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Role of simulated repetitive coughing in mucus clearance

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Role of simulated repetitive coughing in mucus clearance. J.M. Zahm, M. King, C. Duvivier, D. Pierrot, S. Girod, E. Puchelle.
ABSTRACT: The role of repetitive simulated coughing on the clearance of gel mucus simulant was investigated *in vitro*, by using a simulated cough machine. The repetition of cough induced a significant increase ($p<0.01$) in mucus simulant clearance (139.3±78.7 mm) in comparison to a single cough (24.9±27.5 mm). Moreover, the increase in frequency of the repetitive coughing induced a marked and significant increase in mucus simulant clearance (75.4±51.1 mm and 139.3±78.7 mm at 0.1 Hz and 1.6 Hz frequency, respectively). A significant ($p<0.05$) correlation was observed between the percentage increase of clearance and both shear-thinning index ($r=0.62$) and the thixotropic index ($r=0.63$). These results suggest that the shear-dependent properties of mucus, associated with a repetitive coughing, may increase the efficiency of mucus clearance by air flow mechanisms.
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In healthy subjects, the respiratory mucus is cleared out of the lung by ciliary transport. In various respiratory diseases such as chronic bronchitis, cystic fibrosis, bronchiectasis and asthma, hypersecretion of mucus occurs and may induce impairment of mucociliary transport. It has been shown that this deficit in mucociliary transport may be compensated by mucus elimination through the cough mechanism [1, 2]. Mucus transport by ciliary beating, as well as by cough, is dependent on the physical properties of mucus. Among these physical properties, viscosity, elasticity, spinnability and adhesiveness of mucus are of primary importance in establishing the transport mechanism [3–6].

A number of investigators have studied the rheological behaviour of pathological respiratory mucus under steady-state conditions. However, biofluids such as respiratory mucus have been demonstrated to have unsteady rheological properties, such as thixotropy, which corresponds to a decrease of viscosity with time, under the effect of a constant flow rate. Moreover, a decrease of mucus viscosity is observed in parallel with an increase in flow rate of the mucus (shear-thinning effect) [7].

During the expiratory phase of cough, the linear velocity of gas flow ranges up to 5,000 $cm \cdot s^{-1}$ in central airways (for peak volumetric flow rate of 10 $l \cdot s^{-1}$) [8]. Momentum is transferred from gas moving at high velocity to the initially stationary mucus. This transfer induces high flow rates in the mucus blanket. Furthermore, cough is usually characterized by several successive expiratory manoeuvres, each manoeuvre being separated from the others by a time duration which can vary from one patient to another [9]. Thus, both shear-thinning and time-dependent properties of mucus might play an important role in the cough transport mechanism.

The purpose of the present work was to study *in vitro*, using a simulated cough machine, the role of the repetitive coughing in the clearance of mucus gel simulants. We were also interested in the flow induced variations of the mucus physical properties involved in the cough clearance mechanism.

Materials and methods

Simulated cough machine

The main feature of the experimental design was the simulated cough machine which has been described by King *et al.* [4] and is represented in figure 1. An 8 *l* plexiglass tank served as the reservoir of pressurized gas and simulated the capacitative function of the lungs and smaller airways. Cough-type flows were initiated by opening a solenoid valve releasing the pressurized gas. The opening and closure of the solenoid valve was driven by a microcomputer (Apple II). The model trachea was a plexiglass trough of rectangular cross-section, 1 cm high × 2 cm wide × 30 cm long. A simulated cough of intermediate intensity (peak flow rate 8 $l \cdot s^{-1}$) was chosen, generated by a driving pressure of 41.4×10^3 Pa. The number of successive cough manoeuvres and the time duration between each manoeuvre was set by computer software. Three different cough manoeuvres were studied: 1) a single cough; 2) five successive cough manoeuvres at 10 s intervals; and 3) five manoeuvres at 0.6 s intervals. The peak flow rate of each

