
7. Summary

Canine mast cells: distribution, density, heterogeneity, and influence of fixation technique

Mast cells play an important role in physiological and pathological reactions. The present study was carried out to determine the physiological distribution of mast cell numbers and subtypes in the dog according to tissue location, staining and fixation methods and thus helps to evaluate their role in disease.

Tissue samples from stomach, duodenum, lung, lymph node, skin and uterus of thirteen dogs were evaluated. Samples were fixed in formalin as well as in Carnoy's fluid.

Mast cells have been identified with either a metachromatic staining method for mast cell proteoglycans or an enzyme-immunohistochemical staining technique for the detection of the mast cell proteases chymase and tryptase. Protease content was examined with a newly developed double enzyme-immunohistochemical staining technique and it was possible to show both mast cell proteases within one mast cell simultaneously. As a result a so far not described mast cell subtype, the only chymase containing C mast cell could be shown in the dog. This study further provides detailed information about the density of the different mast cell subtypes according to their protease content as well as about the density of formalin sensitive and formalin resistant mast cells, respectively.

The following findings can be summarized:

1. The **average mast cell density** varied between the different organs and between different tissue locations within one organ.

The highest mast cell density was found in the duodenum, followed by stomach, lung, uterus, skin and lymphnode.

Within one organ the highest mast cell density was found in the following tissue locations:

- in the stomach within the apical lamina **mucosa**,
- in the duodenum within the stratum subglandulare of the mucosa,
- in the lung within the lamina propria of bronchi and in the interstitium perivascularly,
- in the uterus within the stratum vasculosum and the stratum longitudinale of the tunica muscularis,
- in the lymph node within the trabecula and the capsule and
- in the skin in the dermis perivascularly.

2. Canine mast cells can be subdivided in formalin sensitive and resistant mast cells, respectively. They are found in all tissue samples examined and not confined to mucosal or connective tissues. Formalin sensitive mast cells are, however, predominant in the mucosa and formalin resistant mast cells are dominant in the intestinal submucosa and in the skin of the dogs.
3. Three mast cell subtypes can be distinguished in canine according to their protease content:
mast cells which contain tryptase (T) but not chymase (**T mast cells**),
mast cells which contain tryptase (T) and chymase (C) (**TC mast cells**) and
mast cells which contain chymase (C) but not tryptase (**C mast cells**).
4. The percentile distribution of these three mast cell subtypes depends on the tissue, the location within the tissue, and the fixation technique used.

After fixation in Carnoy's fluid **more chymase-positive mast cells** and less T mast cells could be detected in stomach, duodenum and lung (except perichondrium), uterus and trabecula and parenchyma of the lymph node.
Periadnexal and perivascular in the skin more C- and T-mast cells were found in sections fixed in Carnoy's fluid.

Independent of the fixation technique used, the **T mast cell** is the predominant mast cell subtype in stomach, duodenum and lung. In uterus and lymph node the **T mast cell** is the predominant mast cell subtype after formalin fixation.
Independent of the fixation technique used, chymase containing mast cells (**TC and C mast cells**) are the predominant mast cell subtypes in skin. After Carnoy's fixation they are predominant in uterus and lymph node.

In some tissues the predominant **mast cell subtype changes** depending on the fixative used.

5. In stomach and duodenum formalin sensitive mast cells can be found among all three mast cell subtypes. In lung and lymph node formalin sensitivity can be found in the majority of the chymase containing mast cell subtypes (**TC and C mast cells**), in the uterus exclusively among Chymase containing mast cells (**TC and C mast cells**) and in skin exclusively among T and C mast cells.
6. Unlike in humans (ALDENBORG and ENERBÄCK, 1994) this study revealed in the majority of canine tissues strikingly more mast cells detectable by methylene blue staining than by the enzyme-immunohistochemical double staining.

The differences established between the examined dogs and the data about heterogeneity in human mast cells indicate species specific differences. It has been shown in this study, however, that different staining and fixation techniques used for the identification of mast cells, can be attributed to methodical differences.

In conclusion the data about mast cell heterogeneity from other species or obtained by

different staining methods are not comparable to data of the dog. Considering these facts the data obtained in this study can be used as reference values to examine the role of mast cells in dogs under pathological conditions.