Immunocytochemical expression of Chromogranin A in mast cells in the canine paranal sinus

I. S. STEFANOV*, A. P. VODENICHAROV¹, M. V. GULUBOVA²

¹Department of Veterinary Anatomy, Histology and Embryology, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, BULGARIA.
²Department of General and Clinical Pathology, Faculty of Medicine, Trakia University, 6000 Stara Zagora, BULGARIA.

*Corresponding author: iv_stefanov@uni-sz.bg

SUMMARY

The aim of the study was to establish the immunocytochemical expression of Chromogranin A in the mast cells in the paranal sinus from 8 dogs, the tryptase expression being considered as specific for mast cells. The immunocytochemical expression of Chromogranin A and tryptase was observed in the granules of mast cells. Chromogranin A positive cells were detected predominantly around the blood vessels, mainly in the subepithelial connective tissue layer, then in the zone of apocrine glands and around the sebaceous glands and poorly in the subglandular connective tissue layer of the sinus wall and its excretory duct. In addition, the Chromogranin A expression was strongly associated with the tryptase presence. These results show that mast cells in the dog paranal sinus express Chromogranin A that may play an important role in the secretory granule biogenesis.

Keywords: dog, paranal sinus, immunocytochemistry, chromogranin A, mast cells.

Introduction

The granin family (Chromogranin A (CgA), 4 Chromogranin B (CgB), Secretogranin (Sg) II, and the less well studied Secretogranins III-VII) comprises a group of acidic proteins that are present in the secretory granules of a wide variety of endocrine and neuro-endocrine cells [6, 22]. There is evidence that granins may be associated to the precursors of biologically active peptides [11, 12, 17]. However, granins cannot be considered simply as precursors of granule cargo. They may act as helper proteins in the packaging of peptide hormones and neuro-peptides [11, 12, 17].

It is well known that mast cells are densely granular, secretory leukocytes that reside in tissues [13, 16]. In response to a variety of stimuli, including immunological challenge, exposure to secretagogues and exposure to physical stimuli, mast cells release preformed, and de novo synthesized, inflammatory mediators into their surrounding tissues. The preformed mediators are housed within membrane-delimited secretory granules. The contents of mast cell secretory granules include inflammatory mediators such as histamine, serotonin, platelet activating factor, some cytokines (TNF-α), and matrix active proteases of the chymase and tryptase families [13, 16].

Data about the contribution of granins to the granularity of secretory immunocytes are too scarce. HAWKINS et al. [5] analyzed the distribution of Chromogranin A in endocrine cells of various species of healthy laboratory animals and supposed that some of immunopositive cells are mast cells. Later, PRASAD et al. [19] established expression of Chromogranin A in mast cells granules and elucidated the control of granule biogenesis in detail.

Because of the lack of studies about the presence of Chromogranin A in canine mast cells, we aimed to determine the immunocytochemical expression of this protein in the mast cells of dogs’ paranal sinus, an organ with certain clinical importance.

Material and Methods

ANIMALS

The paranal sinuses of 8 adult dogs, both male and female, belonging to the following breeds - Rottweiler (n = 4, 1, 2, 5 and 9 years old, respectively), Golden Retriever (n = 1,
1.7 year old), Pitbull (n = 1, 6 years old), Drahthaar (n = 1, 1.5 year old), and mixed-breed dog (n = 1, 9 years old) - were studied. Immediately after death of the animals, due to diseases that had not affected the paranal sinus, samples of 1 cm³ were collected from different parts of the paranal sinus wall in the clinics of the Faculty of Veterinary Medicine, Stara Zagora.

IMMUNOHISTOCHEMISTRY

Immunohistochemical staining for chromogranin A and mast cell tryptase was performed using avidin-biotin-peroxidase complex technique on formalin-fixed and paraffin-embedded tissues. Tissues were cut to 5-6 µm thick sections, which were mounted on glass slides. They were deparaffinised twice in xylene for 1 hour, followed by absolute ethanol, 96°, 70° and 50° alcohol series, each for 10 minutes. The further immunohistochemical procedure was earlier described [4] and was based on the method described by DE VOS et al. [2]. Briefly, the sections were rinsed in 0.1 M phosphate buffer saline (PBS), pH 7.4 and internal peroxidase was blocked by incubation in 1.2% hydrogen peroxide (Peroxidase block K0673, DAKO A/S, Glostrup, Denmark) in methanol for 30 minutes and rinsed in 0.1 M PBS, pH 7.4 for 15 minutes. Immunocytochemical reaction was carried out using primary polyclonal rabbit anti-human chromogranin A (N1535, DAKO) antibody ready to use and monoclonal mouse anti-human mast cell tryptase (N M7052, DAKO clone AA1) diluted to 1:100 for 24 hours at 4°C. After washing in 0.1 M PBS, pH 7.4, the sections were incubated with Dako REAL™ EnVision™ Detection System (Peroxidase/DAB+, Rabbit/Mouse, K5007) for 60 minutes, then visualized with 3,3’ diaminobenzidine (DAB) and counterstained with haematoxylin.

Sections incubated with non-immune sera instead of the primary antibodies were used as negative controls.

