainly related to animal behaviour, espe-

cially to communications among individuals of the same species, as well as to territorial marking behaviour (Donovan, 1969).

The literature data about the presence of mast cells in the end part of the gastrointestinal tract in carnivores are scarce. Myles *et al.* (1995) and Noli *et al.* (2003) have studied only the density of mast cells in the skin zone of the anal canal in dogs and cats, but without PS wall. No studies are available about the shape or dimensions of these cells as well.

The scarce data about the presence and localization of mast cells in the end part of the gastrointestinal tract as well as the lack of information about their shape and dimensions motivated the present study aiming to assist the elucidation of their functional role in that part of canine digestive tract.

MATERIALS AND METHODS

The material for the investigations was obtained from the wall of the anal canals of six mongrel healthy male dogs at the age of 3–4 years. The animals were euthanized with 5% thiopental solution. Pieces of 1 cm² from all parts of the canal's wall were fixed in Carnoy's fixative for 4 hours at room temperature. Then they were dehydrated in ascending ethanol series, cleared in xylene and embedded in paraffin. From them, longitudinal and transverse cross sections of 6 µm, stained with 0.1% aqueous solution of toluidine

blue (pH 3) were prepared.

In the AC wall, a total of 276 fields were studied and within, 8317 mast cells were found out. The number of fields in the PS wall were 198, with 9126 mast cells. All visible cells in the studied fields were counted, including those where the cut had not passed through the nucleus.

The density (number/mm²) and the dimensions of mast cells (in µm) were determined with an micrometre eyepiece, and their shape was determined by light microscopy. The dimensions (length and thickness) and the shape of mast cells were determined only on nuclear cells.

The statistical analysis of data was done by using both the Mann-Whitney test and one-way ANOVA with LSD as post hoc test.

RESULTS

The data for studied parameters are presented in Tables 1 and 2.

Table 1. Area of studied regions of the anal canal wall, number and dimensions of mast cell within

Parameter	PR	IS	ES	GAN	GSUD	GSEB	Total
Area, mm ²	60	60	36	42	36	42	276
%	22	22	13	15	13	15	100
Number	3210	1902	261	1266	715	983	8317
%	39	23	3	15	8	12	100
Number/ mm ²	53.3 ±12.0	31.6 ±5.7*	7.3 ±1.5*** U ₂	28 ±4.2*	20.8 ±1.9** U ₁	23.1 ±1.3*	27.4 ±4.4
Min–max	10–96	11–56	1–15	9–43	15–27	17–28	10.5–4.2
Length, µm	15.2 ±0.3	14.1 ±0.5	16.2 ±0.11	14.3 ±0.5	12.0 ±0.6	13.1 ±0.6	14.2 ±0.5
Min–max	14.3–16.2	12.8–15.6	5.1–17.7	13.1–15.9	10.1–13.4	11.1–4.8	12.8–15.6
Thickness, µm	11.4 ±0.2	7.5 ±0.2	9.2 ±0.3	9.7 ±0.3	8.4 ±0.2	10.1 ±0.2	9.4 ±0.2
Min–max	10.8–11.9	6.9–8.1	8.2–9.8	8.9–10.5	7.8–9.1	7.9–11.5	8.4–10.2

PR –propria; IS – internal anal sphyncter; ES – external anal sphyncter; GAN – anal glands; GSUD – sudoriferous glands; GSEB – sebaceous glands. The data for the number/mm², length and thickness are mean ± SEM. U₁ P< 0.05, U₂ P< 0.01 – statistically significant difference vs PR (Mann-Whitney test); * P< 0.05, ** P< 0.01, *** P< 0.001 – statistically significant vs PR (ANOVA + LSD test).

