Eur Respir J, 1994, 7, 81–87
DOI: 10.1183/09031936.94.07010081
Printed in UK - all rights reserved

European Respiratory Journal
ISSN 0903 - 1936

Mucolytic treatment with N-acetylcysteine L-lysinate metered dose inhaler in dogs: airway epithelial function changes

R.P. Tomkiewicz*, E.M. App**, M. Coffiner†, J. Fossion†, P. Maes†, M. King

Mucolytic treatment with N-acetylcysteine L-lysinate metered dose inhaler in dogs: airway epithelial function changes. R.P. Tomkiewicz, E.M. App, M. Coffiner, J. Fossion, P. Maes, M. King. ©ERS Journals Ltd 1994.

ABSTRACT: N-acetylcysteine L-lysinate Nacystelyn® (L-NAC) is a newly synthesized mucolytic agent, of which the action *in vivo* has not been well defined.

In six healthy mongrel dogs, the rheological properties of mucus, its mucociliary and cough clearability, and the transepithelial potential difference (PD) of the tracheobronchial epithelium were evaluated after placebo and L-NAC metered dose inhaler (MDI) aerosols.

The principal index of mucus rigidity, log G*, decreased at all airway sites with L-NAC administration, *i.e.* the mucus became less rigid and more deformable (the overall change in G* was 0.29 log units, *i.e. ca.* twofold decrease). The viscoelasticity-derived mucus transportability parameters, mucociliary (MCI) and cough (CCI) clearability indices, increased with L-NAC MDI, particularly CCI, which predicts the effect of mucus rheology on cough clearability. PD increased significantly with L-NAC administration at all measurement sites, which appears to be a novel effect for a direct acting mucolytic agent. Tracheal mucus linear velocity (TMV) increased after L-NAC compared with placebo, as did the normalized frog palate transport rate (NFPTR). The increase in NFPTR was greater than that predicted from the mucus rheological properties alone, suggesting that L-NAC still resident in the collected mucus stimulated the frog palate cilia. The index of mucus flux, the collection rate in mg·min^{-1}, was higher with L-NAC compared with placebo.

From our results, we conclude that L-NAC shows potential benefit in terms of improving mucus rheological properties and clearability. It may act, in part, by stimulating the fresh secretion of mucus of lower viscoelasticity. The stimulation of mucociliary clearance could be related to ion flux changes, as indicated by the increase in PD.

Eur Respir J., 1994, 7, 81–87.

*Pulmonary and Cell Biology Research Group, University of Alberta, Edmonton, Canada. **GSF-Research Center for Environment and Health, Munich, Germany. †R&D Dept, S.M.B. Galephar, Brussels, Belgium.

Correspondence: M. King
173 Heritage Med. Res. Ctr.
University of Alberta
Edmonton
Alberta T6G 2S2
Canada

Keywords: Dogs
mucociliary clearance
mucolytic
mucus viscoelasticity
transepithelial potential difference

Received: February 18 1992
Accepted after revision August 8 1993

The surface of the human airway constitutes a passage to the alveoli, where the gas exchange takes place, and also ensures a barrier for the inspired gas. Mucociliary clearance in the airway fulfils an important role in clearing foreign particles deposited on the mucosal surface. Our understanding of the mechanism of this clearance is based on the two-fluid model of mucus [1]. The upper layer of mucus is assumed to be a viscoelastic gel, consisting principally of crosslinked glycoproteins. The lower serous layer, which bathes the cilia, is a sol. The mucus flows on the sol layer and is transported by the beating action of cilia.

When mucus becomes more rigid and more viscous, its transportability by cilia becomes impaired [2]. This usually happens in prolonged inflammatory states, and contributes to the amplification of pathological conditions leading to the destruction of airway ciliated epithelium, which in turn causes further deterioration of mucociliary clearance. Mucolytic therapy in such instances, by breaking inter- and/or intramolecular bonding, liquifies the mucus and ameliorates mucociliary clearance, thus helping to break this vicious circle. However, one has to be cautious not to overliquify the mucus, and thus even impair its actual transportability [3], *e.g.* in the initial phases of acute inflammation. This does not usually happen in patients with chronic bronchitis, but examining a mucolytic agent's influence on individuals with "normal" mucus seems to be warranted, when taking this possibility into account.

