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ORIGINAL PAPERS

THE EFFECT OF SUBCHRONIC EXPOSURE TO THE RUBBER VULCANIZATION FUMES ON GUINEA PIG LUNGS

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Abstract: The influence of 28 days' inhalatory exposure to rubber vulcanization fumes at a concentration of 100 mg/m^3 on guinea pigs' lung morphology was investigated. Focal infiltrations of pulmonary parenchyma with lymphocytes, neutrophilic and eosinophilic granulocytes and macrophages were observed. Lymphatic tissue concentrations having the typical appearance of solitary lymphatic nodules were also seen. The use of the double sequential Alcian blue/safranin O staining method for the identification of the mast cells [MCs] revealed that only Alcian-blue-positive MCs were observed, regardless of the region of the lungs examined, both in control and exposed guinea pigs. No safranin-0-positive MCs were seen.
However, the MCs number increased from 1934±91 cells/mm^3 tissue in controls to a statistically significant ($p<0.05$) 2486±89 cells/mm^3 tissue in exposed guinea pig lungs. It was accompanied by histamine content increase from 1.50±0.06 μg/g wet tissue weight and 2.45±0.18 μg/g wet tissue weight, respectively.
The distribution of the lung MCs varied, showing a statistically significant ($p<0.05$) increase in their number in the intraalveolar septa: from 957±53 to 1369±74 cells/mm^3 tissue and in the peribronchial and peribronchiolar spaces: from 204±36 to 359±42 cells/mm^3 tissue.

INTRODUCTION

Rubber vulcanization fumes are one of many hazardous agents of potentially carcinogenic substances that occur in the workplace atmosphere in rubber factories (11). It was also found (6, 7) that workers exposed to the fumes had a higher prevalence of chronic bronchitis and a positive correlation was detected between the duration of employment in the curing department and such respiratory disease symptoms as

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shortness of breath, chest tightness, reduced forced expiratory volume and forced vital capacity (17).

In the present study the influence of subchronic inhalatory exposure to rubber vulcanization fumes on lung morphology in guinea pigs was investigated. Special stress was laid on the mast cell [MC] morphology, number and distribution in the examined lungs.

Mast cells are considered to be the key cells involved in the pathophysiology of many respiratory diseases. Their activation results in secretion of highly potent biological mediators: histamine, leukotriene C_4, prostaglandin D_2, kininogenase, and chemotactic factors for neutrophils and eosinophils (2, 3). Since the work of Enerback, at least two types of MCs have been described in rats and mice when using the double sequential Alcian blue/safranin O method (4, 5); (i) formaline-sensitive MCs, stained with alcian blue and referred to as mucosal, (ii) formalin-insensitive MCs, stained with safranin O and called connective-tissue mast cells. The mast cell phenotype may, however, vary according to different lungs' physiologic and pathologic conditions (9).

It has been shown recently that in normal guinea pig lungs only Alcian-blue-positive MCs are observed in contrast to rats (1). However, bearing in mind the possibility of phenotypic changes in pathologic conditions our aim was the MCs subpopulation distribution in normal and exposed guinea pig lungs.

MATERIALS AND METHODS

Five male Himalayan guinea pigs, weighing 400—500 g, were exposed to the fumes (100 mg/m^3) released during the rubber vulcanization process. The exposition was performed in dynamic inhalation chambers, 6 hours daily, five times a week, for 28 days. The controls ($n = 4$) inhaled air under the same conditions and period of time.

The lungs were fixed in situ by airway perfusion with Carnoy's fixative at a pressure of 25 cm H_2O for 20 min., then immersed in the fixative for additional 16—24 hours. After slicing, they were dehydrated and embedded in parafin. Five μm sections of tissue were cut and stained with hematoxylin/eosin method for histological examination or with 0.1% Alcian blue in 0.7N MCl, pH 0.5 for 1—2 hours and counterstained with 1% solution safranin O in 0.7N HCl for 5 minutes (4) for mast cell identification. MCs were counted according to the morphometric procedure by Weibel et al. (18). MCs in visceral pleura, alveolar wall, peribronchiolar space and perivascular space were counted in areas corresponding to 0.01 mm^2 at magnification of × 100, and then expressed as a number per mm^3 of the tissue.

