Distribution of mast cells in lung tissues of rats exposed to biomass smoke

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Summary

This study was designed to evaluate the distribution of mast cells in the lung tissues of rats exposed to biomass smoke. Fifty six female Wistar albino adult rats were used. They were divided into two experimental groups (control and biomass smoke-treated), each containing 28 animals. Control rats were not exposed to the biomass smoke at any time during the experiment. Rats in the treatment group were exposed daily (one hour) to biomass smoke for 3, 6 or 9 months. Lung tissues samples were obtained under deep anesthesia from the randomly selected 7 animals in both groups. Lung tissues were fixed in Mota's fixative (BLA) for 24 h and embedded in paraffin. Sections of 6 µm thickness were cut and stained with 0.5% toluidine blue in 0.5 N hydrochloric acid at pH 0.5 for 30 min. The numbers of mast cell in lung tissues of the animals exposed to the biomass for 6 or 9 months were significantly ($P<0.05$) higher than controls. This study showed that long term exposure to biomass smoke was associated with the increased number of mast cells in the lung.

Introduction

Biomass smoke is produced by burning biomass made of animal manure/dung. In some areas, biomass is used, under primitive and inefficient conditions, as the major source of domestic energy. Exposure to biomass smoke, during cooking, baking and heating, in the developing countries was suggested to be a potential risk factor for obstructive airway disease (OAD) and bronchopulmonary disease (*Dennis and Maldonado, 1996*). Several reports suggest that exposure to biomass smoke increases the prevalence of chronic bronchitis and produces lung damage (*Perez-Padilla et al. 1996., Özbay et al., 2001*). Biomass smoke includes NO_2 (oxides of nitrogen), NH_3 (ammonia), hydrogen cyanide, aldehydes, ketones, acrolein, etc. and particles (Pandey et al., 1989).

The role of mast cells (MCs) of the lung tissue has not been studied previously in those exposed to biomass smoke. The MCs in the respiratory tract are now considered to play a pivotal role not only in allergic reaction but also in a number of inflammatory disorders by releasing a variety of biologically active substances, such as histamine, leukotrienes, platelet-activating factors and prostaglandins (*Kaliner, 1985; Marone, 1985*).

MCs play a fundamental role in the initiation of allergic reactions and are believed to play an important role in the pathogenesis of bronchial asthma. Their localization at vascular, mucosal, and connective tissue sites, coupled with their elevated numbers at sites of chronic inflammation, has led to the hypothesis that mast cells are also central to the occurrence of numerous other inflammatory responses (*Katz et al., 1991*) The secretory products of mast cells have important regulatory effects on the immune system (*Vannier et al.,1991*) and on homeostasis in general (*Lewis and Austen 1981*). Mast cells from bronchoalveolar lavage, however, are more sensitive to immunological stimulation and do not require sensitization for maximal response (*Hunt et al. 1991*).

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In previous studies, it has been shown that there is a relation between biomass smoke exposure and chronic obstructive lung disease (*Dennis and Maldonado, 1996; Perez-Padilla et al., 1996; Özbay et al., 2001*). It is also well known that the mast cell is one of the important cells and plays a fundamental role in the pathogenesis of asthma. Therefore, our aim was to investigate the distribution of MCs in lung tissues of rats exposed to biomass smoke.

Materials and Methods

Animals

Fifty six adult inbred female Wistar albino rats (n=7 x 8) weighing about 300 g were obtained from the Laboratory of Animal Science, Medical School, Yuzuncu Yil University, Van, Turkey. The animals were given standard rat pellets (Van Food Factory, Van, Turkey) and tap water *ad libitum*. The rats were housed in individual cages (360 x 200 x 190 mm), each containing 3 animals, 1 month before the start of the experiments. They were divided into two groups (control and biomass smoke-treated), each containing 28 animals. Rats in the treatment group were exposed to the biomass smoke daily (one hour) for 3, 6 or 9 months. Animals in the treated group were exposed to biomass smoke in inhalation chambers equipped with a trap, and designed to sustain dynamic and adjustable airflow. The animals were housed in an individual cage within the inhalation chamber for 1 h/day (from 09.00 a.m. to 10.00 a.m. h). Neither food nor water was given to the animals during the exposure. The control group was housed in identical chambers ventilated with fresh air. There were no deaths in any of the groups. All animals were housed in stainless cages under standard laboratory conditions (light period 07.00 a.m. to 8.00 p.m. h, 21±2°C, relative humidity 55%), and received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health.

