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Effects of salmeterol on cilia and mucus in COPD and pneumonia patients

G. Piatti[a,*], U. Ambrosetti[b], P. Santus[c], L. Allegra[a]

[a] *Institute of Respiratory Diseases, Ospedale Maggiore di Milano, University of Milan, Via F. Sforza 35, 20122 Pad. Litta, Italy*
[b] *Department of Otolaryngology, Ospedale Maggiore, IRCCS, University of Milan, Italy*
[c] *Departmental Unit of Pneumology, Ospedale San Paolo, Milan, Italy*

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Abstract

The present study was performed to evaluate the effects of salmeterol xynaphoate on ciliary beat frequency (CBF) of nasal epithelium and on rheological parameters of tracheobronchial mucus.

We studied 10 steady-state COPD patients, eight patients with community-acquired pneumonia and eight healthy subjects as controls. They underwent a nasal brushing of the inferior turbinate to study the CBF in basal conditions and following application of salmeterol at 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M concentrations directly to the epithelial samples. We also collected sputum samples, in COPD patients only, by the technique for "protected expectoration" for the rheological assessment in basal conditions and following addition of salmeterol at 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M concentrations.

Only samples with basal viscosity values higher than 2000 mPa/s were admitted.

Our results confirmed previous studies that demonstrate a ciliostimulating effect induced by salmeterol. The mean basal CBF was 11.18 ± 0.75 Hz in control subjects, while the pathological subjects showed a markedly lower basal values: 8.64 ± 0.88 Hz ($p = 0.000$) and 8.83 ± 0.68 Hz ($p = 0.000$), in COPD and pneumonia patients, respectively. Salmeterol induced ciliostimulation in both patients groups as well as the healthy controls. The maximum increase in CBF, highly significant, was obtained at 10^{-6} M concentrations of salmeterol, while this effect decreased at lower concentrations. Regarding the action of salmeterol on rheological parameters, a direct effect of salmeterol on mucus cannot been demonstrated and the described beneficial clinical effects on mucociliary clearance occurring in vivo are probably related to an indirect effect of stimulation of ciliary beat.

This preliminary study suggests that, in addition to COPD, salmeterol could be a useful therapeutic agent in pneumonia also, for its positive effect on ciliary movement other than bronchodilation, but this finding needs further investigations.

Keywords: Ciliary beat frequency (CBF); Mucus rheology; Salmeterol xynaphoate; COPD; Community-acquired pneumonia

1. Introduction

Beta-2-agonists long-acting are largely employed in the treatment of COPD and bronchial asthma for their bronchodilator properties, but they can also exert an anti-inflammatory activity [1], reduce bronchial reactivity and modulate mucociliary clearance [2].

Regarding this last point, several studies [3–5] have reported a stimulating effect of beta-2-agonists on ciliary beat frequency (CBF) and mucus clearance but most of studies are based on in vitro data and is still debated whether this can also occur in vivo. Salmeterol xynaphoate (GSK, UK) is a long-acting beta-2-agonist with a prolonged specific binding to the β_2-adrenergic receptor. The present study was performed to evaluate the effects of this bronchodilator agent on CBF in an "ex vivo" model; in addition, we examined the possible direct activity of salmeterol on the rheological parameters of mucus: viscosity (η) and elasticity (G'). In fact, the potential therapeutic role in the enhancement of tracheobronchial clearance in several pathological conditions of the respiratory tract may be equally important as the bronchodilator effect.

* Corresponding author. Tel.: +39 02 5503 4486; fax: +39 02 5032 0628.
E-mail address: gioia.piatti@unimi.it (G. Piatti).

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2. Patients and methods

We enrolled 26 patients, 10 (8M, 2F) affected by COPD in steady-state, 8 (8M, 2F) with community-acquired pneumonia revealed at X-ray and eight healthy subjects (4F, 4M) as controls. None of the subjects suffered of nasal diseases or took medications that could interfere with ciliary motility. They underwent a single nasal brushing (Olympus Optical Co, Hamburg, Germany, 1 mm diameter) of the inferior turbinate to study CBF; when the sample cellular yield was low, the brushing was repeated on the controlateral turbinate. The CBF was studied in basal conditions and following a direct application on this epithelial sample of salmeterol at 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8} M concentrations on the preparation. These concentrations were selected on the basis of previous studies [5,12] and considering that following in vivo inhalation of 50 μ of salmeterol, the estimated lung tissue concentrations are between 10^{-7} [7] and 10^{-8} M [8]. Salmeterol was previously dissolved in methanol and then in Medium 199. After the collection by brushing, ciliated cells were immediately dislodged by gently shaking in Medium 199. A sample of 50 μl containing the fragments of ciliated cells were mixed together with 50 μl of salmeterol and allowed to equilibrate at room temperature for 10 min; instead, in the case of the basal value determination only 50 μl of Medium 199 was added. The slide was placed on a heated microscope stage (22 °C) and observed with interferential contrast of Normarsky (Olympus BH-2); the microscope was connected to a monitor. The monitor possess a visual and auditory marker that pulses at known adjustable frequencies: CBF was calculated by synchronising ciliary motion of the sample with marker pulse frequency and expressed in Hz. We observed that results with this method of counting ciliary beat are overlapping with those obtained recording the movement on videotape and studying the beat frequency with slow-motion analysis.

