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Mast Cells in the Rat Tongue

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The aim of the present study is to determine the quantity, distribution and age differences of the mast cells in rat tongue (in lamina propria and muscle layer). The experiments were carried out on rats of different ages - 20 days, 3 months and 1 year. We used toluidine blue and Bismarck brown staining for visualization of mast cells. We observed more mast cells around nerve bundles and blood vessels. In 20 days and 3 months old rats we observed more mast cells in the deep muscular layer of the tongue compared to lamina propria. The density of mast cells in 1 year old rats was equal in lamina propria and muscle layers. Distribution of mast cells in rat tongue was different in rats of different ages. The total number of mast cells was constant during lifetime; only the distribution changed. Furthermore, we show that staining with Bismarck brown demonstrates more reliable results.

Key words: rat, tongue, mast cells

Introduction

The tongue is an interesting structure in many ways. Substances are commonly administered sublingually, its observation has an important diagnostic value, it is a frequent trauma spot, as well as a target of reflexology manipulation. Mast cells distribution and involvement in processes involving the tongue are therefore interesting to study. The data regarding their normal distribution in the tongue are scarce. Even though rat mast cells differ from human ones, they are still suitable for preliminary testing and control group for experiments with other animals.

It has been previously established, that in the tongue, the mast cell concentration was the same both along its length and in the symmetrical parts of the organ. The concentration of mast cells diminished considerably from the tongue, the duodenum and down to the stomach [14]. Researchers have observed that numerous mast cells were scattered throughout the submucosal region, adjacent to nerve bundles, blood vessels, and skeletal muscle. Mast cells occurred within bundles containing both myelinated and unmyelinated nerves in the rat tongue. Despite this data, no clear evidence for the existence of any specific mast cell distribution in other parts of the animal body has been provided [2].

In mice, mast cells were present in abundance in the tongue, and there was no clear evidence of a difference between numbers of mast cells in animals of different age or

sex [6]. Considerable mast cell heterogeneity exists within the gastrointestinal mucosa of the mouse and indicates that there are both similarities and differences between mouse and rat in the distribution of mast cells and of their granule proteinases [10].

In healthy rats, mast cells were present in abundance in mesenteric lymph nodes and tongue. There was no clear evidence of a difference between numbers of mast cells in young and older animals. There were more mast cells in rats than in mice. Mast cells appear to be more abundant in Wistar rats than in Sprague Dawley rats. Mast cells were hardly seen in dogs and primates except for a few in tongue and sciatic nerve [7].

Mast cell populations in mammals have been recognized as morphologically and functionally heterogeneous. It is currently accepted that mast cell heterogeneity occurs not only in different species but also within the same organ in the same species. Mast cells in the nasal mucosa are essentially unaffected by the polyamine compound 48/80, indicating that they are functionally dissimilar to the connective tissue type mast cells exemplified by those present in the rat tongue [3]. In the rat, the individual mast cell secretory granules may be divided into three subpopulations based on the presence of the specific proteases RMCP-1, RMCP-2, or a variable combination of these two proteases. Mast cells in the tongue only express RMCP-1, both in normal and infected animals [5].

The aim of the present study is to determine the quantity, distribution and age differences of the mast cells in rat tongue, comparing lamina propria and muscle layer.

Materials and Methods

The experiments were carried out on six male and female Wistar rats from each age – 20 days (65-80 g body weight), 3 months (220-350 g) and 1 year (400-450 g). The experimental design was approved by the Research Ethics Committee at the Faculty of Medicine of Trakia University (TrU project number: 1317/2017 MF). All efforts were made to minimize the number of animals used and their suffering. For the histological preparations, the rats were deeply anaesthetized with Ketamine-Xylazine with the usual dosage, and perfused first with 0.05 M PBS followed by 4% PFA in 0.1 M phosphate buffer, pH 7.36. Tongues were dissected, and postfixed in the same fixative overnight at 4 °C. Tissue was paraffin-embedded and sectioned on a conventional microtome in 5 μm sections. We used classical histological staining techniques: H&E, Toluidine Blue and Bismarck Brown [13]. Cells were counted per viewfield at x200 (0,163 m^2) using LAS v.4.12 (Leica, Germany). Mast cell density was expressed as mean number of cells per viewfield. For the statistic evaluation, we counted the cells on 5 different sections per animal, 5 viewfields per slice, for each staining. Raw data were analyzed using GraphPad Prism 6 (GraphPad Inc, USA) with one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. P values < 0.05 were considered statistically significant.

