

## The effect of oral administration of salbutamol on the glycoconjugate composition in goblet cells of the tracheal epithelium in rabbits

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**ABSTRACT:** We verified the influence of salbutamol on changes of glycoconjugates contained in tracheal goblet cells in rabbits by the oral administration of Ventolin™ syrup in the dose of 5 ml. Material for both conventional and lectin histochemistry was collected 15 and 30 minutes post exposure. Gradual decrease of percentage of goblet cells containing acid sialylated glycoconjugates was observed 15 minutes after administration. Thirty minutes after administration, neutral glycoconjugates-containing goblet cells were absent. The proportion of goblet cells containing acid sialylated glycoconjugates reached 50% of the value in control animals. Compared with controls, the changes of the character of the glycoconjugate content in the tracheal goblet cells due to the oral administration of Ventolin™ syrup were statistically significant ( $\alpha \leq 0.01$ ).

**Keywords:** tracheal epithelium; goblet cells; sialylated glycoconjugates; lectin histochemistry; rabbit; Ventolin syrup

### INTRODUCTION

In previous studies, Spahr-Schopfer and Shorten with their co-workers (Spahr-Schopfer *et al.*, 1994; Shorten *et al.*, 1995) evidenced the negative influence of intra-tracheally administered aerosol of Ventolin on the tracheal epithelium using the light microscope. Konrádová *et al.* (1996, 1998) described the ultrastructural alteration of the rabbit tracheal epithelium after the inhalation of Ventolin. The same authors revealed significantly lower degree of alteration of this epithelium after inhalation of the propellants alone, used in the preparation Placebo Inhaler. Thus, the evidence was given that the damage to the tracheal epithelium was mostly caused by the efficient substance proper – salbutamol (Konrádová *et al.*, 1999, 2000c). Significant alteration of the tracheal epithelium was also revealed due to the oral administration of Ventolin (Konrádová *et al.*, 2000a, b). The presence of neutral and acidic sulphated and acidic sialylated glycoconjugates (GCCs) in the tracheal goblet cells was widely evidenced in many species including humans (Lamb and Reid, 1972; Jones and Reid, 1978; Jacob and Poddar, 1982; Spicer *et al.*, 1983; Castells *et al.*, 1990, 1991, 1992, 1994; Jeffery *et al.*, 1992). Jones and Reid (1978) and Jeffery *et al.* (1992) described the conspicuous increase up to total dominance of acidic sulphated GCCs in patients suffering from cystic fibrosis

and bronchitis. Lamb and Reid (1972) and Damjanov (1987) held the decrease of acidic sialylated GCCs and the increase of acidic sulphated ones for a common reaction of the tracheal epithelium to general alteration. Our study dealing with changes of the glycoconjugate composition in tracheal goblet cells in rabbits after the inhalation of Ventolin (Vajner, 1998) confirmed the Lamb's and Reid's and Damjanov's opinions.

Therefore, we decided to verify whether also orally administered Ventolin evoked similar changes of the glycoconjugate composition in the tracheal goblet cells' secretion.

### MATERIAL AND METHODS

Ten SPF-male New Zealand White rabbits (Charles River, Sulzfeld, Germany) of the average body weight  $2010 \pm 211$  g were used in the experiment. Six of them were orally administered with 5 ml of Ventolin™ syrup (Glaxo, Greenford, United Kingdom) containing 2 mg of salbutamol. The dose used followed the arrangement of the previous study (Konrádová *et al.*, 2000a). The material was collected under general anaesthesia (ketamine 35 mg/kg and xylazine 5 mg/kg intramuscularly) and local subcutaneous infiltration of the ventral cervical field with procaine from three

rabbits 15 minutes and from the remaining ones 30 minutes post exposure. Four rabbits served as untreated healthy controls, the material was collected immediately after the induction of anaesthesia.

