

Mast Cells in the Intrapulmonary Airways in Rats of Different Age

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The rat is one of the most frequently used species in experimental models regarding pulmonary fibrotic and allergic processes. The lack of data about the comparison between mast cell distribution in the different layers of intrapulmonary airways, interalveolar septa and pulmonary pleura in normal left lung and that in right lung of rats at different ages motivated us to perform this study. Eighteen rats at the age of 20 days, 3 months and 1 year were used in the study. Tissue pieces were taken from both left and right lung. Sections were stained with Toluidine blue and Bismarck brown. Mast cell number, estimated per field (x200 with area of 0.163 mm²), were specified for different layers of the intrapulmonary large and small bronchi, terminal and respiratory bronchioles, interalveolar septa and pulmonary pleura in both left and right lungs and age dependent peculiarities were identified.

Key words: lung, mast cell, rat

Introduction

The rat is widely used in experimental models regarding pulmonary fibrotic processes, hypoxia and allergic bronchoreactivity [9, 12, 13]. Mast cells (MCs) are one of the main cells involved in the pathophysiology of the mentioned processes, accompanied by increased number of these cells.

It is important to notice that the data about the MC distribution in rat lung are controversial. Some authors reported that in rat, the mast cell number decreased from large to small bronchi, and were not detected in the interalveolar septa [1, 2, 5]. In contrast, other authors established mast cell distribution in the alveolar septa in the same species [3, 6, 13].

Detailed information about the distribution of both metachromatic and serotonin positive mast cells in the intrapulmonary airways, interalveolar septa and pulmonary pleura of cranial lobe in rat right lung has been reported in our previous study [6]. Metachromatic cytoplasmic granules of mast cells together with the histamine-producing capacity and the existence of membrane IgE receptors are fundamental properties of these cells. The metachromatic staining with cationic dyes, for example Toluidine Blue, at low pH enables the identification of glycosaminoglycans such as galactosaminoglycan and heparin, produced by mucosal mast cells (MMC) and connective tissue mast cells

(CTMCs) [4]. Enerback et al. [4], reported that the granules of the rat MMCs, like those of the few CTMCs in the subserosal layer of the organs, stained strongly with Toluidine Blue.

The aim of the study was to compare the number of mast cells in the different layers of intrapulmonary airways, interalveolar septa and pulmonary pleura in both left and right lungs of normal rats at different ages.

Materials and Methods

Animals

In this study we used six male Wistar rats for each age group (20 day-old, 3 month-old and 1 year old), housed under a 12/12 h light/dark cycle and given a standard diet and tap water *ad libitum*. The procedures were performed according to the Bulgarian laws about the animal care (Ordinance 20 of 01.11.2012 on the minimum requirements for the protection and welfare of experimental animals and requirements to objects for use, cultivation and/or delivery) and the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The animals were anesthetized with ketamine and xylazine (90 mg/kg + 10 mg/kg, IP), then transcardially perfused with 4% paraformaldehyde in phosphate-buffered saline (PBS).

The cranial and caudal lobes of right lung as well as cranial part of left lung from each animal were removed, immersed in the same fixative for 24 h, washed in PBS, dehydrated, cleared in xylene and embedded in paraffin.

Histochemistry for detection of metachromatic mast cells using Toluidine blue

Cross sections of 5 μm thickness from the tissue specimens were cut and mounted on gelatin-coated glass slides, deparaffinised in two changes of xylene, and rehydrated through descending ethanol concentrations. The sections were immersed in buffered solution of Toluidine blue (pH=3).

After Toluidine blue staining additional Bismarck brown staining was performed on serial sections in order to confirm the mast cell presence. The adjacent sections were deparaffinised, rehydrated through descending ethanol concentrations and immersed in a staining solution of 500 mg Bismarck brown in 80 ml 96% ethanol and 10 ml 1N HCL for two hours at room temperature. After three changes of 70% ethanol for differentiation, the sections were counterstained by immersion in Mayer's haematoxylin, then rinsed in tap water, cleared in xylene and coverslipped in Entelan [10].

Statistical analysis

The number of mast cells was estimated on two microscopic fields (X200 with area of 0.163 mm^2) from the sections of the three lung lobes from each animal using a light microscope (LEICA DM1000) equipped with a digital camera (LEICA DFC 290). The data for mast cells density (number per field) were processed using GraphPad Prism 6 for Windows (GraphPad Software, Inc., USA) via one-way ANOVA followed by Tukey-Kramer's post-hoc test. P-values of less than 0.05 were considered statistically significant. The data are presented as mean \pm SD.