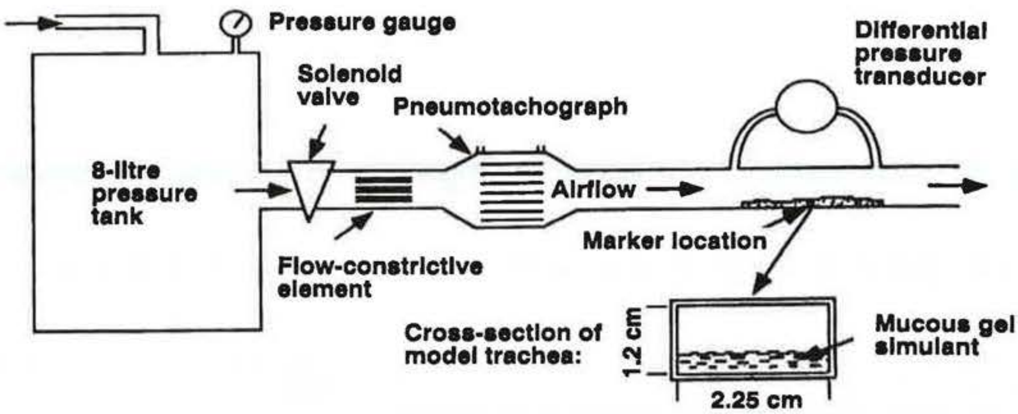

Fig. 1. – Schematic representation of the simulated cough machine.

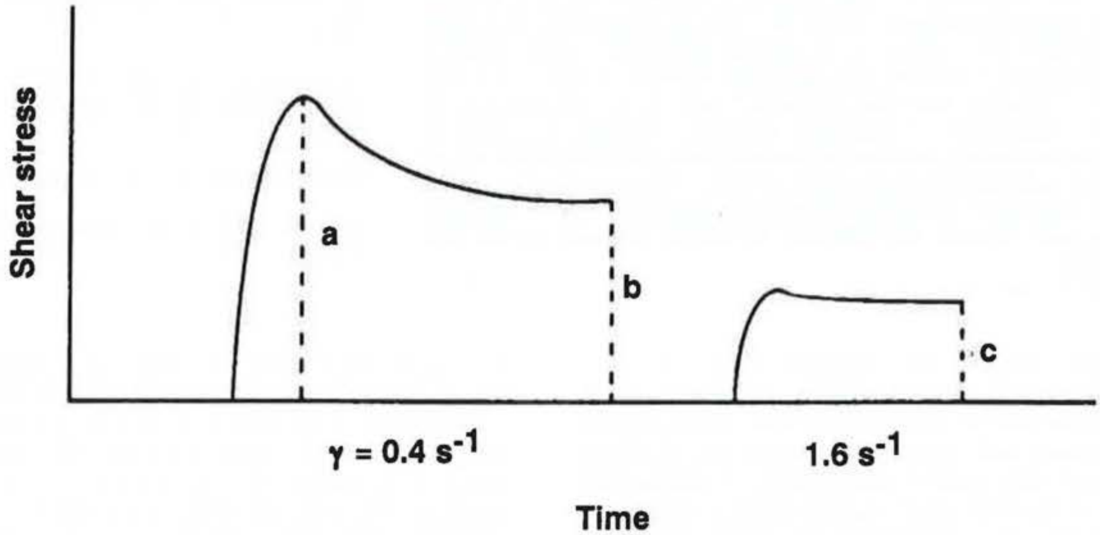

Fig. 2. – Schematic representation of the viscoelastometer output for a mucus-like gel: shear stress *vs* time for two applications of steady shear. a/b: thixotropic index (ratio of peak to steady-state viscosity at 0.4 s^{-1}); b/c: shear-thining index (ratio of steady-state viscosities at 0.4 and 1.6 s^{-1}, respectively).

manoeuvre was the same (8 $l \cdot s^{-1}$), whatever the cough frequency.

Mucus simulants

Gels of similar rheological properties to respiratory tract mucus were made from mixtures of two industrial gums: guar gum (Viscogum HV 300A, SATIA) and scleroglucan (Actigum CS 11, SATIA). Twelve different gels were prepared by adding an appropriate volume of saline solution (0.9 =% NaCl) to pre-weighed mixtures of the two gums (Viscogum 0.25% and 0.5% w/v and Actigum varying from 0.5–1.75% w/v). After dissolution, 0.2 ml per 10 ml of Sörensen buffer (Na borate/ HCL, pH 9) was added to crosslink the guaran portion of the gel.