The middle portions of tracheae between the 15th and 20th tracheal rings were formalin-fixed, paraffin-embedded, and cut at 5–7  $\mu\text{m}$ . The combined staining method of Alcian Blue (AB) at pH 2.5 followed by PAS-reaction according to Mowry and Winkler (1956) was used to reveal both total acidic and neutral glycoconjugates. Selective staining of acidic sulphated GCCs was obtained using AB at pH 1.0 (Kiernan, 1981). To detect sialylated GCCs directly, the methods of *in situ* lectin histochemistry were used. At first, we used the Sata's modification (Sata *et al.*, 1990) of digoxigenin-labelled lectin reaction, visualised by the alkaline phosphatase (AP)–X-phosphate (BCIP)–nitroblue tetrazolium (NBT) system (Boehringer Mannheim Biochemica, Germany). *Maackia amurensis* agglutinin (MAA) and *Sambucus nigra* agglutinin (SNA) were used. The reaction of *Tritrichomonas mobilensis* lectin (TML) (Calbiochem, La Jolla, USA) was also employed (Babál *et al.*, 1994, 1995, 1996a, b), visualised in the same way as MAA- and SNA-reactions. We evaluated only goblet cells containing well-developed granules with the positive reaction in each method used. Their marginal sections as well as differentiating secretory elements were not included. Using all given methods, 398, 330, and 191 goblet cells were evaluated in control and experimental animals 15 and 30 minutes post exposure, respectively.

For statistical evaluation, relative values of the six categories of goblet cells, revealed by individual methods,

were evaluated by the chi-square test of homogeneity in frequency tables, using the Yates' correction in low frequencies when appropriate. To evaluate the necessary number of evaluated goblet cells in individual categories, the minimal expected frequency was stated. If this minimal expected frequency had been within the range 2 to 5, Fisher exact test was performed (Tables 2  $\times$  2). The equivalency of sialylated glycoconjugate-detecting methods was tested by the paired *t*-test, median (sign) test, and Wilcoxon's paired test.

The experimental procedures were approved by the Animals Protection Expert Commission of the Faculty.

## RESULTS

The tracheae of both control and treated rabbits were lined with the pseudostratified columnar ciliated epithelium composed mostly of ciliated, goblet, and basal cells. The height of the epithelium was approximately 30  $\mu\text{m}$ . The distribution of secretory elements was irregular.

Using conventional histochemical methods, the secretory elements revealed typical staining patterns according to the type of GCCs they contained. Mucous granules were stained either with the PAS-reaction only or with some proportion of the Alcian Blue up to pure blue (Figure 1). We also encountered cells containing PAS-positive granules located mostly basally and pure blue granules located apically. The appearance of goblet cells reacting with lectins used was the same in both control and treated rabbits. The positive reaction of MAA was featured with the

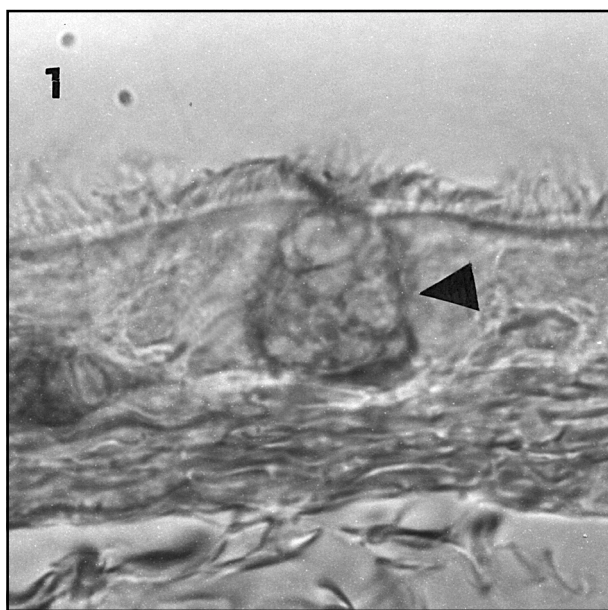


Figure 1. Goblet cell (arrow) containing voluminous mucous granules with acidic glycoconjugates in the rabbit tracheal epithelium 30 minutes after oral administration of 5 ml of Ventolin<sup>TM</sup> syrup. Alcian Blue pH 2.5 + PAS. Enlarged 100 times

