

BACKGROUND: Increased secretion of mucus is a hallmark of many respiratory diseases and contributes significantly to the airflow limitation experienced by many patients. While the current pharmacological approach to reducing mucus and sputum production in patients is limited, clinical studies have suggested that drugs which inhibit the cyclooxygenase and/or 5-lipoxygenase enzymatic pathways may reduce secretory activity in patients with airway disease.

Aim: This study was performed to investigate the effects of indomethacin (cyclooxygenase inhibitor) and Bay x1005 (5-lipoxygenase inhibitor) on MUC5AC release from human airways *in vitro*.

Methods: An immunoradiometric assay was used to determine the quantities of MUC5AC present in the biological fluids derived from human airways *in vitro*. The measurements were made with a mixture of eight monoclonal antibodies (MAbs; PM8) of which the 21 M1 MAb recognized a recombinant M1 mucin partially encoded by the MUC5AC gene.

Results: The quantities of MUC5AC detected in the biological fluids derived from human bronchial preparations were not modified after treatment with indomethacin (cyclooxygenase inhibitor) and/or an inhibitor of the 5-lipoxygenase metabolic pathway (BAY x1005).

Conclusion: These results suggest that the cyclooxygenase and 5-lipoxygenase metabolic pathways play little or no role in the release of MUC5AC from human airways.

Key words: MUC5AC mucin, Human airways, Secretion, Indomethacin, BAY x1005

MUC5AC mucin release from human airways *in vitro*: Effects of indomethacin and Bay X1005

Patricia Roger¹, Jean-Pierre Gascard¹,
Jacques Bara², Vincent Thomas de Montpreville³
and Charles Brink^{1,CA}

Laboratoire de Pharmacologie Pulmonaire, CNRS-ESA 8078¹ and Anatomopathologie², Hôpital Marie Lannelongue, 133 av. de la Résistance, 92350 Le Plessis Robinson, France, and ³INSERM U482, Hôpital Saint Antoine, 184 rue du Faubourg Saint Antoine, 75012 Paris, France

^{CA} Corresponding Author

Tel: +33 01 40 94 28 00

Fax: +33 01 46 30 12 08

E-mail: charlesbrink@hotmail.com

Introduction

An increase in the quantities of the metabolites of the cyclooxygenase and 5-lipoxygenase enzymatic pathways has been demonstrated in sputum derived from asthmatic patients^{1,2} and patients with chronic bronchitis.^{3,4} In clinical studies,⁵ involving patients treated with inhibitors of these enzymatic pathways, a significant reduction in sputum volume has been reported. These observations suggest that eicosanoids may be involved in airway inflammation and modify the secretory activity in the human respiratory tract.

While inhibition of eicosanoids has been reported to alter a number of physiological responses in the lung,⁶ there is limited information available concerning such inhibition on secretory activity in human airways.^{7,8} Initial reports have indicated that exposure of human airway cultured cell explants to indomethacin caused an increase in mucus secretion, when the incorporation of ³H-glucosamine was used as the index of glycoprotein secretory activity.⁸ However, these data were obtained in tissues constantly exposed to hydrocortisone and therefore the direct

effects of indomethacin may be compromised by the continuous steroid exposure. In fact, steroid inhalation or injection has been reported to significantly reduce sputum production in asthmatic patients² and in isolated human airways, steroids also significantly reduced glycoprotein secretion.⁹ These data suggest that further studies concerning the effects of inhibition of the cyclooxygenase and/or lipoxygenase pathways on secretory activity may be warranted since there remains considerable controversy concerning such inhibition in the human respiratory tract.

The aim of this investigation was to examine the effects of indomethacin and BAY x1005 (5-lipoxygenase inhibitor) on MUC5AC mucin release from human airways *in vitro*.

Materials and methods

Tissues

Human lung tissues were obtained from patients who had undergone surgery for lung carcinoma. Tissues

were obtained from the resected lung at a distance from the tumor area. Subsequently, the bronchi (third to sixth generation bronchi) were dissected free from the parenchyma tissue and washed with Tyrode's solution, pH 7.4 as previously described by Labat and coworkers.¹⁰ Bronchial tissues were cut as rings (3–6 mm internal diameter and 145 ± 33 mg wet weight). A total of 55 bronchial preparations were examined from lungs of 15 different patients.

Functional protocols

Human bronchial preparations were placed in separate wells of a 24-well plate (Costar, UK) containing 1 ml of Tyrode's solution and equilibrated for 1 h at 37°C in a humidified incubator (5% CO₂/air). At the end of this equilibration period, the medium was exchanged and fresh Tyrode's solution previously warmed at 37°C was added. After 1 h the bronchial fluids were collected (Period I; basal release). Subsequently, fresh Tyrode's solution or Tyrode's solution containing either indomethacin (INDO; 1.7 μM), BAY x1005 (5-lipoxygenase inhibitor; 1 μM) or the combination (INDO, 1.7 μM/BAY x1005, 1 μM) were added for 30 min to the bronchial preparations. After this period the preparations were then exposed to the drugs for 1 h (Period II; treatment). The bronchial fluids were collected at the end of Period II. In protocols involving ATP the preparations were challenged with ATP (0.1 mM) during Period II (1 h). The fluids (Periods I and II) were collected and stored at -20°C until analysis.