Rheological properties of mucus simulants

The viscoelastic properties of the gels were analysed with a steady-shear viscoelastometer SEFAM [10] at 0.4 s^{-1} and 1.6 s^{-1} shear rate. A shear-thinning index, corresponding to the ratio of the viscosity measured at 1.6 s^{-1} to the viscosity measured at 0.4 s^{-1}, was calculated.

Under shearing, characteristic curves of shear stress *versus* time are observed for respiratory mucus [7]. A transitory "overshoot" is followed by a steady-state. The ratio of the amplitude of the shear stress overshoot to the steady-state shear stress (at 0.4 s^{-1}) was calculated and considered as a thixotropic index (decrease of viscosity with time). The two indices of unsteady viscosity behaviour are illustrated in figure 2 .

Clearance measurements

For each measurement, 3 ml of mucus simulant gel was spread over a fixed area of the bottom surface of the model trachea to produce a mean depth of 1 mm. Five marker particles were placed in the gel and their positions before and after the simulated cough were recorded. Cough clearability was defined as the mean distance travelled for the particles.

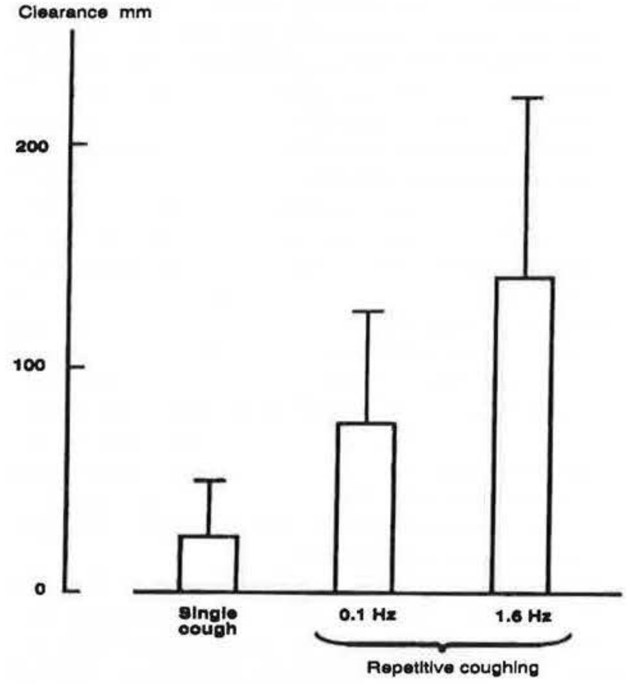

Fig. 3. – Mucus simulant clearance for three different cough manoeuvres: 1) single cough; 2) five cough manoeuvres at 0.1 Hz; and 3) five cough manoeuvres at 1.6 Hz.

Statistical treatment of data

The data set consisted of observations of cough clearability on 12 gels. A paired t-test was employed to determine the differences in cough clearability. A linear regression test was used to describe the relationship between cough clearability and the rheological variables.

Results

We observed that the rapid repetition of cough (*i.e.* 1.6 Hz) induced a significant increase ($p<0.01$) in mucus simulant clearance (139.3±78.7 mm) in comparison to a single cough (24.9±27.5 mm). Moreover, the increase in frequency of the repetitive coughing resulted in a marked and significant increase ($p<0.01$) in mucus simulant clearance (75.4±51.1 mm and 139.3±78.7 mm at 0.1 Hz and 1.6 Hz, respectively) (fig. 3).

For the 12 gel mucus simulants, the shear-thinning index ranged from 1.7–3.9. A high value of this index corresponds to a high decrease of viscosity, in parallel with an increase of the shear rate applied to the simulant. The thixotropic index ranged from 1–1.8, indicating that the time dependence of viscosity varied from one mucus simulant sample to another.