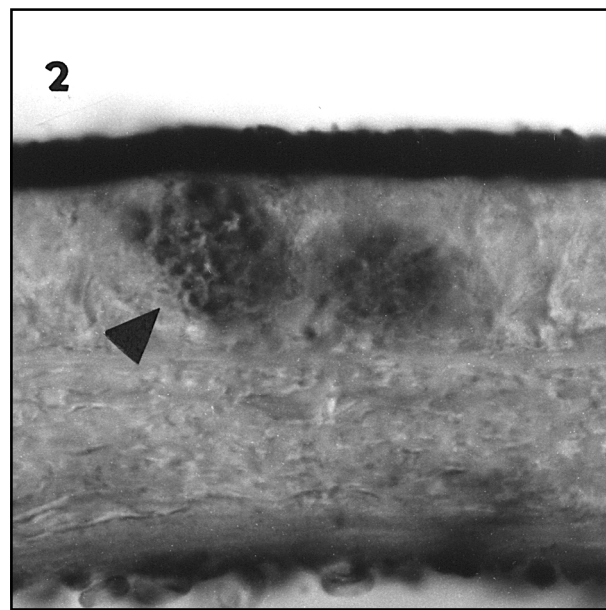


Figure 2. Goblet cell (arrow) containing mucous granules with acidic sialylated glycoconjugates in the rabbit tracheal epithelium 15 minutes after oral administration of 5 ml of Ventolin<sup>TM</sup> syrup. TML reaction. Enlarged 100 times

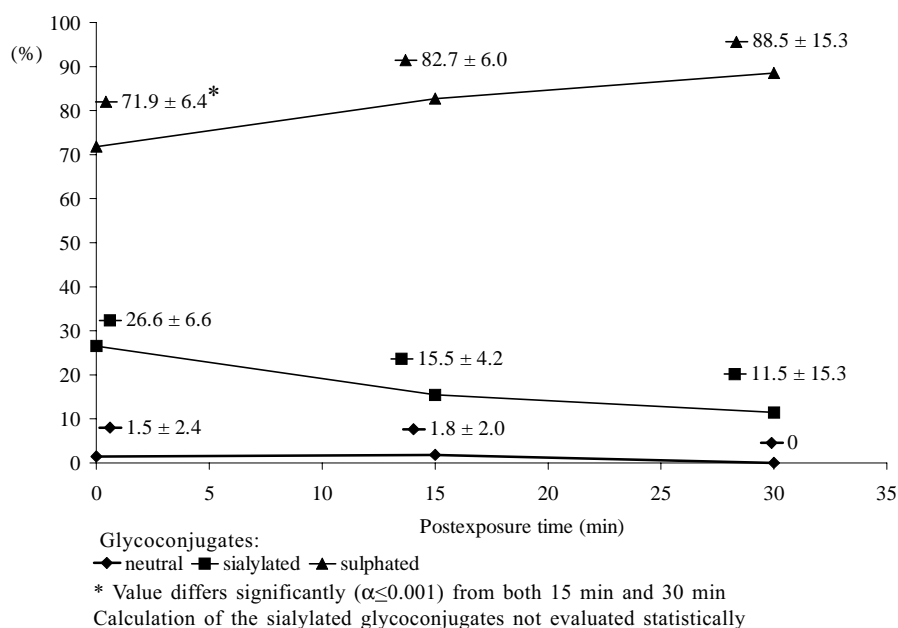


Figure 3. Changes in percentage of goblet cells in the tracheal epithelium containing different glycoconjugates after oral administration of 5 ml of Ventolin™ syrup. Conventional histochemistry

intensive staining of mucous granules in a goblet cell, either in the whole volume of a granule, or as a densely contrasted ring. The ciliary border was always densely stained, too. In some goblet cells, a web-like MAA-positive structure was encountered surrounding the nucleus as well as a dense lamellar structure following the shape of a nucleus mostly at one-fourth of its circumference. SNA-reaction depicted individual mucous granules as tiny rings, staining of the ciliary border was restricted to the close vicinity of apical surfaces of goblet cells. TML reacted in a similar way as MAA, but not so intensively (Figure 2).