Histological procedure

At the end of the experiment, the rats were anesthetized with an intraperitoneal injection of chloral hydrate (6 ml of 7% chloral hydrate/kg body wt) and sacrificed by decapitation. Lung tissue samples were taken from the randomly chosen seven rats in each group for histological examination at the beginning, and at 3, 6 or 9 months of the experiments. Lung tissues were fixed in Mota's fixative (BLA) for 24 h, and embedded in paraffin. Sections of 6 μm thickness were cut and stained with 0.5% toluidine blue (Gurr, England) in 0.5 N hydrochloric acid at pH 0.5 for 30 min (*Enerback, 1986*).

Microscopic Examination

Microscopic examination was carried out at a magnification of 400 and the counts of mast cells were determined per square millimeter by using a standardized ocular grid. Intact or partially degranulated mast cells were counted. The density and distribution of mast cells were examined in the sections stained with toluidine blue. Tissue sections were examined under light microscopy (x400) and the number of mast cells counted in random high-power fields using a Nikon Optiphot 2 light microscope incorporating a square graticule in the eyepiece (eyepiece x10, objective x40, a total side length of 0.225mm). MC density was assessed by counting the number of cells in 200 high power fields in lung tissue preparations of each group. The MC density in each site was calculated and recorded as MC numbers /mm^2. The tissue compartments used to record the MC distribution in the lung were the subpleural and interalveolar connective tissues, peribronchiolar areas, perivascular regions, alveolar septa and alveolar space.

Statistics

Student's t-test was used for comparing groups.

Results

At the end of the experiment, no morphological differences were found in the mast cells of control (Fig. 1) and experimental group in the lung parenchyma.

However, these cells were extremely large in the subpleural area. The MCs varied in shape from ovoid to elongate and were predominantly located in the intralung bronchial lamina propria, subpleural, and alveolar wall and septa. The majority of MCs contained many strongly stained granules, which frequently obscured the nucleus (Fig. 2). Some of the MCs in the lung tissues of the biomass experimental group were degranulated.

The number of MCs in the lung tissue of both groups is shown in Table 1. Although numbers of MCs were counted in the intralung bronchial lamina propria, subpleura, and alveolar wall and septa region of lung tissues, because the distribution of MCs in 3 regions were linear in both groups, their means are shown in the table as the numbers of MCs in the lung tissues. The numbers of mast cell in lung tissues of the animals exposed to the biomass smoke for 6 or 9 months were significantly ($P<0.05$) higher than controls.

Discussion

Many pathological characteristics of lung disease, such as asthma, can be attributed to the actions of mast cell mediators (*Kaliner 1985; Holgate & Kay, 1991*). Therefore, lung diseases increase the number of MCs. MCs in asthmatic lung or such conditions secrete a greater amount of histamine and other mediators than in healthy individuals, thus causing constriction of airways. Mast cells also play a role in the etiology of many inflammatory diseases especially of the lung and intestine. Ercan et al. (1997) suggested that under stressful conditions the number of mast cells in the mucosa of the urinary bladder are increased. In addition to their role in hypersensitivity responses, mast cells can be involved in the generation of the immune response in the respiratory tract, in the central arm of the immune response where histamine is a potent modulator (*Rocklin et al, 1978*), and also at the effector stage of cytotoxicity (*McDermott et al, 1982*). Goto et al.(1984) suggested that after intratracheal administration of bleomycin, lung histamine levels increased as much as 14-fold by day 50. Pulmonary mast cells changes were present early in the fibrotic process, and by day 14 the mast cell density in the parenchyma was 10 times higher than normal. Such an increase of the mast cells was found to be coherent with that in chronic bronchitis (*Pesci et al., 1994*), fibrosis (*Goto et al., 1984*), asthma (*Casala et al., 1987*) or when exposed to radiation (*Bjermer et al., 1993*).

The current experiment indicated (Table 1) that biomass-smoke exposure for 6 or 9 months

Table 1. Number of mast cells in the lungs rat*.

	Groups (n=28)	
Exposure time of biomass smoke (Month)	Control	Experimental Groups
0	25.58±4.3 [a]	25.07±4.3[a]
3	24.81± 2.4[a]	27.05±2.4[a]
6	26.10 ±1.8[a]	31.74±3.1[b]
9	25.32±3.2[a]	37.92±5.6[c]

* All samples stained with toluidine blue, pH 0.5.

Values are mean ± Standard deviation (SE) (Experimental groups n = 7, control n = 7)

[a,b,c] Mast cells density in the second and third biomass smoke-exposed rats was significantly greater than that in control specimens ($p<0.05$).