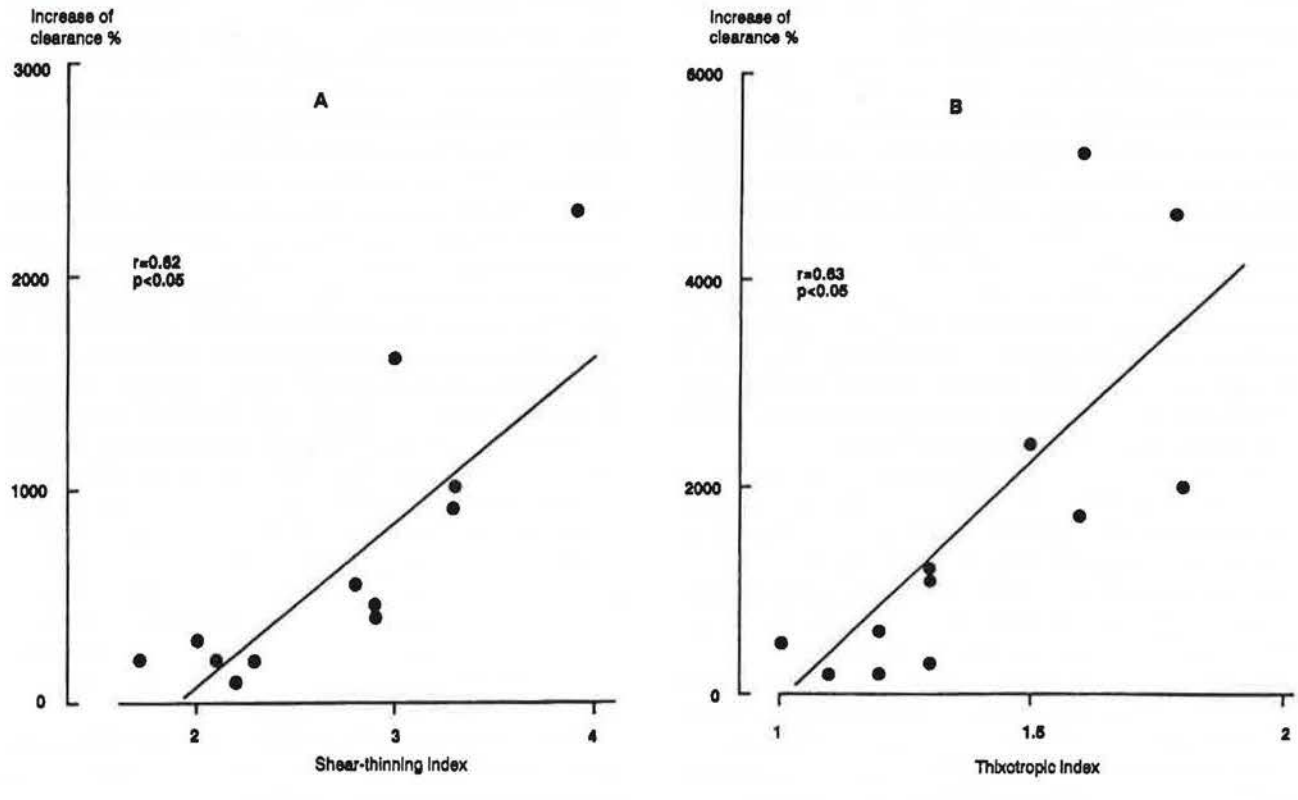

Fig. 4. – Relationship between the percentage of clearance increase

$$\frac{(\text{clearance at 1.6 Hz - clearance with a single cough}) \times 100}{\text{clearance with a single cough}}$$

and: A) the shear-thinning index; and B) the thixotropic index.

Figures 4A and 4B show the relationship between the percentage of clearance increase induced by a repetitive cough (in comparison to a single cough) and both the shear-thinning index ($r=0.62$; $p<0.05$) and the thixotropic index ($r=0.63$, $p<0.05$). A mucus highly sensitive to shear (*i.e.* exhibiting a high decrease in viscosity under shearing) is better transported by the repetitive coughing mechanism. Moreover, the decrease of mucus viscosity with time also enhanced the cough clearance.