There has always been a constant search for improved mucolytics, with increased effectiveness for patients with chronic impairment of mucociliary clearance, as in chronic bronchitis. Our aim was to study a newly derived compound, N-acetylcysteine L-lysinate (L-NAC), a salt of the classic mucolytic agent N-acetyl-L-cysteine [4]. The questions we have asked regarded not only the action of this mucolytic agent on the rheological properties of mucus, but also addressed functional changes in airway epithelium, since both contribute to mucociliary clearance. We analysed the viscoelastic properties of mucus,

its secretion rate, and its mucociliary transport *in vivo* and *in vitro*. In addition we measured the transepithelial potential difference (PD) as an *in vivo* index of epithelial ion transport and indicator of epithelial "health", and examined the interrelations between these various measurements.

Methods

Study Design

Six healthy mongrel dogs, weight 18.9–28.5 kg were studied on two occasions. On one day, the dogs received a placebo metered dose inhaler (MDI) aerosol, followed 60–70 min later with administration of L-NAC MDI aerosol.

Three sprays of placebo or L-NAC, 2 mg of N-acetylcysteine L-lysinate per spray, were applied at the end of expiration through the endotracheal tube, making the total delivered dose of L-NAC 6 mg.

As a control, to test the effects of repeated experimentation (anaesthetization with pentobarbital, intubation and bronchoscopy, with measurements of transepithelial potential difference and linear velocity of mucus, as well as mucus collection), on a different day, the dogs received three consecutive administrations of a 5-min Ringer's solution aerosol, at intervals of one hour.

Transepithelial potential difference measurements and mucus collections from mainstem bronchi, lower trachea, and the subglottic area were performed after each aerosol application, *i.e.* placebo or L-NAC MDI. Measurements of the linear velocity of mucus, and recordings of respiratory flows and pressures for computation of pulmonary mechanics were also made. The study was approved by the University of Alberta Animal Welfare Committee.

L-NAC and placebo MDI were supplied by Galephar S.A. (Brussels, Belgium). The placebo MDI consisted of Span 85, 1% by wt, and a mixture of freons (F11, F114 and F12). The L-NAC MDI was composed of the same ingredients as in the placebo formulation, plus 2 mg·100 mg^{-1} of N-acetylcysteine L-lysinate (Nacystelyn®). The L-NAC MDI was designed to deliver 150 puffs per container, containing 2 mg of active ingredient per puff.

Procedures and techniques

The dogs were anaesthetized with sodium pentobarbital (25 mg·kg^{-1} *i.v.,* supplemented as required), placed supine, and intubated with a shortened No. 9 endotracheal tube. Ventilatory mechanics (lung resistance (R_L), respiratory frequency (*f*), and tidal volume (V_T)) were determined from measurements of airflow and volume (Fleisch No. 1 pneumotachograph, connected with an integrator) and oesophageal pressure (5 cm balloon and Validyne transducer). V_T was determined from the integrated flow tracings. R_L was computed as the ratio of transthoracic pressure and airflow differential at mid-tidal volume [5]. Heart rate was determined by means of an electrocardiographic (ECG) monitor.

Transepithelial potential difference (PD). An agar bridge technique under bronchoscopic guidance was used. Two microelectrodes filled with 3% agar in Ringer's solution were connected with a common Ringer's solution bath and KCl saturated agar bridges leading through 3 M KCl solution to calomel half-cells. These were connected to a grounded electrometer (Fisher Accumet 950) and subsequently connected to an IBM PC AT computer. The measuring electrode was guided by bronchoscopy and carefully contacted with the epithelium. The reference electrode was placed in the subcutaneous space on the upper interior side of the hind leg, isoelectric with the subepithelial space of the airways [6].

Transepithelial PD measurements were always carried out at the same comparable levels, *i.e.* in the subglottic region, just below the inlet of the endotracheal tube; in the lower trachea 2–3 cm above the main carina; in the mainstem bronchi 1–2 cm below the main carina, and were always made prior to tracheal mucus linear velocity measurements, in order to minimize the degree of mechanical manipulation of the epithelium just before the bioelectrical measurements. Directly before each measurement, the microelectrodes and agar bridges were connected to a common Ringer's solution bath; the electrode pairs were allowed to differ by 1 mV maximally.