Table 2. Area of studied regions of the perianal sinus, number and dimensions of mast cell within

Parameter	PR	ES	GAP	GSEB	Total
Area, mm ²	60	42	60	36	198
%	30	21	30	19	100
Number	4095	2561	1032	1438	9126
%	45	28	11	16	100
Number/mm ²	67.1±12.4	59.8±12.5	18.5±4.1**U ₁	43.3±7.0	47.2±9.0
Min-max	39-110	25-97	5-30	24-63	23-75
Length, µm	14.6±0.3	15.9±0.2	14.3±0.4	12.4±0.6	14.3±0.4
Min-max	13.7-15.2	15.2-16.6	13.3-15.7	10.9-13.7	13.3-15.3
Thickness, µm	10.5±0.2	8.8±0.2	10.1±0.5	9.5±0.5	9.6±0.4
Min-max	9.9-11.1	7.8-8.9	8.8-11.2	8.0-10.7	8.6-10.5

PR –propria; ES – external anal sphincter; GAP – apocrine glands; GSEB – sebaceous glands. The data for the number/mm², length and thickness are mean ± SEM. U₁ P< 0.05 – statistically significant difference between GAP and GSEB (Mann-Whitney test); ** P< 0.01 – statistically significant difference between GAP and GSEB (ANOVA + LSD test).

The highest mast cell density (Table 1) per 1 mm² was that in AC propria – 53.3 ± 12.0/mm², followed by the internal anal sphincter – 31.7 ± 5.0, anal glands (28 ± 4.2 /mm²), sebaceous glands (23 ± 1.3 /mm²) and sudoriferous glands (20.8 ± 1.9/mm²). The least number of mast cells was detected in the external anal sphincter – 7.3 ± 1.5/mm². The greatest mast cell length was observed in the external anal sphincter – 16.2 ± 0.11 µm, followed by that in the propria – 15.2 ± 0.3 µm, anal glands – 14.3 ± 0.5 µm, internal anal sphincter – 14.1 ± 0.5 µm, sebaceous glands – 13.1 ± 0.6 µm and sudoriferous glands – 12.0 ± 0.6 µm. The greatest thickness was observed in the propria mast cells – 11.4 ± 0.2 µm, followed by that in sebaceous glands – 10.1 ± 0.2 µm, anal glands – 9.7 ± 0.3 µm, the external anal sphincter – 9.2 ± 0.3 µm, sudoriferous glands – 8.4 ± 0.2 µm and the internal anal sphincter – 7.5 ± 0.2 µm (Fig. 1).

Table 2 shows that the highest mast cell density per 1 mm² in the PS wall was found out in the propria (67.1 ± 12.4 /mm²), followed by the external anal sphincter (59.8 ± 12.5/mm²) and seba-

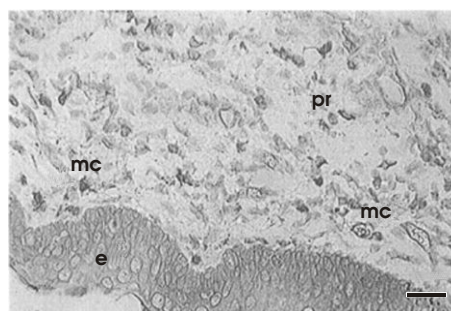


Fig. 1. Mast cells (mc) in the propria (pr) of the wall of the anal canal: e – *Lamina epithelialis mucosae*. Bar = 30 µm.

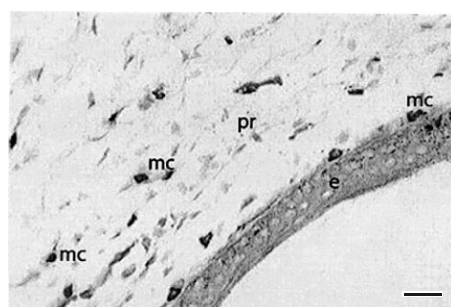


Fig. 2. Mast cells (mc) in the propria (pr) of the wall of perianal sinuses: e – *Lamina epithelialis mucosae*. Bar = 30 µm.

ceous glands layer ($43.3 \pm 7.0/\text{mm}^2$), whereas the lowest density was observed in apocrine glands layer ($18.5 \pm 4.1/\text{mm}^2$). In PS fields, the length of mast cells was the highest in the external anal sphincter – $15.9 \pm 0.2 \mu\text{m}$, with thickness of $8.8 \pm 0.2 \mu\text{m}$. The highest thickness was that of mast cells in the propria – $11.4 \pm 0.2 \mu\text{m}$, followed by the thickness of cells in the apocrine glands layer – $10.1 \pm 0.5 \mu\text{m}$ and sebaceous glands – $9.5 \pm 0.5 \mu\text{m}$, and the thinnest cells were found out in the external anal sphincter – $8.8 \pm 0.2 \mu\text{m}$ (Fig. 2).