Results

The results show interesting trends in the mean count of mast cells in different ages, layers, and stains. We observed that numerous mast cells in rats were scattered throughout the lamina propria (**Fig.1**) and in the deep muscular layer (**Fig. 2**). We observed more mast cells in the loose connective tissue around nerve bundles and blood vessels. Mast cells were observed as solitary cells or in groups of several cells.

Distribution of mast cells was different between 20 days, 3 months and 1 year old rats. In 20 days and 3 months old rats we observed more mast cells in the deep muscular

layer of the tongue compared to lamina propria. The density of the mast cells in 1 year old rats was equal in lamina propria and muscle layers. At the age of 1 year the quantity of the mast cells in both layers was without statistical difference (**Fig. 3**). The mean number of mast cells per viewfield was around 8 (8,45-8,77) in all ages (**Table 1**). The total number of mast cells does not change during lifetime, it remains the same, however the distribution changes.

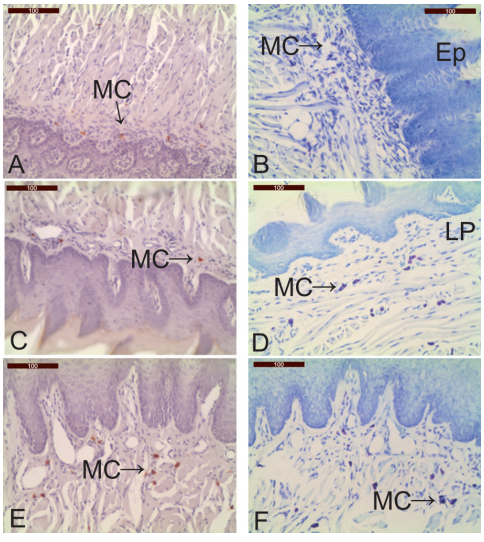

Fig. 1. Mast cells in lamina propria of rat tongues stained with Toluidine Blue (B, D, F) and Bismarck Brown (A, C, E) form 20d, 3m, 1y rats. MC - mast cells, Ep- epithelium, LP- lamina propria. Scale bar = 100 μm.

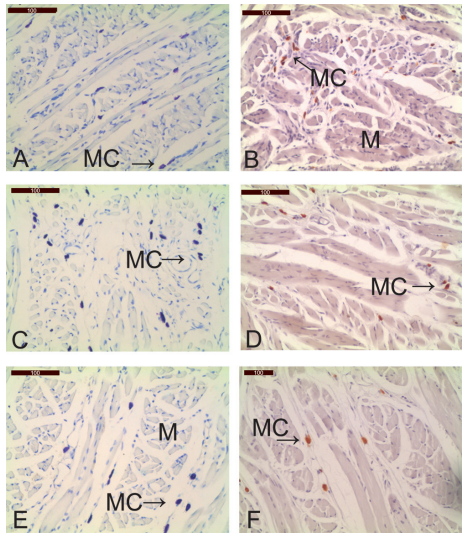

Fig. 2. Mast cells in muscle layer of rat tongues stained with Toluidine Blue (A, C, E) and Bismarck Brown (B, D, F) form 20d, 3m, 1y rats. MC – mast cells, M - muscle layer. Scale bar = 100 μm.

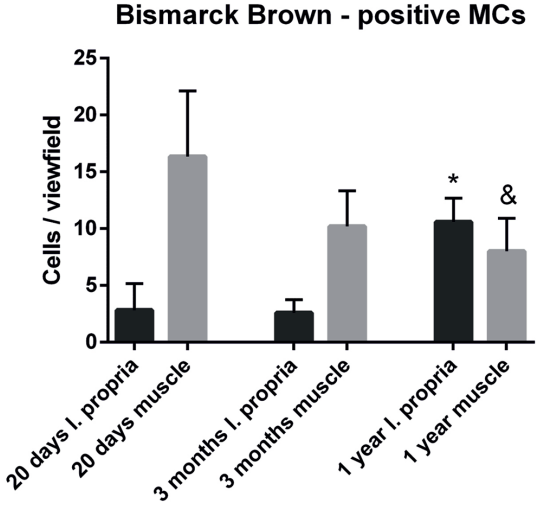

Fig. 3. One-way analysis of variance (ANOVA) with Tukey's post-hoc test for multiple comparisons of mast cell counts of different ages (data from analysis of Bismarck brown staining only). *$p<0.01$ (3 months lamina propria vs. 1 year submucosa); &$p<0.01$ (20 days muscle vs. 1 year muscle)