Measurements of CBF were always performed by the same observer. Investigator was blinded to the source of epithelial cells, and the dose of salmeterol used at each experiment was unknown at the time of observation.

Only large groups of cells in continuous strips were examined an average of 10 random areas were considered. The mean value of CBF was calculated for each concentration of salmeterol.

All data were statistically evaluated by *t*-test for paired data between values recorded at each concentration of salmeterol and the basal values in the same group of subjects and by *t*-test for comparison of CBF values in pathological subjects versus the control subjects. Values of $p < 0.05$ were considered to be significant.

In COPD patients we also collected sputum samples by the technique for "protected expectoration" for rheological assessment so that the mucus is collected after rinsing the oral cavity with water and after placing odontological rolls at the output of the salivary glands [9]. Both baseline and post-salmeterol rheological assessments were carried out on the freshly collected samples within 10 min; salmeterol had previously been dissolved in methanol and then in distilled water and added directly to the different aliquots of mucus sample, in a volume relationship of 1:15, to reach the final concentrations ranging from 10^{-5} to 10^{-8} M. Measurements were performed with a portable ESLAB Mucometer (Milan, Italy) that measures the dynamic viscoelasticity of mucus with the forced oscillation method [10]. Viscosity (η) was recorded in mPa/s and elasticity (G') in mPa. We evaluated only samples with basal viscosity values higher than 2000 mPa/s.

3. Results

All brushings showed multiple fragments of ciliated cells and all patients were evaluable. Table 1 shows the mean CBF values at different concentrations of salmeterol. Both COPD and pneumonia patients showed significantly reduced CBF values compared to those of the control subjects ($p = 0.000$, $t = 5.878$ and $p = 0.000$, $t = 5.688$, respectively). The mean basal CBF was 11.18 ± 0.75 Hz in control subjects, while that in pathological subjects was 8.83 ± 0.68 Hz in pneumonia patients and 8.64 ± 0.88 Hz in COPD patients.

Our results, obtained in an "ex vivo" model, confirmed previous findings [5,11,12] obtained on cell cultures that salmeterol stimulates the activity of ciliated cells. Both in COPD and in pneumonia patients the maximum increase of CBF was obtained at the 10^{-6} M concentration. The stimulating effect decreased at lower concentrations, and CBF reached values similar to basal conditions at 10^{-8} M concentrations, in control subjects and in pathological subjects. Conversely, in pneumonia patients, at this concentration, CBF was still greater than basal value, although not statistically significant.

The 10^{-5} M concentration resulted excessive and ciliotoxicity occurred: in fact, in this case, we observed a high

Table 1
Mean (±S.D.) values of CBF in Hz obtained in pneumonia, COPD patients and healthy subjects before and after addition of salmeterol at different concentrations; *p* values represent a comparison between recorded data at different concentrations and basal values in the same group of subjects

Basal	10^{-6} M	10^{-7} M	10^{-8} M
Healthy subjects			
11.18 ± 0.75	$12.69 \pm 0.84^{**}$ $p = 0.000$ $t = -9.259$	11.38 ± 0.76 $p = 0.443$ $t = -0.832$ NS	10.95 ± 0.86 $p = 0.178$ $t = 1.565$ NS
Pneumonia patients			
8.83 ± 0.68	$11.57 \pm 1.30^{**}$ $p = 0.004$ $t = 5.024$	$10.26 \pm 1.26^{*}$ $p = 0.04$ $t = -2.764$	9.44 ± 0.82 $p = 0.192$ $t = -1.508$ NS
COPD patients			
8.64 ± 0.88	$10.72 \pm 0.96^{**}$ $p = 0.000$ $t = -9.411$	$10 \pm 0.95^{**}$ $p = 0.000$ $t = -6.391$	8.92 ± 0.86 $p = 0.054$ $t = 2.217$ NS

NS = not significant.
* $p < 0.05$.
** $p < 0.01$.