Histamine concentration in the lungs was also estimated using the spectrofluorometric method (12) and expressed as a μg/g wet tissue weight.

RESULTS

In the controls the lungs were morphologically intact (Fig. 1). The use of the double sequential Alcian blue/safranin O staining method for the identification of the mast cells revealed that only Alcian-blue-positive MCs were observed, regardless of the region of the lungs examined. No safranin-O-positive MCs were seen.

In the guinea pigs exposed to the rubber vulcanization fumes focal infiltration of pulmonary parenchyma with lymphocytes, neutrophilic and eosinophilic granulocytes and macrophages was observed. Lymphatic tissue concentrations having the typical appearance of solitary lymphatic nodules were seen (Fig. 2). Around those lymphatic nodules only a few MCs were observed (Fig. 3). They had the same staining properties as MCs in the controls; only Alcian-blue-positive MCs were observed.

The lung MC counts in unexposed guinea pigs amounted to a mean number of 1934 ± 91 cells/mm^3 tissue. The histamine content was found to be 1.50 ± 0.06 μg/g wet tissue weight. Most of the lung MCs were found in the intraalveolar septa — 957 ± 53 cells/mm^3 tissue. They were also observed in the visceral pleura — 560 ± 58 cells/mm^3 tissue, in the peribronchial and peribronchiolar space — 204 ± 36 cells/mm^3 tissue, and in the perivascular space — 212 ± 38 cells/mm^3 tissue (Table 1).

After subchronic exposure to the rubber vulcanization fumes the lung MCs number and histamine content increased significantly ($p < 0.05$), thus 2486 ± 89 cells/mm^3 tissue and 2.45 ± 0.18 μg/g wet tissue weight, respectively (Table 1). The distribution of the lung MCs in exposed guinea pigs varied, showing a statistically significant ($p < 0.05$) increase in the intraalveolar septa up to 1369 ± 74 cells/mm^3 tissue and in the peribronchial and peribronchiolar spaces up to 359 ± 42 cells/mm^3 tissue. The changes in the MC number observed in the visceral pleura, estimated to be 498 ± 48 cells/mm^3 tissue and in the perivascular space amounted to 260 ± 32 cells/mm^3 tissue, were not statistically significant ($p > 0.05$) (Table 1, Fig. 4).

DISCUSSION

Our findings revealed that after the inhalatory exposure of guinea pigs to rubber vulcanization fumes at a concentration of 100 mg/m^2 for

TABLE 1. The number of mast cells per mm^3 tissue and histamine content in μg/g wet tissue weight in the lungs of guinea pigs; normal (n=5), and exposed to rubber vulcanization fumes [28 days, conc. 100 mg/m^3] (n = 4). Data expressed as a mean ± standard error of the mean. Statistical significance ($p < 0.05$) marked by *

Group	Visceral pleura	Intraalveolar septa	Peribronchiolar space	Perivascular space	Total	Histamine
Normal	560±58	957±53	204±36	212±38	1934±91	1.50±0.06
Exposed	498±48	1369±74 *	359±42 *	260±32	2486±89 *	2.45±0.18 *