Results

In this study toluidine blue staining enabled to mark and count metachromatic MCs in the different layers of intrapulmonary airways as well as in pulmonary pleura from

the left lung (LL), cranial (CrLRL) and caudal lobes of right lung (CaLRL) in rats at different ages (**Fig 1**). To confirm the presence of MCs, Bismarck brown staining was performed (**Fig 2**). In this regard, adjacent sections were used, where the same MCs were demonstrated by both methods (**Fig.1** and **Fig. 2**).

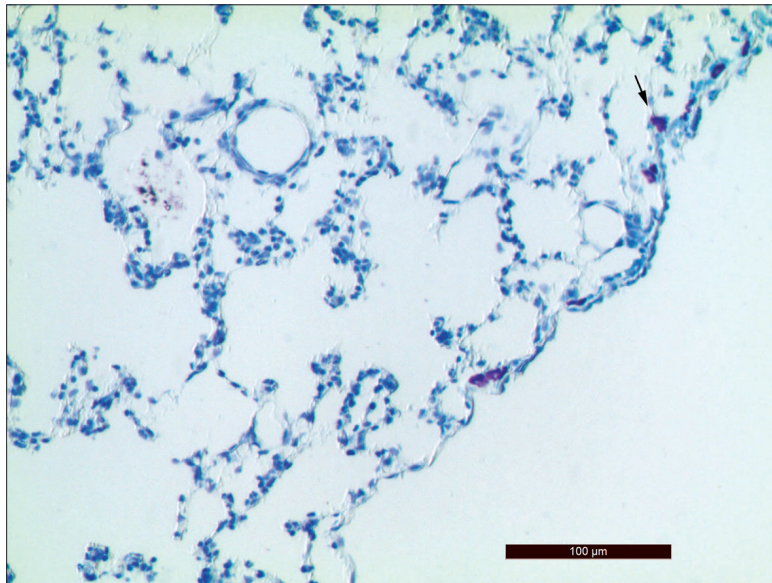


Fig. 1. Metachromatic MCs (arrow) in the visceral pleura of cranial lung lobe in rats at the age of 3 months. Toluidine blue staining. Bar = 100µm.

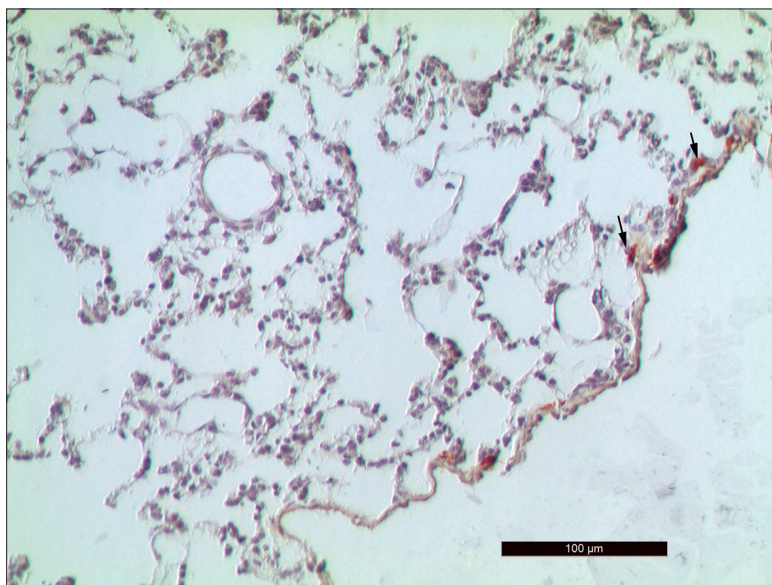


Fig. 2. MCs visualized by Bismarck brown staining (arrows) in the visceral pleura of cranial lung lobe in rats at the age of 3 months. Bar = 100µm.

In the rat, the intrapulmonary airways are devoid of cartilaginous structures; large bronchi represented those of the first bronchial generation and the others were considered to be small bronchi (second generation) which continue with the terminal and respiratory bronchioles. Mast cells were counted in the airways propria bordered by the base of the epithelium and muscular layer, also in the muscular and adventitial layer. In general, mast cells were located in lamina propria predominantly of large and small bronchi, but rarely in propria of bronchioles, near blood vessels in the adventitia. The most MCs were found in the adventitial and muscle layers of the airways, than in the propria. The least MCs were found in the respiratory bronchioles and interalveolar septa.