In healthy control rabbits,  $1.5 \pm 2.4\%$  of goblet cells containing mucous granules with neutral GCCs and  $71.9 \pm 6.4\%$  of goblet cells with acidic sulphated GCCs were revealed (Figure 3). We calculated the percentage of the goblet cells containing acidic non-sulphated GCCs as  $26.6 \pm 6.4\%$ . TML-positive sialylated GCCs were found in  $26.6 \pm 11.5\%$ , MAA-positive ones in  $27.9 \pm 8.4\%$ , SNA-positive GCCs in  $2.3 \pm 2.9\%$ , and both MAA- and SNA-positive GCCs in  $30.2 \pm 9.6\%$  of goblet cells (Figure 4).

Fifteen minutes after the administration of 2 mg of salbutamol,  $1.8 \pm 2.0\%$  of GC containing neutral GCCs and

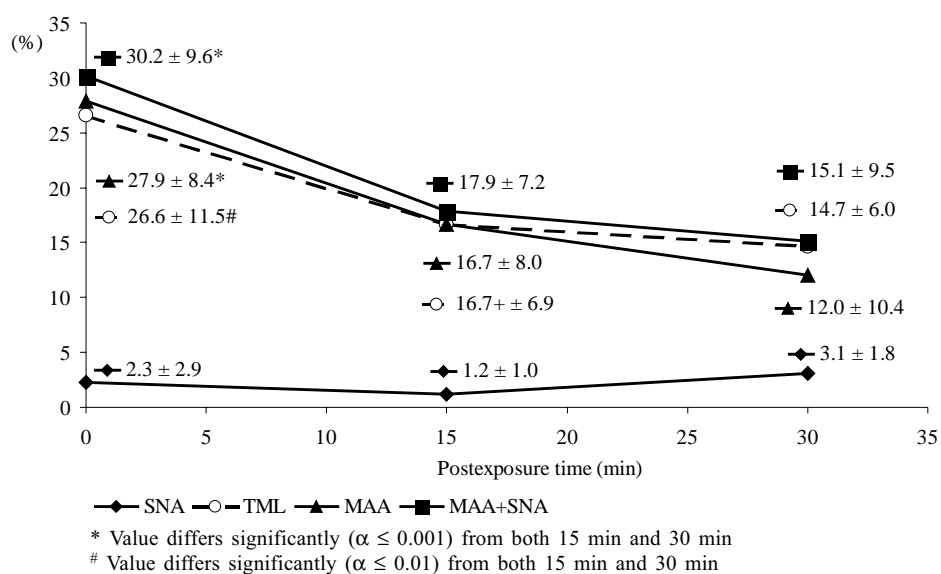


Figure 4. Changes in percentage of goblet cells in the tracheal epithelium containing sialylated glycoconjugates after oral administration of 5 ml of Ventolin™ syrup. Lectin histochemistry

82.7 ± 6.0% of goblet cells with acidic sulphated GCCs were found (Figure 3). We calculated the percentage of the goblet cells containing acidic non-sulphated GCCs as 15.5 ± 4.2%. TML-positive sialylated GCCs were found in 16.7 ± 6.9%, MAA-positive ones in 16.7 ± 8.0%, SNA-positive GCCs in 1.2 ± 1.0%, and both MAA- and SNA-positive GCCs in 17.9 ± 7.2% of goblet cells (Figure 4).

Thirty minutes after the administration of 2 mg of salbutamol, no goblet cells containing neutral GCCs and 88.5 ± 15.3% of GC with acidic sulphated GCCs were found (Figure 3). We calculated the percentage of the goblet cells containing acidic non-sulphated GCCs as 11.5 ± 15.3%. TML-positive sialylated GCCs were found in 14.7 ± 6.0%, MAA-positive ones in 12.0 ± 10.4%, SNA-positive GCCs in 3.1 ± 1.8%, and both MAA- and SNA-positive GCCs in 15.1 ± 9.5% of goblet cells (Figure 4). The number of goblet cells found within one tracheal section was notably lowered. Rarely, small groups of 2 to 3 goblet cells were revealed.

