MASTOCYTOMA WITH CUTANEOUS INVOLVEMENT IN A PITBULL TERRIER - A CASE REPORT

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Summary

Mast cell proliferations are more common in dogs than in humans. Mastocytoss is a rare disorder characterized by mast cell proliferation. Of the different clinical types, the localized variety called mastocytoma is even rarer and constitutes only about 15% of the total cases of cutaneous mastocytoses. Throughout the literature, the majority of reports are of a generalized variety called urticaria pigmentosa. An 11 year old castrated female pitbull terrier has shown nodular and ulcerative cutaneous lesions on the thorax and rhinostilus, with a history of one year. First clinical and dermatological examinations pointed towards a dermatological condition. The dog was treated locally with topic corticosteroids. After 10 months of unsuccessful therapy, a cutaneous biopsy was sampled, embedded in paraffin, sectioned at 5µ. Sections were stained with usual hematoxilin – eosin, toluidine blue at pH3, alcian blue – safranin at pH1,42 methods, and also with immunohistochemical methods using anti – vimentin, anti – CD117, and anti – mast cell tryptase (MCT) antibodies in LSAB+ system. Macroscopic examination of the tumor revealed nodular ulcerated lesion with areas of necrosis and hemorrhage. Morphological examination revealed large areas of round tumor cells accompanied by areas of necrosis, with normal suprajacent epidermis and annexes. Tumor cells had granular cytoplasm, mostly orthocromatic for toluidine blue staining. Using alcian blue – safranin, tumor cells were found to have granules into their cytoplasm, mostly alcian positive and mixed, but some of the tumor cells had safranin positive granules within the cytoplasm. Tumor cells were positive for vimentin with strong cytoplasmic pattern and the inflammatory infiltrate cells were negative. Tumor cells were weakly positive with cytoplasmic pattern for CD117, and positive positive, with cytoplasmic pattern for MCT. The histochemical and immunohistochemical findings support the pathologic diagnosis of mastocytoma.

Key words: canine, mastocitoma, mast cell tryptase, histochemistry, immunohistochemistry

Mast cell proliferations are more common in dogs than in humans; mastocytoses are a rare group of disorders of mast cell proliferation. Of the different clinical types, the localized variety called mastocytoma is even rarer and constitutes only about 15% of the total cases of cutaneous mastocytoses in the fog. Throughout the literature, the majority of reports are of a generalized variety called urticaria pigmentosa. In dogs, mast cell tumor or mastocytoma is one of the most common types of skin neoplasms, accounting for between 16 to 21% of all skin tumors and 11% to 27% of all malignant skin tumors (1). Most mastocytomas arise from the dermis and subcutaneous tissues; widely metastatic mastocytosis is not

common (less than one-tenth that of cutaneous cases). Disseminated mastocytosis without an associated cutaneous mastocytoma is reported infrequently in dogs (2). Histological grading is widely used for prognosis analysis, but its prognostic impact remains debatable (3). According to the internationally adopted system, MCTs are usually graded as well differentiated (grade I), moderately differentiated (grade II) or poorly differentiated (grade III) tumors.

Alcian blue safranin staining is the most important cytochemical methods for identification of the mast cell. The method was described for the first time by Spiecer and modified by Worthington, Baley, Csaba. This staining identifies only mast cells and gives information about the ratio between sybtypes of cytplasmic granules. Toluidin blue was first described by Paul Erlich and identifies mast cell because of the high content of GAG and metachromatic properties. Citoplasmatic granules are even metachromatic in red-purple or ortochromatic in blue.

Immunohistochemistry has become a practical and widely used tool for diagnosis in human pathology since the 70's. However, its application in veterinary diagnostic pathology has not been so common, especially due to the lack of specific antibodies. The proto-oncogene c-kit is known to play a critical role in mast cell development and tumors, localized to human chromosome 4,6 encodes a transmembrane receptor, CD117/c-kit, belonging to the class III receptor tyrosine kinase family, which includes the receptor for colony-stimulating factor 1, and the platelet-derived growth factor receptors type A and B.

Monoclonal antibodies to MC tryptase effectively stained both canine and human mast cells and were also used for the identification of MCs. Human mast cell tryptases comprise a family of trypsin-like neutral serine proteases that are predominantly expressed in mast cells. Mast cell tryptase is capable of degrading vasoactive intestinal peptide and activating prekallikrein as well as generating kinins, all important mediators involved in bronchoconstriction and airway hyperresponsiveness, which are major contributors to allergic airway disease (2). Mast cell tryptase also exhibits mitogenic effect on human airway smooth muscle cells, and human lung and dermal fibroblasts. Mast cell degranulation and thereby release of mast cell tryptase as well as histamine, leukotrienes and cytokines into the surrounding tissue is a pivotal event in an inflammatory response and seems to play an important role in host defense against pathogens identification of mast cells through staining of tissues with antibodies specific for human mast cell tryptase has been useful in identification of focal and diffuse MC infiltrates in primary MC disease and mastocytosis.