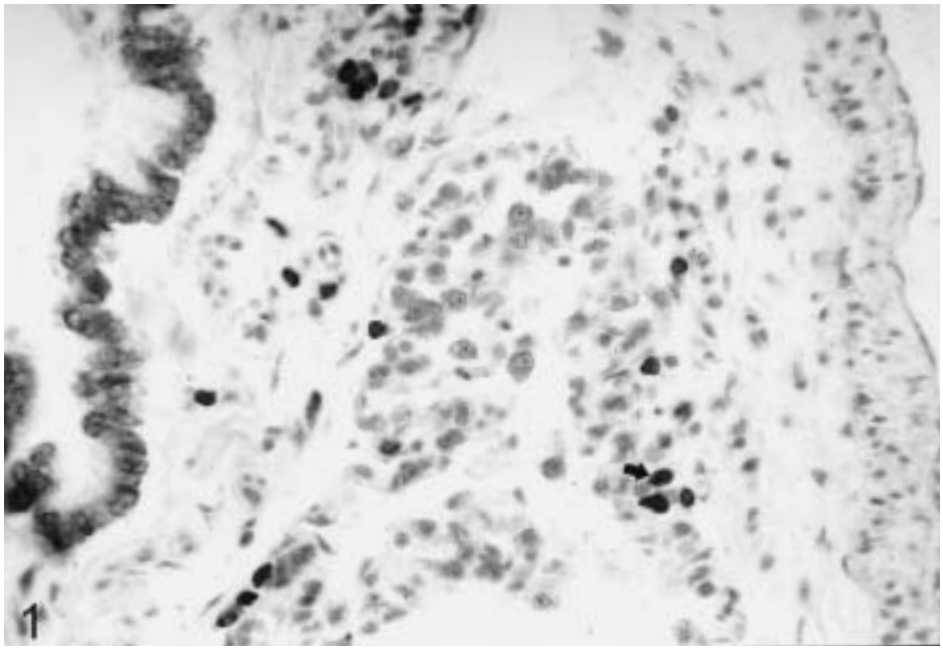

Fig.1. Toluidine blue staining, lung tissues of rats control group, mast cells (arrows), x360.

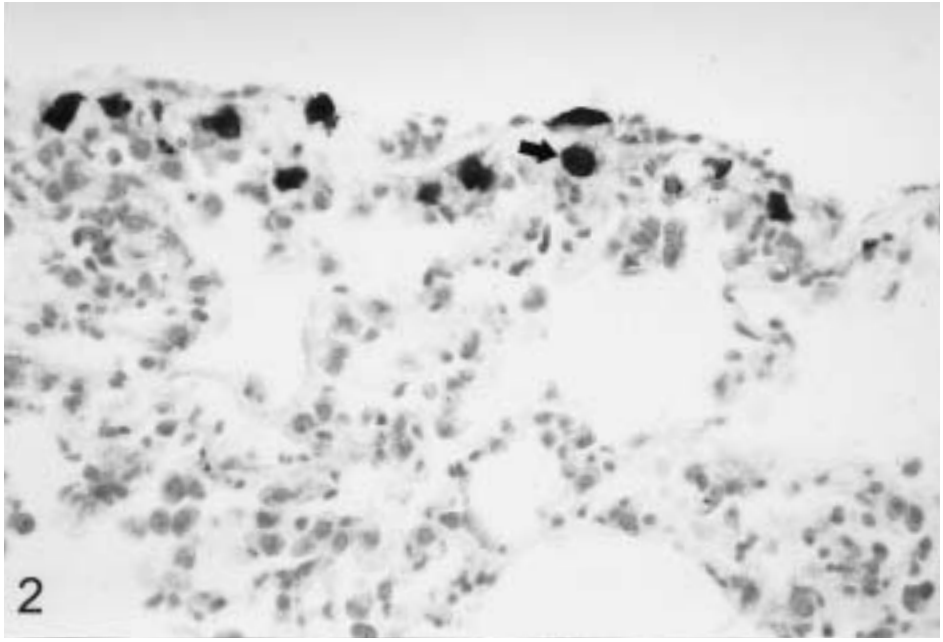

Fig. 2. Toluidine blue staining, lung tissues of rats 6 months biomass-exposed group, mast cells (arrows), x360

increased the number of MCs in the lung of rats. Our study is the first one to show a linear increase in the lung tissue mast cells with the elapse of time. In a quantitative and qualitative study by Fox et al., normal human lungs had an average concentration of 350 mast cells/ mm^2 of alveolar wall whereas abnormal lungs showed an average of 523/mm^2 (*Fox et al. 1981*). Warton et al. (1986) identified that the numerical density of MCs per square millimeter of alveolar wall in tissue section was 299±258 mast cells / mm^2 in normal and 366±260 in asthmatic lung. Pesci et al. (1994) found, 15.51±13.39 and 2.9±3.65, the numbers of mast cell per square millimeter of epithelium in patients with chronic bronchitis and normal control subjects, respectively.

It is concluded that long-term biomass smoke exposure increases the number of MCs in the lung tissue, which might lead to lung diseases such as chronic bronchitis, fibrosis and asthma.

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