Tracheal mucus linear velocity (TMV). Direct evaluation of mucociliary clearability *in vivo*, TMV in mm·min^{-1} was determined by bronchoscopic observation of charcoal marker particle (Sigma Cat. No. C 5260) transit times (leading edge) in the lower trachea [7, 8]. The initial placement of charcoal was at approximately the same level of lower trachea that mucus was collected from. Transit times were generally 10 min, but occasionally less, depending on the rapidity with which the charcoal front approached the inlet of the endotracheal tube.

Mucus collection and analysis. Mucus collection from mainstem bronchi and lower trachea was performed using a modified cytology brush technique, by placing a cytology brush (No. 151 Mill Rose, Mentor, OH, USA), guided by a bronchoscope against the wall of the airway, and removing the brush once it was covered with sufficient mucus for analysis [8, 9]. The sampling time was normally 10 min, and usually resulted in a harvest of 1–10 mg of mucus. For collection of mucus from the subglottic intubation area, the endotracheal tube (ETT) collection technique [10] was employed. Mucus collection rates (wt·min^{-1}) were used as an index of flux [8, 9], and indirectly of secretion rate. Mucus collections and PD measurements were carried out at comparable levels, but on opposite sides to avoid interference. For each experiment, the mucus samples were frozen at -80°C for further analysis.

The magnetic microrheometer technique was used to measure the viscosity and elasticity of microlitre quantities of mucus [11]. A 100 µm steel ball was care-fully positioned in a 1–10 µl sample of mucus, and motion of this sphere under the influence of an electromagnet was used to determine the rheological properties of the mucus.

For that purpose, the steel ball was imaged through a microscope to a pair of photocells, the output of which was amplified and transmitted to an oscilloscope. By plotting the displacement of the ball against the magnetic driving force, the viscoelastic properties of the mucus were computed.

The parameters of mucus viscoelasticity determined were mechanical impedance, *i.e.* G*, reported here in the log scale, expressing the vector sum of "viscosity+elasticity", and loss tangent, *i.e.* tan δ, reflecting a ratio of "viscosity/elasticity" at low (1 rad·s^{-1}) and high (100 rad·s^{-1}) frequency [11]. Two derivative parameters, mucociliary clearability index (MCI) and cough clearability index (CCI), were computed from *in vitro* relationships derived from model studies of clearance [12–14]. The MCI, indicating clearability by normalized ciliary function, was computed from G* and tan δ at 1 rad·s^{-1}, and the CCI was computed from G* and tan δ at 100 rad·s^{-1}. Both indices relate negatively with log G*; MCI also relates negatively with tan δ, but CCI relates positively with it. Their respective formulas are as follows:

$$MCI = 1.62 - 0.22 \times \log G^*_{1} - 0.77 \times \tan \delta_{1} \quad (1)$$

$$CCI = 3.44 - 1.07 \times \log G^*_{100} + 0.89 \times \tan \delta_{100} \quad (2)$$

For samples of mucus larger than 2 mg, mucus hydration and percentage solids content were calculated by evaporation to dryness (microwave 30 min at 750 W), and gravimetry (Mettler analytical balance).

Normalized frog palate transport rate (NFPTR). NFPTR was used as a standard *ex vivo* technique defining the inherent "transportability" of mucus, independent of any systemic influence on ciliary function. A sample of mucus, *ca.* 1 µl, was placed on the mucus depleted leopard frog (*Rana pipiens*) palate, and the average transport rate of this sample normalized to the transport rate of collected endogenous frog mucus [15]. In order to prevent possible interpretive problems due to effects of residual L-NAC in the mucus, placebo samples were studied first, as was the case with the *in vivo* experiment.

Statistical methods

Statistical analysis was performed using the StatView II statistics package (Abacus Concepts, Berkeley, CA, USA) and a Macintosh II computer (Apple Computer, Cupertino, CA, USA). All results are presented as mean±standard deviations (SD), unless otherwise indicated. Comparisons between placebo and L-NAC were made using paired t-tests. In all cases $p<0.05$ was considered significant.

Results

Control experiment - administration of Ringer's solution

On a separate experiment day, each dog was exposed to three successive inhalations of Ringer's solution aerosol at intervals of 1 h by spontaneous breathing, 5 min per exposure. The effects of the aerosol and the repeated experimental manipulation on tracheal mucus viscoelasticity (log G*) and transepithelial potential difference (PD) are shown in figure 1. Although there was a tendency for PD to fall and log G* to rise over the course of the experiment, there was no significant change in either parameter. There was also no significant change in TMV or mucus collection rate over the course of the 3 h (not illustrated).