DISCUSSION

The present study provides for the first time a detailed information about the number, shape and dimensions of mast cells in the walls of the AC and PS in the dog.

The light microscopy data showed that the highest number of mast cells was that in AC and PS propria and that the ratios vs the other studied areas were significant. It could be noted that the number of mast cells per 1 mm^2 in PS propria was higher than that in AC propria, but the difference was not statistically significant ($P > 0.05$). A similar finding was observed with mast cell number in the external anal sphincter in both studied parts, and the ratio was almost eight times higher in favour of PS with statistically significant difference ($P < 0.001$).

In our view, the data about mast cell number in the different glandular layers in AC and PS walls are also remarkable. In both AC and PS, the numbers varied in a wide range – $18.5 \pm 4.1/\text{mm}^2$ (for apocrine glands) vs $43.3 \pm 7.0/\text{mm}^2$ (for sebaceous glands). The comparative analysis of data for sudoriferous and sebaceous glands in both areas however showed that between AC and PS, there was a statisti-

cally significant difference only in mast cells numbers, situated in the respective layers of sebaceous glands – $23.1 \pm 1.3/\text{mm}^2$ vs $43.3 \pm 7.0/\text{mm}^2$; $P < 0.05$.

The number of mast cells in the AC propria observed by us ($53.3 \pm 12.0/\text{mm}^2$) was significantly higher than numbers reported by other authors (Myles *et al.*, 1995; Noli *et al.*, 2003) in the skin AC zone ($27 \pm 5 /\text{mm}^2$). In our opinion, this difference could be mainly attributed to the used fixative. It is generally accepted that after fixation with formalin, few mast cells are visualized (up to 50%) than when using the Carnoy's fixative, a fact, reported for the first time by Enerbäck (1966) and subsequently confirmed by other authors (Wingren & Enerbäck, 1983; Marshal *et al.*, 1987; Ghanem *et al.*, 1988).

The relatively high mast cells numbers in the propria of both AC and PS is supporting the findings of others (Xu *et al.*, 1993; Hill & Martin, 1998) about a similar distribution in the intestinal wall. Therefore, we also assume that the probable role of mast cells could be related not only to local homeostasis, but also with their involvement in immune response against foreign agents, commonly present in this part of the digestive tract.

As to the mast cells' shape, our observations are in agreement with other investigators, having studied mast cells in mammalian tubular organs. Their data showed that in muscles, mast cells are mainly elongated and in the other parts of the wall – with an oval shape. As a rule, elongated cells are less thick (Wingren & Enerbäck, 1983; Xu *et al.*, 1993; Vodenicharov *et al.*, 2005). Our results allowed us to assume that the elongated shape of mast cells, located in muscle layers, was due to muscle cells contractility and the relatively little interspace among them.

The higher mast cells number in *M. sphincter ani externus* of PS wall compared to their number in the corresponding muscle in AC wall, presumes an involvement of these cells in the regulation of collection and particularly in the discharge of anal sac secretions via release of substances, influencing the motorics of muscle cells. This hypothesis of ours is supported by the data of Vodenicharov *et al.* (2005) about mast cells in the muscle lining the porcine ureter, where several biologically active substances as histamine and vasoactive intestinal polypeptide (VIP), with a definite role in smooth muscle cell motility were found out.

In conclusion, the observed features in the distribution, shape and dimensions of mast cells in those two important parts of canine gastrointestinal tract add to the knowledge on this animal species. Considering the functional role and the clinical significance of AC and PS, it is evident that further studies on the histo- and immunocytochemical traits of mast cells are necessary with regard to further revealing their importance at the end part of the digestive tract.

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