However some differences in the distribution of lung MCs were detected according to both: the place – left lung or cranial and caudal lobes of right lung and the age of animals.

I. Comparing the mast cell number in the wall of airways with different diameters, alveolar septa and pulmonary pleura in cranial lobe of rat right lung

I.1. In rats at the age of 20 days

After comparing the mast cell number in the airways with different diameters, alveoli and pulmonary pleura, the following results were detected:

In the whole wall of the airways of CrLRL from 20-day-old rats, the highest MC number was estimated in large bronchi (LBr) (14.50 ± 2.6), followed by small bronchi (SBr) (2.9 ± 0.5), interalveolar septa (1.5 ± 0.52) and pleura (1.2 ± 0.39). In terminal (TBol) and respiratory bronchioles (RBol), MCs were not detected. Significant difference between alveolar septa and pleura was not found.

In the different layers of LBr wall, the MC number observed in the propria (3.5 ± 0.52) was significantly lower than in the adventitial (6.5 ± 0.52) and muscular layer (5.5 ± 0.52). MC amount in muscular layer was equal to that in adventitial layer.

In contrast to LBr, in the wall of SBr, MCs were not detected in the propria. Their number in muscular layer (1.17 ± 0.39) was equal to that in the adventitial layer (1.75 ± 0.45) but was smaller than in the same layers of LBr.

I.2. In rats at the age of 3 months

Unlike 20-day-old rats, in the whole wall of the airways of cranial lobe of right lung from 3-month-old rats, the highest MC number was estimated in pleura (8.5 ± 0.52), followed by LBr (6.08 ± 0.51), SBr (3.75 ± 0.45) and alveolar septa (1.25 ± 0.45). Similar to 20-day-old rats, in terminal and respiratory bronchioles MCs were not observed. MC number in the muscle layer (4.83 ± 0.39) of LBr was significantly higher than in the same layer (1.17 ± 0.39) of small bronchi, but in adventitial layer (1.25 ± 0.45) of LBr their number was lower than in the small bronchi (2.5 ± 0.51).

I.3. In rats at the age of 1 year

In this group of animals, like 3-month-old rats, MC number was highest in the pleura (10.50 ± 0.52). MC amount in the whole wall of LBr (4.5 ± 0.67) and SBr (4.0 ± 0.42) as well as in terminal bronchioles (4.5 ± 0.52) was similar but higher than in respiratory bronchioles (1.83 ± 0.39) and alveolar septa (1.83 ± 0.39). In the respiratory bronchiole wall and alveolar septa equal number of MCs was detected.

II. Comparing the mast cell number in the wall of airways with different diameters, alveoli and pulmonary pleura in caudal lobe of right lung

II.1. In rats at the age of 20 days

In the whole wall of the airways of caudal lobe of right lung from 20-day-old rats, the most MCs were estimated in LBr (5.5 ± 0.52), but less – in the alveolar septa (1.5 ± 0.52) and pleura (1.5 ± 0.52) (with the same number of mast cell). We found more MCs in the muscle layer (4.00 ± 1.04) than in adventitial one (1.5 ± 0.52). In SBr, terminal and respiratory bronchioles MCs were not detected.

II.2. In rats at the age of 3 month

The most MCs were estimated in pleura (10.50 ± 0.52), followed by LBr (7.00 ± 1.04) and SBr (4.67 ± 1.16), terminal bronchioles (1.17 ± 0.39) and alveolar septa (1.50 ± 0.52). In the propria of all airways and in the whole wall of respiratory bronchioles MCs were not observed. In the wall of LBr and SBr, the MC number was highest in the adventitial layer (5.50 ± 0.52 and 3.08 ± 0.90 , respectively), followed by that in muscle layer (1.5 ± 0.52 and 1.58 ± 0.51 , respectively). In terminal bronchioles these cells were located in the muscle layer (1.17 ± 0.39) only.

II.3. In rats at the age of 1 year

MC number was highest in the pleura (9.00 ± 1.04). MC amount in the wall of LBr (4.67 ± 0.49) and SBr (5.00 ± 0.43) was similar but higher than alveolar septa (1.25 ± 0.45). In the terminal and respiratory bronchioles' wall, MCs were not observed.