Placebo vs *L-NAC experiments*

Pulmonary function tests: There was no change in the ventilatory mechanics or the heart rate between pre- and postaerosol for either placebo or L-NAC. Pre- L-NAC administration, RL was 0.67±0.21 *versus* 0.66±0.20 cm $H_2O \cdot l^{-1} \cdot s$ post- L-NAC aerosol. Breathing frequency (*f*) and (VT) were, respectively, 14.6±13.9 pre *versus* 15.3±12.3 breaths·min^{-1} post and 0.33±0.15 pre *versus.* 0.32±0.15 *l* post L-NAC administration.

The other results are presented by the site of measurements, *i.e.* mainstem bronchi (MBR), lower trachea (LT) and subglottic area (SG). These are summarized in table 1.

Transepithelial potential difference (PD). There was a gradual increase of negative values of PD from MBR, to LT to SG, both with placebo and L-NAC administration. With L-NAC administration, significant increases of PD were recorded in all of these locations, *i.e.* from -46.3±3.4 to -51.1±3.5 mV in SG, from -25.3±3.4 to -31.6±4.3 mV in LT, and from -18.3±1.6 to -22.2±2.4 mV in MBR (table 1).

Mucociliary clearance: The direct *in vivo* measurement of mucociliary clearance, *i.e.* tracheal mucus linear velocity (TMV), measured from a starting point in lower trachea, increased with L-NAC administration compared with placebo: 16.9±4.9 *versus* 12.7±1.3 mm·min^{-1}. Also, the *ex vivo* indicator of transportability, normalized frog palate transport rate (NFPTR) of mucus from mainstem bronchi increased with L-NAC aerosol from 0.73±0.26 to 1.18±0.36.

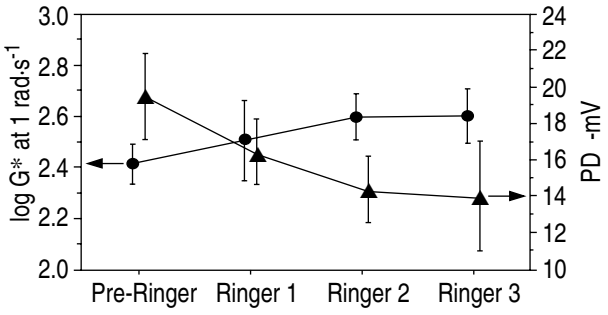

Fig. 1. – Mucus rigidity (G* at 1 rad·s^{-1}, logarithmic scale —●—) and transepithelial potential difference (PD, -mV —▲—) (mean±SEM) in lower trachea of anaesthetized, spontaneously breathing dogs subjected to repeated Ringer's aerosol solution administration and experimental manipulation over the course of 3 h.

Table 1. – Mucus epithelial function variables by airway site

Treatment	n	PD mV	TMV mm·min^{-1}	NFPTR	log G* at 1 rad·s^{-1}	MCI	CCI	Solids %	MCR mg·min^{-1}
Subglottic area (SG)									
Placebo	6	-46.3±3.4	-	-	1.78±0.32	0.90±0.13	1.53±0.38	10.5±1.3	5.56±1.32
L-NAC	6	-51.1±3.5	-	-	1.36±0.42	1.03±0.14	2.02±0.57	11.1±2.2	5.99±1.98
p-value		0.0005			0.014	0.027	0.017	0.574	0.467
Lower trachea (LT)									
Placebo	6	-25.3±3.4	12.7±1.3	-	1.92±0.22	0.87±0.10	1.45±0.28	15.2±4.0	2.15±1.46
L-NAC	6	31.6±4.3	16.9±4.9	-	1.68±0.17	0.96±0.05	1.78±0.24	12.2±2.0	6.93±3.29
p-value		0.023	0.075		0.185	0.176	0.176	0.142	0.028
Mainstem bronchi (MBR)									
Placebo	12	-18.3±1.6	-	0.73±0.26	1.89±0.21	0.91±0.07	1.54±0.31	12.7±3.5	1.77±1.02
L-NAC	12	-22.2±2.4	-	1.18±0.36	1.63±0.28	0.94±0.10	1.86±0.37	13.2±2.2	7.18±5.70
p-value		0.0001		0.0009	0.009	0.415	0.021	0.634	0.007

Data are presented as mean±SD. Comparisons between placebo and L-NAC were made using paired t-tests. L-NAC: N-acetylcysteine L-lysinate; PD: transepithelial potential difference; TMV: tracheal mucus linear velocity; NFPTR: normalized frog palate transport rate; log G*: mechanical impedance, index of mucus rigidity; MCI: mucociliary clearability index; CCI: cough clearability index; MCR: mucus collection rate.