The different stains used showed some difference in the demonstrated numbers of mast cells. Toluidine blue staining produced a great variability of the numbers of mast cells in lamina propria and muscle layer (**Table 1**). Bismarck Brown staining, on the other hand, demonstrated more reliable results compared to Toluidine blue staining, mainly because of the more consistent results obtained.

Table 1. Mean count of mast cells in different ages (20d, 3m, 1y rats) and layers (lamina propria and muscle layer) and stains (Toluidine Blue and Bismarck Brown).

Numb. of mast cells in one visual area, mag. X20							
stain	Bismark Braun		Toluidin Blue				
layer	lam.pr.	muscl.	lam.pr.	muscl	maen lam.pr.	mean muscl.	mean all
age							
20 d	2,833333		7,4285714		5,130952381		
		16,33333		8,5		12,41666667	8,77381
3 m	2,6		5,2857143		3,942857143		
		10,2		15,375		12,7875	8,365179
1 y	10,6		6,5714286		8,585714286		
		8		7,7143		7,857142857	8,221429
mean quantity of mast cells in one visual area at x20 during lifetime							8,453472

Discussion

It has been previously described, that the total number of mast cells was similar in the superficial layers of all oral tissues studied. There were more mast cells in the deeper than in the superficial portions of the tongue. Mast cells with staining characteristics and size similar to those observed in the intestinal mucosa (MMCs) were found together with 'classical' connective tissue mast cells (CTMCs). The results suggest that the mast cell population of oral mucosal tissues of the rat contains both CTMC- and MMC-like cells [8]. The mast cell population is very dynamic and can respond to different stimuli – a considerable increase of density of mast cells in rat mucosae was reported after photodynamic therapy [11].

The close anatomical associations suggest communication between nerve fibers and immune cells, which can be crucial for maintaining mucosal homeostasis and for ensuring an appropriate response to injury [4]. That could be part of the morphological substrate of the reflexogenic response after tongue manipulations (like acupuncture). Also the involvement of mast cells with branches of the trigeminal nerve is not to be ignored. TN stimulation has been shown to result in MC activation and oral vascular permeability, suggesting that MC inhibitors may be used for the treatment of oral inflammatory diseases [1].

Some age differences in the distribution of oral mast cells have been previously reported. It has been demonstrated that the total number of mast cells in the tongue, buccal mucosa, and gingival mucosa was significantly higher in the juvenile than in the adult rats. In the buccal and gingival mucosa, more than twice as many mast cells were found in the young animals [9].

The results obtained in our study indicate that both staining used by us (Toluidine blue and Bismarck brown) were suitable for visualizing the mast cells, however the Bismarck brown showed better grouping of the results, which could be due to lower specificity of the Toluidine Blue for mast cells and the staining of eosinophil and plasmatic cells alongside mast cells. The quantity of mast cells per visual area was constant in all samples but the distribution changed with age. Young animals have lower number of mast cells in lamina propria than in the muscle layer. This distribution could be a normal feature for the growing organism. The older animals have equal distribution as their structural development is completed.

A comparative characterization of the oral mucosa in various animals is needed to identify the best animal model(s) for nonclinical evaluation of sublingual immunotherapy products. The oral mucosae of minipigs and monkeys are closest to that of humans, and the immune networks are quite similar between all rodents and non-rodents. That data also support the use of rats and minipigs to perform biodistribution and safety studies of sublingual products [12]. Interesting area for our future work could be studying the effects of the acupuncture on the rat tongue.

Conclusions

Distribution of mast cells in rat tongue was different in rats of different ages. The total number of mast cells does not change during lifetime; however, the distribution in lamina propria and the muscle layer changes significantly. The staining with Bismarck brown demonstrated more reliable results.

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