



*Original Contribution*

**HISTOCHEMICAL INVESTIGATION OF THE GLANDULAR AND RESPIRATORY EPITHELIUM IN THE MICE TRACHEA AND LUNG AFTER ORAL ADMINISTRATION OF AMBROXOL**

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**ABSTRACT**

In this investigation we aimed to determine the effect of the mucosecretolytic Ambroxol on the epithelial structural units in the trachea and lungs of conventional mice from ICR line.

Paraffin sections from control and experimental mice were subjected to Mowris alcian blue histochemical staining followed by a PAS reaction. A differentiation of the acid mucopolysaccharides in histochemical tests with alcian blue at pH 1.0 and pH 2.5 was conducted. The histochemical reactivity was determined using light microscopy analysis and subsequently photo-documented.

After the oral administration of 3.5 mg ambroxol structural changes in the glandular and coating epithelium were not found. The secreted glucoseaminoglycans did not show any changes but the secretion in the trachea and lungs was influenced at different rates.

**Key words:** mouse, ambroxol, trachea, lung, histochemistry

**INTRODUCTION**

One of the most frequently used mucolytical agents in the human medicine clinical practice is Ambroxol. It possesses a secretomotorial activity over the secretory and ciliary epithelium, decreases the obstruction of the small aeriferous ducts and increases the amount of the secretory IgA. Only a few studies investigate its influence over the tracheal epithelium in rabbits (6, 10).

Having in mind this data we set ourselves to determine the influence of ambroxol over the epithelial structural units in the middle and caudal parts of the respiratory system in mice.

**MATERIALS AND METHODS**

For the conduction of this study we used materials from 30 conventional mice from ICR line with weight between 18 and 22 g (equal count from both gender). They were divided into a control group of 10 not treated mice and an experimental one with 20 mice

(equally divided from both gender). Every experimental mouse was orally treated with 0.5 ml Mucosolvan solutio (Boehringer Ingelheim International GmbH, Ingelheim, Germany). This dose of Mucosolvan solutio contains 3.5 mg of ambroxol (2-amino-3,5-dibromo-N-[trans-4 hydroxy cyclohexyl] benzylamin). Thirty minutes after the treatment with Mucosolvan solutio, from every mouse (from both groups) material for histological investigation was taken. The material was taken postmortally after an anaesthesia with aether pro narcosi. From every mouse was taken whole trachea (right after the larynx) with lungs. The trachea was in rough guess dissected into cranial, middle and caudal segment. The obtained samples were fixated in 10 % neutral formalin and the fixating mixture of Karnua. The fixated materials were processed following conventional histological methods. A part of the obtained single and serial paraffin sections after their staining with hematoxylin (Erich)-eosin, were used for the preparation of durable histological specimens. Over the rest of the sections (fixated in Karnua mixture) several histochemical tests has been conducted. A combined Mowris alcian blue histochemical staining followed by a PAS reaction has been conducted for the simultaneous determination of the neutral and the acid

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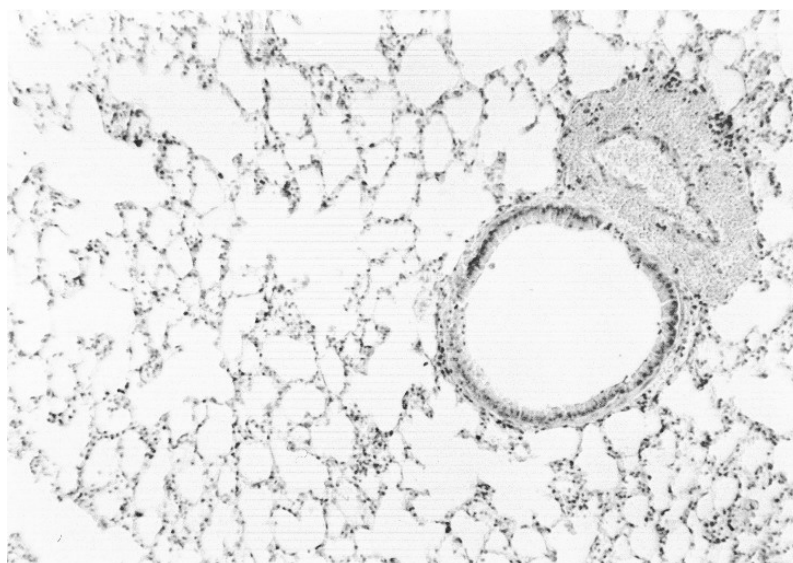
glycoseaminoglycans (8). For the differentiation of the acid glucoseaminoglycans we conducted two histochemical tests with alcian blue at pH 1.0 and pH 2.5 (2). With a light microscope NU – 2 (Carlzeiss Jena, Germany) was conducted an analysis of the microstructural condition of

the examined organs. The manifested histochemical reactivity was reported and photodocumented. The data of the cytochemical reactivity was presented in **Table 1**.