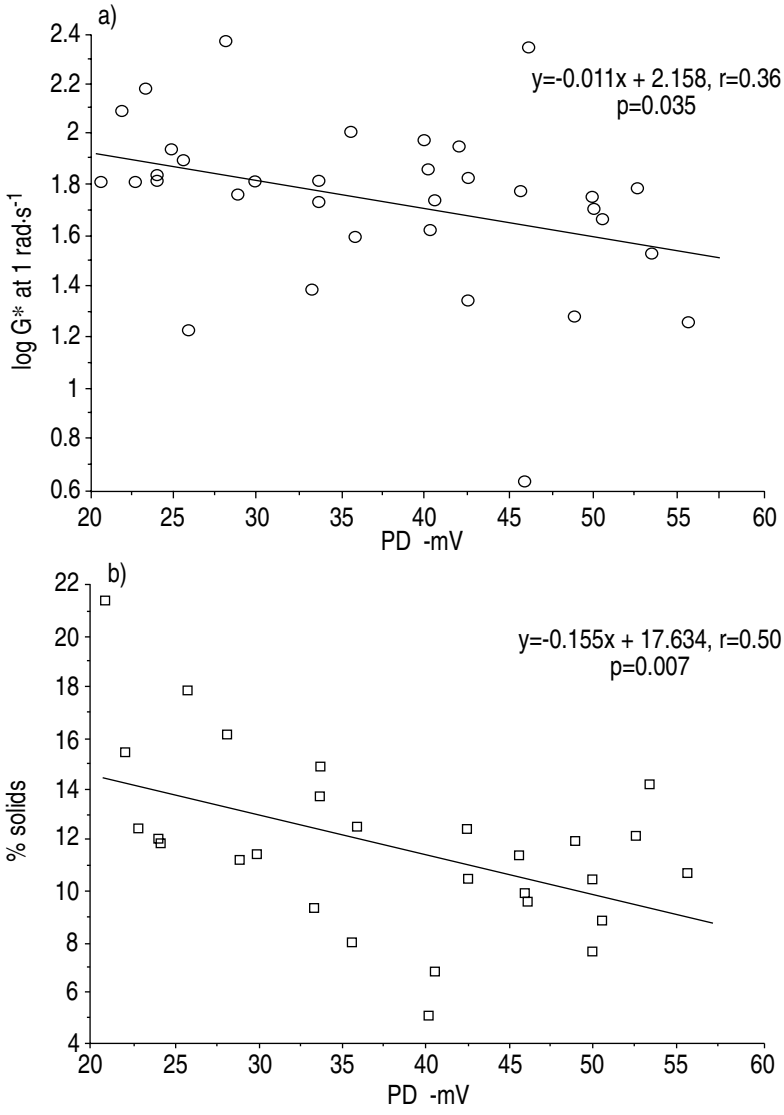

Fig. 2. – Mucus rigidity (logarithm of G* at 1 rad·s^{-1}); and b) % solids content *versus* transepithelial potential difference (PD -mV) for tracheal and subglottic regions. Note that the axes do not extend to zero.

Mucus rheology. The principal index of mucus rigidity, G*, decreased at all airway sites with L-NAC aerosol administration, *i.e*. the mucus became less rigid and more deformable. These changes were significant for the 12 MBR and the 6 SG mucus samples analysed; in LT the same tendency was observable. There was a significant negative correlation between log G* and -PD, as indicated in figure 2a.

As a consequence of the relative changes in mucus viscoelasticity, both of the derived mucus transportability parameters, MCI and CCI, also increased with L-NAC administration (table 1), particularly CCI, which predicts the effect of mucus rheology on cough clearability.

Mucus volume and hydration: Mucus collection rates expressed in mg·min^{-1} from MBR and LT were significantly higher after L-NAC administration compared with placebo, whereas mucus solids content (% dry weight) did not change significantly at any measurement site. There was a significant negative correlation between mucus solids content and airway PD, as illustrated in figure 2b.