STATISTICAL ANALYSIS

The density of mast cells was estimated by a light microscope (ZEISS Primo Star, Germany), camera (Progress, Capture 2.6 - JENOPTIK) and software analysis programme (Soft Imaging System GmbH). Data for number / mm² are given as mean ± standard deviation. Statistical data processing was done using Data Analysis tool and Student’s t-test by means of the Stat Most for Windows software. Differences were considered as significant when p values were less than 0.05.

Results

In this study, it was observed that regardless of the content of immunopositive granules in the cytoplasm, the nuclei of positive cells always exhibited a negative reaction (figure 1). CgA positive cells with strong and moderate immunoreactivity were mainly localized in the subepithelial connective tissue layer especially under the basal lamina and in the vicinity of blood vessels- arterioles, venules and capillaries compared to the 2 other layers, the zone of apocrine glands and the subglandular connective tissue layer (p < 0.001) (Table I). CgA positive cells were observed in the interstitial connective tissue around the apocrine glands and next to the blood vessels, as well (figure 1). In the subglandular connective tissue, between the apocrine glands and external anal sphincter muscle, immunopositive cells were found predominantly around the blood vessels of all types (small arteries and veins, arterioles, venules and capillaries). CgA immunoreactive cells were detected in the adventitia of vessels but they were not located in the intima. In addition, the frequencies of positive CgA and tryptase cells were also markedly higher in the periphery of apocrine glands than in the subglandular connective tissue (p < 0.001) (Table I). CgA positive cells with strong to moderate reactivity were also determined between sebaceous acini of the paranal sinus excretory duct in the subepithelial, subglandular layer and in the interstitial connective tissue.

The morphology and the localisation of these cells matched with that of the perivascular mast cells that were identified by carrying out the immunocytochemical reaction for tryptase on serial sections (figure 2). Consequently, the differential distribution of the tryptase positive mast cells accordingly the layers of the paranal sinus was similar to that of CgA positive cells (Table I). Nevertheless, the number of tryptase positive mast cells was lower than the number of CgA immunoreactive cells in all layers of paranal sinus and statistically significant differences were established for the subepithelial connective tissue layer and the zone of apocrine glands (p < 0.01) (Table I).
Discussion

In the present study, the chromogranin A immunopositive mast cells were detected in all layers of paranal sinus wall for the first time. As it is well known granins are involved in granulogenesis in neuroendocrine cells [11, 12]. For the first time, PRASAD et al. [19] determined the expression and function of granins in the mast cells in detail. The authors demonstrated the expression of granins in these cells, and imply an important role for these proteins in the secretory immunocytes. There is evidence that mast cells have hormonal properties and that they are related to the dispersed neuroendocrine system, but these relationships are obscure and require further investigations. For example, substantial quantities of somatostatin and substance P-like substances have been found by radioimmunoassay in rodent mucosal mast cells [3] and messenger RNA for preproenkephalin has been shown to be present in mast cell lines [15]. Additionally, normal and/or neoplastic human mast cells have reacted immunohistochemically to regulatory peptides such as anti-ACTH, anti-leu-enkephalin, and anti-met-enkephalin, but they did not react to antisera for neuron specific enolase or CgA [7, 20].

The biological roles of granin proteins are not well understood. Several ideas, which are not mutually exclusive, have been proposed for the molecular functions of granins. It is clear that CgA and CgB act as cargo precursors that are proteolytically cleaved to form bioactive peptides [18]. In addition to being sources of granule content in the form of proteolytically derived peptides, chromogranins have also been considered as chaperone secretory granule components [9, 18]. It is known that chromogranins act as cholesterol binding proteins, and a model for their role in the cholesterol sequestration events that participate in granulogenesis has been derived from several studies [8]. This fact explains the localization of most mast cells around the blood vessels observed in the present study. One common observation for several granins suggests that their overexpression result in the expansion of the granule compartment [11, 14]. Both their proposed roles as precursors of granule cargo, and as regulators of granule membrane dynamics, would be consistent with these observations of granulogenic capacity. In a further, intriguing, set of publications, the CgA and CgB proteins have been described as low affinity, high capacity, calcium buffering proteins that associate with intracellular calcium channels in the endoplasmic reticulum and in the Golgi apparatus. In this context, the calcium reservoir that is provided by CgA appears to play a feedback role in regulating the open status of these calcium permeant channels [1, 10]. Although the enigmatic granin proteins should therefore be considered as components of the secretory apparatus in mast cells, the data in the study of PRASAD et al. [19] also suggest potentially wider functions as regulators of organelle ion channels.

The higher number of Chromogranin A positive cells than the tryptase positive cells in the wall of paranal sinus may be explained by the presence of endocrine cells which are different from mast cells. This finding correlates with the results of a previous study where we establish that some of the cells in the connective tissue layers of the organ express the 3 β-hydroxysteroid dehydrogenase [21].

As a conclusion, for the first time, the immunocytochemical expression of Chromogranin A in mast cells in the canine paranal sinus has been detected. These findings strongly suggest that Chromogranin A may play an important role...
in the secretory granule biogenesis in canine mast cells. The presence of granins in mast cells granules may be used as a basis for further detailed elucidation of granule formation mechanism leading to the creation of more effective methods against the pathological processes that are common in this organ. Moreover, the mechanisms that control the biogenesis and abundance of these granules are also of therapeutic interest, but remain poorly studied [19].

References