Immunoradiometric assay (IRMA)

M1 mucin isolated from an ovarian mucinous cyst fluid was used as standard. Eight MAbs raised against epitopes associated with the peptide core of M1 mucin allowed the detection of mucins.^{11,12} These anti-M1 mucin MAbs are IgG₁. An immunoradiometric assay was performed using a mixture of eight anti-M1 mucin MAbs (PM8), which included both 1–13 M1 and 21 M1 MAbs.

A solid-phase double-antibody-sandwich immunoradiometric assay (IRMA) was used as previously described.¹³ Polystyrene stars (Oris Industrie, Saint Quentin en Yvelines, France) were coated with the 1–13 M1 MAb (10 μg/ml in 0.01 M PBS, pH 7.4) by incubating for 2 h at room temperature, rinsed three times with PBS-Tween 20 (0.1%) and subsequently incubated in PBS-BSA (1%) overnight at 37°C. After several washings, stars were dried at 40°C and stored at 4°C until used. The M1 mucin standard (10 μg/ml), as well as human bronchial fluids were serially diluted in PBS-Tween 20 (0.1%). A volume of 300 μl of each dilution was added to the 1–13 M1 MAb coated-stars and incubated overnight at 37°C. Stars were then

washed with PBS-Tween 20 (0.1%) and incubated with PM8 MAbs previously radiolabelled with ¹²⁵I (5×10^5 cpm/ml) overnight at 37°C. Subsequently, the stars were washed and the radioactivity was measured in a gamma counter (Wizard Model 147005). The concentration of mucin contained in the human bronchial fluids was estimated from the IRMA standard curve obtained with the M1 mucin standard. Mucin concentrations were determined in bronchial fluids of 55 preparations from 15 human lung samples.

Calculation

The concentration of MUC5AC mucin detected in the fluids derived from human bronchial preparations by the IRMA are expressed as MUC5AC mucin (μg/ml) and were estimated from the M1 standard curves. All results are means ± SEM. Statistical analysis was performed using the Student's *t*-test. A value ($P < 0.05$) was taken as an indication of significance.

Results

The basal release of MUC5AC mucin from human bronchial preparations was not significantly altered during the experimental protocol under control conditions (Table 1, Period I *vs* Period II). When bronchial preparations were exposed to either INDO (1.7 μM) or the 5-LO inhibitor, (BAY x1005; 1 μM), the MUC5AC mucin released from bronchial tissues was not significantly different from those quantities detected either during control conditions (Period II) or when compared with data obtained during Period I (Table 1). Treatment of human bronchial preparations with the drug combination (INDO/5-LO) also did not significantly modify the release of MUC5AC mucin when results were compared with appropriate control values (Table 1). In contrast, ATP (0.1 mM) significantly increased the detection of MUC5AC (approximately 3-fold, when compared either with results obtained during Period I or with control values, Period II).

Discussion

The cyclooxygenase inhibitor, INDO and/or the 5-LO inhibitor, BAY x1005 did not significantly alter the basal release of MUC5AC mucin from human airways *in vitro*. The data suggest that metabolites of these enzymatic pathways may not be involved in the local regulation of human respiratory tract goblet cell secretory activity.

Marom and coworkers⁸ have shown that INDO provoked a small but significant increase in the

Table 1. Detection of MUC5AC in biological fluids derived from human airways *in vitro*: effects of INDOMETHACIN and 5-LIPOXYGENASE inhibition

Treatment	[conc]	MUC5AC ($\mu\text{g/ml}$)	
		Period I	Period II
Control ($n=15$)		0.45 \pm 0.18	0.41 \pm 0.13
INDO ($n=7$)	1.7 μM	0.36 \pm 0.03	0.35 \pm 0.10
5-LO ($n=7$)	1 μM	0.43 \pm 0.10	0.33 \pm 0.07
INDO/5-LO ($n=6$)	1.7 μM /1 μM	0.61 \pm 0.19	0.51 \pm 0.21
ATP ($n=7$)	0.1 mM	0.39 \pm 0.09	1.12 \pm 0.19*†

Detection of MUC5AC mucin release from human bronchial preparations *in vitro* using an immunoradiometric assay (IRMA) and pooled anti-M1 MAbs (PM8). Data are presented as means \pm SEM and n indicates the number of lung samples obtained from different patients.

* Indicates values different from results obtained in Period I ($P < 0.05$).