Discussion

The answers to principal questions addressed in this study are presented in table 2, which summarizes the overall effects of the L-NAC MDI aerosol administration on airway epithelium, combining the results of measurements from all sites. As expected of a mucolytic agent, we observed a decrease in viscoelasticity of the mucus (0.29 log units at 1 rad·s^{-1}, or approximately a factor of two reduction in viscosity and elasticity). A classical explanation of the mechanism of this action would be a cleaving of some of the disulphide bonds between mutually interacting glycoprotein chains forming the network of the mucus blanket. Alternately, it is possible that L-NAC prevents the formation of S-S bonding in newly secreted mucus, as has been suggested for N-acetylcysteine (NAC) [16].

From previous studies [17, 18], we know that there exists an optimal range of mucus viscosity for the most effective ciliary transport, and liquifying a normal mucus beyond that range brings its ciliary transportability back in the direction of baseline, or even lower. This was not the case in the present study, however, since the possibility of overliquification of the mucus, which would have showed up in terms of suboptimal transport, was not observed, despite the fact that healthy animals were used. This may indicate that even a normal mucus has some potential for the improvement of its ciliary transportability by decreasing its viscosity.

Overall, far from leading to suboptimal clearance by overliquification, administration of L-NAC improved mucus clearability more than that predicted by the change in rheology alone. The increase in NFPTR in this study (*ca*. 60% over baseline) was even greater than we could predict from the mucus rheological properties alone, where the increase in predicted transportability, MCI, was only 8%. From this, it can be deduced that the increase in NFPTR was probably the result of ciliostimulation, as well as due to a contribution from improved rheological properties of the mucus. We would suggest that L-NAC still resident in the collected mucus resulted in stimulation of the frog palate cilia. Ciliostimulation has been reported at low concentrations of N-acetylcysteine [19].

There was also an increase in airway transepithelial PD with L-NAC administration; an increase in lumen-negative PD could be due to an increase in active ion transfer (current), or an increase in epithelial resistance. Although we could not separate these two possibilities in the *in vivo* experiments that we conducted, there is no reason to suppose that an acute treatment with a substance such as L-NAC could lead to increased membrane resistance, leaving an increase in active anion transport as the most likely mechanism to account for the change in PD. This was not anticipated, since one might not expect a direct acting mucolytic agent to influence epithelial ion transport. The increase in PD with L-NAC treatment was not, however, accompanied by a significant change in mucus water content. This might be due to the insensitivity of the method, but it could also be a real effect. It is possible that the increase in PD, which probably indicates an increase in active chloride ion secretion, could affect the periciliary layer more than the mucus itself, accounting for the increase in NFPTR and TMV beyond what would have been expected for the change in mucus viscoelasticity alone.

Neither the change in log G* nor the changes in PD can be attributed to the effects of time and repeated experimental manipulation, since these effects (fig. 1) were nonsignificant and of opposite tendency to those seen with administration of active drug. Also, in line with previous studies from our laboratory and from others [20–22], increasingly negative values of transepithelial PD, were recorded from mainstem bronchi to the subglottic region. Mucus viscoelasticity, indicated

Table 2. – Mucus epithelial function variables: overall results

	PD mV	TMV mm·min^{-1}	NFPTR	log G* at 1 rad·s^{-1}	MCI	CCI	Solids %	MCR mg·min^{-1}
Placebo	-27.0±12.0	12.7±1.3	0.73±0.26	1.87±0.24	0.90±0.09	1.51±0.31	12.8±3.6	2.8±2.0
Nacystelyn®	-31.8±12.4	16.9±4.9	1.18±0.36	1.58±0.31	0.97±0.11	1.88±0.40	12.4±2.3	6.8±4.4
n	24	6	12	24	24	24	24	24
p-value	0.0001	0.075	0.0009	0.0001	0.013	0.0002	0.625	0.0005

Data are presented as mean±SD. Comparisons between placebo and Nacystelyn® were made using paired t-test. For abbreviations see legend to table 1.

by log G*, and solids content were similar for MBR and LT samples, and significantly lower for the SG mucus. Administration of L-NAC MDI aerosol did not alter the basic distribution or gradation of either PD, log G*, or % solids along the tracheobronchial tree, which indicates that the drug acted similarly in all of the sites tested. These physiological trends are illustrated in figure 2, showing a gradual decrease of log G* (fig. 2a), as well as a decrease of solids content (fig. 2b), with increasingly negative PD within the trachea. These correlations can be rationalized, if it is assumed that the increasingly negative PD represents increasing active chloride secretion by the epithelium, and increased hydration of the mucus towards the subglottic region [20].