**Table 1.** Data for the cytochemical reactivity in the trachea and lungs in mice treated with ambroxol

Test	MAB/PAS			AB (pH 1.0)			AB (pH 2.5)		
	G	GC	E	G	GC	E	G	GC	E
Trachea	+++	+(-)	+(-)	-	-(+)	-	+++	+(-)	+(-)
Bronchi:									
<i>main</i>	+++	+(-)	-(+)	-	-	-	+++	+(-)	-(+)
<i>big</i>	+++	+(-)	-(+)	-	-	-	+++	+(-)	-(+)
<i>middle</i>	+++	-	-	-	-	-	+++	-	-
<i>small</i>	+++	-	-	-	-	-	+++	-	-
Bronchioles:									
<i>terminal</i>	-	-	-(+)	-	-	-	-	-	-(+)
<i>respiratory</i>	-	-	-(+)	-	-	-	-	-	-(+)
Alveolar ducts	-	-	-	-	-	-	-	-	-
Alveoli	-	-	-	-	-	-	-	-	-

MAB – Mowris alcian blue; PAS – Periodic acid-Schiff; AB – Alcian blue; G – Glands; GC – Goblet cells; E – Covering epithelium; (-) – Lack of reactivity; (+) – Weak reactivity; (++) – Middle reactivity; (+++) – Well expressed reactivity.



**Figure 1.** Lungs. The lumen of the terminal bronchus, the respiratory bronchioles, the alveolar ducts and the alveoli do not possess secretion and has a negative epithelial histochemical reactivity. Staining – MAB/PAS; Magnification – 100X.

## RESULTS AND DISCUSSION

It is taken for granted that the basic sources of the secretion covering the mucosal surface in the middle and caudal parts of the respiratory system are the glands in the trachea and the bronchi as well as the scattered among the respiratory epithelium goblet cells. The investigation of these epithelial structural units in the experimental mice (30 minutes after the reception of 3.5 mg ambroxol), did not found any changes in their microstructure.

After the completion of all the histochemical tests and the comparison of the results from the control and the experimental group, we determined that there is no change in the type and the kind of the excreted secretion. 30 minutes after the reception of 3.5 mg ambroxol, the glandular cells of the tracheal glands didn't possessed reactivity to the applied tests. In contrast to them, the secretion in the lumen of the glands possessed a well pronounced reactivity to MAB/PAS and AB

(pH 2.5), but to AB (pH 1.0) there wasn't any reactivity (**Table 1**). Almost all of the goblet cells were empty 30 minutes after the treatment. Only some of them with preserved secretion had a positive alcian staining after MAB/PAS and AB (pH 2.5) and a lack of reactivity after AB (pH 1.0). The tracheal lumen was empty and only small areas of the ciliary part of the respiratory epithelium were covered with remains of thin layer of secretion, that reacted histochemically positive like the secretion in the glands. We determined the same condition and reactivity in all the branches of the bronchial tree as the one in the trachea. Lumen without content and a lack of epithelial histochemical reactivity were also present in the bronchioles, the alveolar ducts, and the alveoli of the experimental mice (**Figure 1**).

The determined in the present study results confirms the position of other authors (1, 3, 7, 9) on the type and kind of secretion in the trachea and lungs in other animal species. Our results and those obtained by other authors (5, 6, 9, 10) disproves the position of (4) that the mucolytics influences on the secretion formation and the type of secretion in the respiratory tract. Our results confirms the determined from (10) facts about the mucinal histochemistry of the goblet cells in the rabbits trachea.

On the basis of the obtained results we think, that the oral application of 3.5 mg ambroxol in mice do not provokes structural changes, and do not alters the composition of the secretion but possesses a mucolytical and secretory effect in their respiratory ducts. We recommend its investigation over domestic animals and pets.

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