The aim of this study consists of a case report of a tumor in a 11 years old female dog with a nodular and ulcerative cutaneous lesion about one year before diagnostic.

Materials and methods

Case presentation. An 11 year aged female pitbull terrier, castrated, previously (at the age of 3 months) diagnosed with canine demodicosis treated and healed both clinically and parasitologically, with good nutrition and growth conditions has presented with nodular and ulcerative cutaneous lesions on the thorax and rhinostilus, with a history of one year. After 10 months of unsuccessful therapy, a cutaneous biopsy was sampled and sent to the histopathology lab for examination. Firstly, the biopsy was fixed in formlin for 48 hours, included in parafin and sectioned at 3 microns. The slides were colored with standard method hematoxilyn eosin to establish the morphopathological diagnosis. Aditional slides were syained with cytochemical staining alcian blue safranin and toluidin blue for identification of the mast cells. Immnuhistochemical methods were performed with tree antibodies vimentin clone V9, CD117 clone c-kit and mast cell tryptase, clone AA1. Antigen retrival for vimentin was performed for 5 minute by heating in citrate buffer, incubating with primary antigen for 30 minutes, and applying the working system EnVision. Antigen retival for CD117 was performed by heating with buffer citrate pH 6.0 for 20 minute, dilution used 1:500 and incubation at room temperature for 30 minute with primary antibody. Working system used was EnVision+ for 30 minute at room temperature. Antigen retrival for mast cell tryptase was performed using microwave in buffer based on EDTA, pH 9.0, incubating with primary antibody and the working system was the same. Visualization for all antigen was prtfomrd with 3,3 diaminobenzidine. Endogenous peroxidase activity was blocked by immersing slides in oxygenated water 3% for 5 minutes for all antibodies used. The counter staining was performed with Lille's modified hematoxilyn. The mounting was in permanent environment and visualized with the Nikon Eclipse E600 microscope. Pictures were taken in JPEG format.

Results and discussions

First clinical and dermatological examinations pointed towards a dermatological condition, and the dog was treated locally with Veteusan, Cicatrisol, Nidoflor and Imaverol, successively, and generally with immunostimulatorsand vitamins and minerals. A cutaneous raclate was prelevated and cultured on Laborand culture medium. The examination for parasites (*Demodex*, *Sarcoptes*, *Cheyletiella*) and mycetes (*Microsporum*, *Trichophyton* and *Candida*) were negative. Macroscopic examination of the biopsy reveled nodular ulcerated lesion, with the dimensions of 2x1,5x0,5 cm, white – reddish on section, with areas of necrosis and haemorrhage. Hematoxilyn eosin staining establish a large areas of round tumor cells, with mild atypia and high mitotic count, accompanied by areas of necrosis, with normal suprajacent epidermis and adnexes. Tumor cells were medium in size, oval and polygonal in shape, with centrally located nucleus, and the cytoplasm was pale stained with eosin, with granular diffuse pattern in some

cells. Inflammatory infiltrate consisting mainly of lymphocytes was focally found, mainly around hair follicles. Toluidin blue staining was performed at pH3, and showed that tumour cells present granular cytoplasm, mostly orthochromatic. It must be noticed that all tumor cells were stained with this method, without significant differences between individual cells. Alcian blue safranin stained tumor mast cell in a heterogeneous manner. The large majority of tumor cells were intensely staine in blue with alcian blue, and this applies mainly to the superficial part of the tumor. In the deeper part, were found scattered safranin positive mast cells, stained with cytoplasmic granular pattern. Taken together, the morphologic and histochemical findings, support the diagnosis of mastocytoma. Next, we analyzed the expression of some molecular markers, based on the fact that histochemical methods are useful nut not enough specific. Vimentin was positive in all tumor cells with diffuse cytoplasmic pattern and the inflammatory infiltrate cells were negative. Tumor cells staining with CD117 were weakly positive with cytoplasmic pattern but for MCT were intensely positive, with cytoplasmic pattern. Looking for the distribution of positive tumor cells, we found the most frequently, CD117 positive cells are found in the supertficial area of the tumor, and mast cell tryptase, despite positive in the entire tumor mass, was more intensely expressed in the deepest part, close to the proliferation front.

Conclusions

There was the first time when an examination of the tumor of a dog was performed with method specific to humans; we used examination for parasites, mycetes, histochemical and immunohistochemical methods.

There are few reports in literature for this type of tumor. We used standard method hematoxylin – eosin, but the diagnosis was established by histochemical and immunohistochemical methods when all of the tumor cells were intensely positive for generic marker of mast cell.

Despite canine mastocytoma is a well known entity, it should be stressed on the possibility to diagnose such tumors in human, based on histochemical and immunohistochemical methods.

References

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