In both experimental groups, goblet cells were mostly found as being voluminous and communicating widely with the tracheal lumen by their dome-shaped apical portions.

The statistical significance of differences between individual groups of goblet cells was given in Figures 3 and 4. The minimal expected frequency was greater than 5 in all evaluated contingency tables except PAS-positive- and SNA-positive-goblet cells where it varied from 2 to 6. Differences between individual sialylated-glycoconjugate detecting methods were not statistically significant.

## DISCUSSION

The methods used allowed us to give the proportion of acidic sialylated glycoconjugates as either the difference of the percentage of total acidic and acidic sulphated glycoconjugates, or the direct sum of MAA- and SNA-detected glycoconjugates, or the direct result of TML detection. MAA reacted with terminal N-acetylneuraminic acid  $\alpha(2-3)$  glycosidically linked to galactose, SNA reacted with terminal N-acetylneuraminic acid  $\alpha(2-6)$  glycosidically linked to galactose or N-acetylgalactosamine, and TML possessed the exclusive affinity to various modifications in linkages of both N-acetylneuraminic and N-glycolneuraminic acids.

In accordance with many authors (Lamb and Reid, 1972; Jones and Reid, 1978; Jacob and Poddar, 1982; Spicer *et al.*, 1983; Castells *et al.*, 1990, 1991, 1992, 1994; Mandal and Mandal, 1990; Jeffery *et al.*, 1992), we found both neutral and acidic sulphated and acidic sialylated glycoconjugates in the mucous granules of goblet cells in the tracheal epithelium of the control rabbits. In the experimental group, we identified the same glycoconjugates. The data obtained by both conventional and lectin histochemistry were almost identical.

Gradual decrease of percentage of goblet cells containing acid sialylated glycoconjugates was observed 15 minutes after administration. Thirty minutes after administration, neutral glycoconjugates-containing goblet cells were absent. The proportion of goblet cells containing acid sialylated glycoconjugates reached 50% of the value in control animals. Compared with controls, the changes of the character of the glycoconjugate content in the tracheal goblet cells due to the oral administration of Ventolin<sup>TM</sup> syrup were statistically significant ( $\alpha \leq 0.01$ ). The change of percentage of goblet cells containing individual kinds of glycoconjugates as well as the presence of goblet-cell groups gradually followed the post-exposure time. The dynamics of changes in percentage of MAA + SNA-positive goblet cells followed the same dynamics in TML-positive goblet cells. The only exception was the percentage of goblet cells containing the glycoconjugates with the terminal  $\alpha(2-6)$ -linked N-acetylneuraminic acid that waived around its medium value and did not reach the zero point.

The change towards the dominance of acidic sulphated glycoconjugates was in accordance with studies of many authors on the influence of some noxae affecting the terminal glycosylation (Lamb and Reid, 1972; Jones and Reid, 1978; Damjanov, 1987; Jeffery *et al.*, 1992) and also with our previous study (Vajner, 1998). However, the reaction of goblet cells 30 minutes after inhalation of salbutamol was deeply pronounced, featured especially by the selective release of all sialylated glycoconjugates.

Ultrastructural findings in the tracheal epithelium after the oral administration of salbutamol (Konrádová *et al.*, 2000a, b) indicated the overstimulation of majority of secretory elements resulting even in their damage and degeneration. Fifteen minutes post exposure, the injury to the tracheal epithelium was considered moderate. Thirty minutes post exposure, the signs of regeneration, resulting in secretory cells' hyperplasia, were noticed. The release of the goblet cells' secretion was rapid (Konrádová *et al.*, 2000a, b) during the first phase. The signs of regeneration during the second phase were followed by the advanced change of the glycoconjugate content of goblet cells.

Based on our recent results, we arrived at a conclusion that the oral administration of salbutamol affected the composition of glycoconjugates contained in goblet cells of the tracheal epithelium in rabbits less severely and evoked a delayed reaction than the inhalation administration 30 minutes post exposure.

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