III. Comparing the mast cell number in the wall of airways with different diameters, alveoli and pulmonary pleura in left lung in rats

III.1. In rats at the age of 20 days

In the whole wall of the airways the highest MC number was estimated in LBr (10.08 ± 0.99), followed by SBr (2.17 ± 0.38), alveolar septa (1.50 ± 0.52) and pleura (1.08 ± 0.29). In the alveolar septa and pleura the MC amount was almost the same. In the wall of terminal and respiratory bronchioles MCs were not detected. In the adventitial layer of LBr (5.50 ± 0.52) MCs were lower than in propria (1.08 ± 0.29) and muscle layer (3.5 ± 0.52) but significantly more than in the adventitial layer of SBr (1.08 ± 0.29).

III.2. In rats at the age of 3 month

The most MCs were estimated in pleura (8.5 ± 0.52), followed by LBr (4.42 ± 0.51) and SBr (3.50 ± 1.57), terminal bronchioles (2.58 ± 0.67) and alveolar septa (1.17 ± 0.39). Like the caudal right lung lobe, in the propria of all airways and in the whole wall of respiratory bronchioles MCs were not observed.

Unlike the caudal right lung lobe, in the wall of LBr, the MC number was highest in the muscular layer (2.92 ± 0.51), but in SBr and terminal bronchioles similar MC number was observed in the muscle (1.50 ± 0.52 and 1.08 ± 0.29 , respectively) and adventitial layers (2.00 ± 1.04 and 1.50 ± 0.52 , respectively). The highest amount of MC was observed in the muscle layer of LBr than in the same layer of SBr and terminal bronchioles.

III.3. In rats at the age of 1 year

MC number was highest in the pleura (8.92 ± 2.43), followed by the wall of LBr (6.50 ± 2.61) and SBr (3.42 ± 0.67), terminal (1.33 ± 0.65) and respiratory bronchioles (1.17 ± 0.39), alveolar septa (1.58 ± 0.51). There is not significant difference between the amount of MCs in the terminal and respiratory bronchioles, and alveolar septa as well. In the terminal bronchioles MCs were located in their adventitial layer only, but in the respiratory ones – in the muscle layer only.

Discussion

In this study, toluidine blue staining was successfully used for demonstration of metachromasia and identification of lung mast cells which is in agreement with the findings of Enerback et al. [4]. The distribution of toluidine blue stained MCs in the wall of intrapulmonary airways as well as in the interalveolar septa and pulmonary pleura was compared between left lung and cranial and caudal lobes of the right lungs in rats at different ages, for the first time. To confirm the presence of MCs Bismarck brown staining was used, known as an alternative to the Toluidine blue staining [10]. For

that purpose, serial sections were used, where the same MCs were identified by both methods.

The localization of rat lung cells predominantly in the muscular and near the blood vessels of adventitial layer can be explained with the ability of these cells to synthesize mediators such as serotonin [6, 7] and nitric oxide [8], responsible for smooth muscle cell contraction and relaxation. This means that mast cells may regulate the function of the smooth muscle layer of airways with different diameters and the same layer of blood vessels.

Rat lung mast cells and their mediators are widely studied in experimental models regarding pulmonary fibrotic and allergic processes associated with increasing mast cell density [1, 2, 5]. But the information about the normal rat mast cell distribution in the left and right lungs and also in different lung lobes is scarce. In this regard, we undertook this study to establish the MC density in the different lobes of normal rat lungs that can be used as reference values. In general, we agree with the authors reported that in the rat, the mast cell number decreased from large to small bronchi [1, 2, 5].

The data about the mast cell distribution in rat lung are controversial, for example, some authors did not describe mast cell in the interalveolar septa [1, 2, 5]. The current study showed that single mast cells are located in the interalveolar septa in lungs of rats at different ages which confirm the results of other studies where the same mast cell distribution was described [3, 6, 13].

Based on both the results of studies above and our findings we tried to give more detailed data regarding the distribution of lung mast cells in normal rat. Firstly, the normal distribution of mast cells in the different layers of the airways' wall, pulmonary parenchyma and visceral pleura in left and right lungs was elucidated; secondly, lung MC distribution in rats at different ages was established; thirdly, it was clarified whether MCs localize in the interalveolar septa of normal rat lungs or not.

Conclusions

The mast cell populations in the left and right lung of the rat are different in terms of both their distribution along the bronchial tree and visceral pleura, and their distribution during the ages. Our findings could be very useful in planning experimental morphological studies using rat lungs. The results showed that in such studies the same parts of lungs should be collected from the control and experimental animals to be sure that the accurate results will be received.

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