† Indicates values different from controls (Period II; $P < 0.05$). The 5-LO inhibitor was BAY X1005.

release of radiolabeled glycoprotein from cultured human bronchial explants. These investigators demonstrated that the release of radiolabeled glycoprotein increased approximately 30% when tissues were exposed to INDO. In contrast, the data (present report) demonstrated that release of the specific MUC5AC mucin found in goblet cells of the surface epithelium was not modified by INDO. While an explanation for these differences is not readily apparent, one possibility may be that the labeling of the glycoprotein pool with ^3H -glucosamine represents a number of different mucins, some of which may not be altered by INDO treatment. In addition, the labeled glycoprotein pool may also represent mucins derived from different secretory elements in the respiratory tract (goblet cells and/or submucosal glands). Furthermore, in the previous reports^{7,8} the effects of INDO were obtained in tissues constantly exposed to hydrocortisone. The relative contribution of INDO to the increase glycoprotein release may therefore be difficult to determine. In contrast, MUC5AC mucin is primarily associated with human goblet cells in the epithelium,¹⁰ these data suggest that the goblet cell release of this specific mucin may not be modified by metabolites of the cyclooxygenase enzymatic pathway.

Marom and coworkers⁸ also reported that inhibition of the 5-lipoxygenase enzyme either by ETYA, nordihydroguaiaretic acid or α -naphthol decreased mucus glycoprotein release. Since these inhibitors are known to have anti-oxidant effects as well as inhibit other enzymes, interpretation of these results may be compromised by the selectivity of these compounds. However, these observations indirectly suggested that products of the 15-lipoxygenase metabolic pathway may be responsible for the alterations in mucus glycoprotein. Hunter and coworkers¹⁴ showed that epithelial cells in culture released predominantly products of the 15-lipoxygenase enzymatic pathway. Thus the small decrease in release of

mucin⁸ may be due to the mobilization of arachidonic acid via this latter pathway. While 15-HETE is known to increase both secretion (hillock formation) and tracheal fluid in the dog,¹⁵ there are no reports dealing with inhibition and secretory activity associated with the 15-lipoxygenase metabolic pathway in human airways.

In conclusion, these data suggest that although MUC5AC from the goblet cells in the surface epithelium was not modified by INDO and 5-LO inhibition, the release of MUC5AC by goblet cells may be affected by metabolites of the 15-lipoxygenase enzymatic pathway. However, further work will be necessary to confirm this latter suggestion when specific inhibitors of this pathway become available.

References

- Inoue H, Aizawa H, Koto H, Miyagawa Y, Ikeda T, Shigematsu N. Effect of cyclooxygenase inhibitor on excessive sputum. *Fukuoka Acta Med* 1991; **82**:177-80.
- Shimura S, Sasaki T, Sasaki H, Takishima T. Chemical properties of bronchorrhea sputum in bronchial asthma. *Chest* 1988; **94**:1211-5.
- Zakezewski JT, Barnes N, Costello JF, Piper PJ. Lipid mediators in cystic fibrosis and chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1987; **136**:779-82.
- Zakezewski JT, Barnes N, Piper PJ, Costello JF. The detection of 5-lipoxygenase and cyclooxygenase products in sputum of patients with chronic bronchitis and bronchiectasis. *Prostaglandins* 1987; **33**:663-74.
- Tamaoki J, Chiyotani A, Kobayashi K, Sakai N, Kanemura T, Takizawa T. Effect of indomethacin on bronchorrhea in patients with chronic bronchitis, diffuse panbronchiolitis, or bronchiectasis. *Am Rev Respir Dis* 1992; **145**:548-52.
- Douglas JS, Brink C. Histamine and prostanoids. *Am Rev Respir Dis* 1987; **136**:S21-S24.
- Shelhammer JH, Marom Z, Kaliner M. Immunologic and neuropharmacologic stimulation of mucous glycoprotein release from human airways *in vitro*. *J Clin Invest* 1980; **66**:1400-8.
- Marom Z, Shelhammer JH, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways *in vitro*. *J Clin Invest* 1981; **67**:1695-702.
- Marom Z, Shelhammer J, Alling D, Kaliner M. The effects of corticosteroids on mucous glycoprotein secretion from human airways *in vitro*. *Am Rev Respir Dis* 1984; **129**:62-5.
- Labat C, Bara J, Gascard J P, Sossé-Alaoui H, de Montpreville V T, Yeadon M, Brink C. M1/MUC5AC mucin released by human airways *in vitro*. *Eur Respir J* 1999; **14**:365-9.

11. Bara J, Gautier R, Daher N, Zaghouani H, Decaens C. Monoclonal antibodies against oncofetal mucin M1 antigens associated with precancerous colonic mucosae. *Cancer Res* 1986; **46**:3983-9.
12. Bara J, Gautier R, Le Pendu J, Oriol R. Immunochemical characterization of mucins. Polypeptide (M1) and polysaccharide (A and Le^b) antigens. *Biochem J* 1988; **254**:185-93.
13. Bara J, Gautier R, Mouradian P, Decaens C, Daher N. Oncofetal mucin M1 epitope family: characterization and expression during colonic carcinogenesis. *Int J Cancer* 1991; **47**:304-10.
14. Hunter JA, Finkbeiner WE, Nadel JA, Goetzl EJ, Holtzman MJ. Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc Natl Acad Sci* 1985; **82**:4633-7.
15. Johnson H G, McNee M, Sun F F. 15-hydroxyeicosatetraenoic acid is a potent inflammatory mediator and agonist of canine tracheal mucus secretion. *Am Rev Respir Dis* 1985; **131**:917-22.

**Received 12 December 2000;
Accepted 4 January 2001**