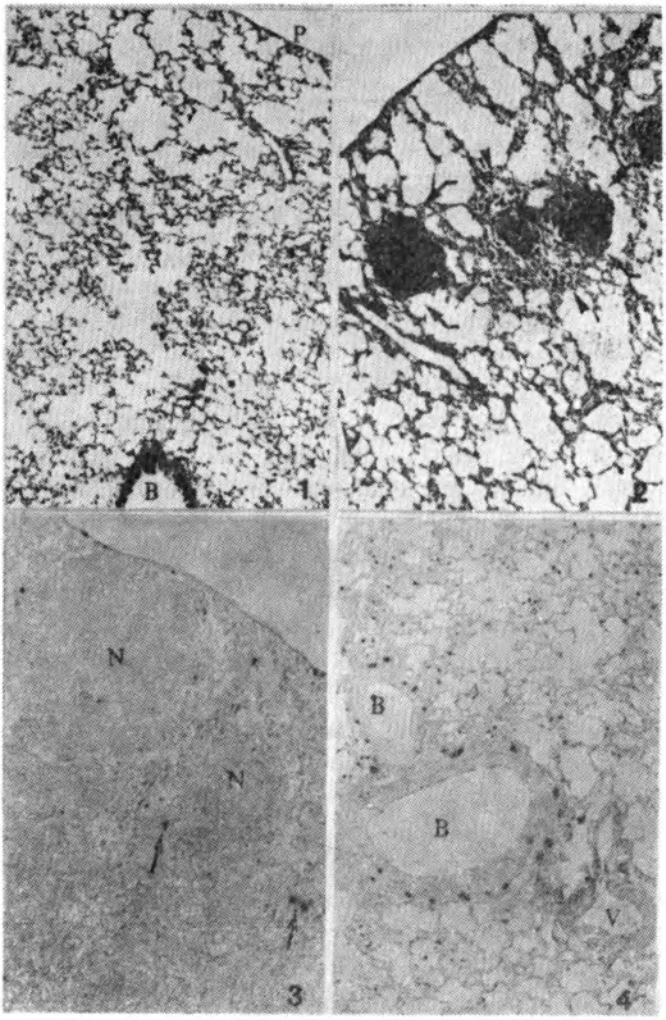

Fig. 1. Photomicrograph of a section of the control lung, showing pleura (P), a bronchiole B), many alveolar ducts, sacs and alveoli. Hematoxylin and eosin staining, ×40.

Fig. 2. Lymphatic tissue concentrations (bend arrows) in the pulmonary parenchyma of the exposed guinea pig. A number of inflammatory cells (arrowheads) are also seen. Hematoxylin eosin staining. ×60.

Fig. 3. Interstitial inflammatory infiltration (arrowheads) and lymphatic tissue concentration (N) in the lung of exposed guinea pig. Note that only few mast cells are seen (arrows). Alcian blue and safranin 0 sequential staining. ×100.

Fig. 4. A great number of mast cells around the bronchiole (B), in the perivascular space (V) and in the alveolar wall of the lung of exposed guinea pig. Alcian blue and safranin 0 sequential staining. ×100.

28 days, pathomorphological signs of lung lesions could be observed. Parenchymal infiltrations with inflammatory cells and an increased number of mast cells were the predominant features.

The mucosal surface of the airways is the first site of contact of inhaled toxic agents. Among various cells lining this surface, MCs are considered essential because of their immediate secretion of many potent biological mediators, an activity which leads to a recruitment of secondary cells such as macrophages, neutrophils and eosinophils (3, 8, 19).

The vulcanization processes generate vapours and gases which may have potential irritant and carcinogenic properties. The presence of benzene, benzo[a]pyrene and nitrosamines in the curing fumes, amongst others, has been confirmed (11).

The increase in the total number of MCs indicates that their proliferation takes place in the course of subchronic exposure to rubber curing fumes. No phenotypic changes were observed; only Alcian-blue-positive mast cells were present irrespective of the region and exposition type studied.

The most significant increase was observed in the intraalveolar septa and in the peribronchial and peribronchiolar space. However, it was shown that exposure to nitrosamines may lead to pleural and peritoneal hemorrhages, and in some cases it may produce cancer in a variety of organs, including the respiratory tract (15). An increased number of MCs was found at an early stage of some chemically-induced animal cancers (10, 14).

It cannot be excluded, however, that the observed parenchymal infiltrations with inflammatory cells were secondary, due to the bacterial infections, as an impaired resistance to Listeria monocytogenes was discovered in mice exposed to benzene, even at very low concentrations (16). An increased susceptibility to pneumonia was also demonstrated in rabbits exposed to benzene (20).

Preliminary data yielded by the present study have revealed the harmful effects on guinea pig lungs following subchronic exposure to rubber curing fume. Further studies have been launched to investigate the problem in more detail.

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