The increased index of mucus flux (collection rate) might also indicate that L-NAC acts, in part, by stimulating fresh mucus secretion of a lower viscosity. This would be consistent with the reported action of N-acetylcysteine [16, 19]. Increased mucus secretion did not constitute any problem here, since inherent mucus clearability (NFPTR or MCI) was increased, and there was no indication of mucus overloading. The more than two-fold increase of mucus collection rate that we observed (from 2.8±2.0 to 6.8±4.4 $mg \cdot min^{-1}$ table 2) cannot be solely ascribed to the 30% increase of TMV (from 12.7±1.3 to 16.9±4.9 $mm \cdot min^{-1}$). Because of such a discrepancy in the ratios of these two increases, it is unlikely that the increased TMV is the only reason for the increased mucus flux.

The distribution of aerosol in humans using L-NAC MDIs of the same design has recently been studied [23]. It was found that for a delivered dose of 2 mg L-NAC per puff, approximately 12% was delivered to the lungs by inhalation through the mouth. In our study, the oropharyngeal region was bypassed, hence the lung deposition was probably higher. The concentration or dose of L-NAC to reach the tracheobronchial surface was not determined in this study, however, an upper limit estimate of L-NAC concentration in airway mucus can be made, if it is assumed that all of the L-NAC delivered to the lungs ends up in the airway surface fluid lining the trachea and major bronchi (300 cm^2 surface, 10 µm depth). Thus, a delivered dose of 0.6 mg (three puffs at 10% deposition efficiency) diluted in an estimated airway surface fluid volume of 0.3 ml, would give rise to a maximal concentration (before diffusion into tissue) of 2 $mg \cdot ml^{-1}$ or *ca*. 10^{-2} M.

L-NAC is relatively neutral from the point of view of pH, since its pKA is 6.4. The MDIs contain a non-aqueous vehicle, and the pH of the delivered aerosol is not relevant. However, when the L-NAC arrives on the tracheobronchial surface, it must dissolve in the airway surface fluid in order to act. The pH of airway mucus is not well defined, but is probably in the range 6–8 [24, 25]. Hence, the dissolution of L-NAC in airway surface fluid is not likely to cause any disturbance of the pH of the periciliary milieu. Also, the mucus has a substantial buffering capacity [26], which would minimize any local disturbances in pH occurring during the dissolution of the drug. Furthermore, the pH of the Ringer's solution aerosol used in control experiments is 5.5, but this does not appear to present any major problem, since it has no buffering capacity. In toxicological studies (Cazin, Lille, France - unpublished results), L-NAC demonstrated very low toxicity, comparable to N-acetylcysteine, and pilot clinical studies have shown the absence of any bronchospastic effect, even in patients with bronchial hyperreactivity [4].

In conclusion, our study supports the possible benefit of this new mucolytic agent, as already indicated in pilot clinical studies in chronic bronchitis patients, which have shown an increase of mucociliary clearance measured by radiolabelled particle clearance (Robience and Godart, Hornu, Belgium - unpublished results). The changes in mucus physical properties and ciliary and predicted cough clearability described in the present study are desirable in airway diseases characterized by elevated mucus viscoelasticity; however, the clinical effectiveness of L-NAC might be dependent on at least some degree of preserved functionality of the ciliary apparatus in the impaired lungs, since the treatment seems to induce an increased volume of secretion. Finally, in further investigations, a direct comparative study with the classic mucolytic N-acetylcysteine, incorporating the evaluation of ciliary beat frequency, appears to be warranted.

References

1. Lucas AM, Douglas LC. – Principles underlying ciliary activity in the respiratory tract. II. A comparison of nasal clearance in man, monkey and other mammals. *Arch Otolaryngol* 1934; 20: 528–541.
2. King M. – Mucus, mucociliary clearance and coughing. *In*: Bates DV, ed. Respiratory Function in Disease. 3rd Edn. Philadelphia, Saunders, 1989; Chapter 3.
3. Puchelle E, Zahm JM, Polu JM, Sadoul P. – Drug effects on viscoelasticity of mucus. *Eur J Respir Dis* 1980; 61 (Suppl. 110): 195–208.
4. Duchatelet P, Songore D, Ravez P, Robience YJ. – Influence du lysinate de N-acetylcysteine en aerosol doseur sur les parametres spirographiques. *Acta Ther* 1987; 13: 579–586.
5. Amdur MO, Mead J. – Mechanics of respiration in unanesthetized guinea-pigs. *Am J Physiol* 1958; 192: 364–368.
6. App EM, King M. – Airway transepithelial electric potential difference measurements *in vivo* guided by bronchoscopy in animals and in man. *Eur Respir J* 1989; 2: 278s.
7. King M, Phillips DM, Gross D, Vartian V, Chang HK, Zidulka A. – Enhanced mucus clearance with high frequency chest wall compression. *Am Rev Respir Dis* 1983; 128: 511–515.
8. King M, Kelly S, Cosio M. – Alteration of airway reactivity by mucus. *Respiration Physiol* 1985; 62: 47–59.
9. King M, Engel LA, Macklem PT. – Effect of pentobarbital anesthesia on rheology and transport of canine tracheal mucus. *J Appl Physiol: Respirat Environ Exercise Physiol* 1979; 46: 504–510.
10. Rubin BK, Ramirez O, Zayas JG, Finegan B, King M. – Collection and analysis of respiratory mucus from individuals without lung disease. *Am Rev Respir Dis* 1990; 141: 1040–1043.
11. King M. – Magnetic microrheometer. *In*: Braga PC,

Allegra L, eds. Methods in Bronchial Mucology. New York, Raven Press, 1988; pp. 73–83.
12. King M. – Relationship between mucus viscoelasticity and ciliary transport in guaran gel/frog palate model system. *Biorheology* 1980; 17: 249–254.
13. King M, Brock G, Lundell C. – Clearance of mucus by simulated cough. *J Appl Physiol* 1985; 58: 1776–1782.
14. King M. – Role of mucus viscoelasticity in cough clearance. *Biorheology* 1987; 24: 589–597.
15. Rubin BK, Ramirez O, King M. – The mucus depleted frog palate as a model for the study of mucociliary clearance. *J Appl Physiol* 1990; 69: 424–429.
16. Ventresca GP, Cicchetti V, Ferrari V. – Acetylcysteine. *In*: Braga PC, Allegra L, eds. Drugs in Bronchial Mucology. New York, Raven Press, 1989; pp. 77–102.
17. Shih CK, Litt M, Khan MA, Wolf DP. – Effect of nondialyzable solids concentration and viscoelasticity on ciliary transport of tracheal mucus. *Am Rev Respir Dis* 1977; 115: 989–995.
18. King M, Gilboa A, Meyer FA, Silberberg A. – On the transport of mucus and its rheologic simulants in ciliated systems. *Am Rev Respir Dis* 1974; 110: 740–745.
19. Iravani J, Melville GN, Houstmann G. – N-acetylcysteine and mucociliary activity in mammalian airways. *Drug Res* 1978; 28: 250–254.
20. Boucher RC, Stutts MJ, Gatzy JT. – Regional differences in bioelectric properties and ion flow in excised canine airways. *J Appl Physiol: Respirat Environ Exercise Physiol* 1981; 51: 706–714.
21. App EM, King M. – Tracheal mucus rheology and potential difference in two day old puppies. *Biorheology* 1990; 27: 515–526.
22. App EM, Zayas JG, King M. – Rheology of mucus and epithelial potential difference: small airways *vs* trachea. *Eur Respir J* 1993; 6: 67–75.
23. Hardy JG, Everard ML, Coffiner M, Fossion J. – Lung deposition of a Nacystelyn® metered dose inhaler formulation. *J Aerosol Med* 1993; 6: 37–44.
24. Boat TF, Mathews LW. – Chemical composition of human tracheobronchial secretions. *In*: Dulfano MJ, ed. Sputum: Fundamentals and Clinical Pathology. Springfield, IL, CC Thomas, 1973; pp. 243–274.
25. Lutz RJ, Litt M, Chakrin LW. – Physical-chemical factors in mucus rheology. *In*: Gabelnick HL, Litt M, eds. Rheology of Biological Systems. Springfield, IL, Thomas, 1973, pp. 119–157.
26. Holma B, Hegg PO. – pH and protein dependent buffer capacity and viscosity of respiratory mucus. Their interrelationships and influence on health. *Sci Total Environ* 1989; 84: 71–82.