Discussion

The role of cough in tracheobronchial clearance has been demonstrated *in vivo* by Camner *et al.* [1] and Puchelle *et al.* [2]. By using a simulated cough machine, King *et al.* [4, 5] studied, *in vitro,* the role of the rheological properties in mucus clearance by a single cough. The results reported in the present study suggest that the cough clearance is influenced by both the number of successive cough manoeuvres and the time duration between each cough manoeuvre. These mechanisms induce modifications of the mucus physical properties which are time and shear dependent. During cough, momentum must be transferred from gas moving at high velocity to the initially stationary mucus adhering to the airway wall. This coupling is the result of a two-phase flow [11–13] and is influenced by the physical properties of the mucus cleared by cough [14].

Respiratory mucus is a non-Newtonian fluid, *i.e.* its viscosity varies with the applied shear rate. Mucus viscosity may range from 1 Pa.s to 100 Pa.s at low shear rate (1 s^{-1}) and its magnitude is about 10^{-1} Pa.s at high shear rate (–100 s^{-1}). During cough, the airflow in the airways induces shear rates in the mucus which may range well over 1,000 s^{-1}. Thus, due to the mucus shear-thinning properties, the first cough manoeuvre induces a decrease of viscosity and the following successive cough manoeuvres mobilize a thinner less viscous mucus. As reported by King *et al.* [5], there is a negative relationship between mucus viscosity and cough clearance. Therefore, a less viscous mucus is better transported than a highly viscous mucus.

Mucus also shows time-dependent properties, *i.e.* a decrease of viscosity is observed with time when a constant shear rate is applied (thixotropic properties). This typical behaviour has been well-documented and may have different characteristics according to the applied shear rate [7]. At low shear rate, a classical viscoelastic behaviour is obtained. Increasing the shear rate leads to the well-known "overshoot" behaviour, typical of the transient response of a large class of biological fluids. At very high shear rate, the overshoot is confined to very early times close to the start of the shear rate application. Such properties are interpreted as resulting from flow-induced modifications of the internal structure of these elasto-thixotropic systems [15]. Cough is a transient mechanism which induces high shear rates in mucus and consequently a high decrease of viscosity with time.

The difference in clearance obtained between close coughing manoeuvres (1.6 Hz) and separated coughing manoeuvres (0.1 Hz) can be explained by the reversible change of the mucus inner structure. Puchelle *et al.* [7] showed that increasing the rest time between two successive shear rate steps leads to the partial recovery of the mucus initial viscosity. Therefore, in the case of rapid successive cough manoeuvres, the mucus viscosity remains low from one cough to the other and the mucus transport is easier. On the other hand, when a large time duration occurs between each cough manoeuvre, the mucus has enough time to recover its inner structure and the viscosity increases towards its initial value between each cough. We would therefore conclude that respiratory mucus with high shear-thinning properties and high thixotropic properties is better transported by the cough mechanism. Moreover, several successive cough manoeuvres are more efficient than a single cough or successive cough manoeuvres with a rest time of at least several seconds between each.

The effect of a single cough manoeuvre (24.9±27.5 mm) is greater than one fifth of the effect of five successive cough manoeuvres at low frequency (75.4±51.1 mm). This indicates a decreasing differential effect of successive cough manoeuvres. This is to be expected because after each successful cough manoeuvre (*i.e.* one producing significant clearance), the average depth of mucous gel is reduced, and this factor alone has been shown to diminish the effectiveness of cough clearance [4]. This consideration in no way invalidates the comparison between five successive coughs at low and high frequency. On the contrary, because of the counteracting effect of decreasing gel depth, the frequency effect is if anything underestimated.

In our study, the trachea was modelled as a solid tube with a constant peak air flow in order to only obtain clearance changes dependent on cough frequency and mucus rheology. However, during cough, the trachea can contract and relax, due to tracheal wall flexibility. The effect of airway wall flexibility on the clearance of mucus-like gels has been analysed by Soland *et al.* [16] who demonstrated that the efficiency of cough clearance is directly related to airway wall flexibility, the higher the flexibility, the more efficient the clearance. In fact, the rapid successive cough manoeuvres that we carried out are somewhat different from that existing *in vivo* where there is a decrease in peak flow rate per cough in parallel with the fall in lung volume. This decrease in peak flow may lead to a decrease in cough clearance. Inasmuch as we aimed to define the frequency dependent rheological parameters involved in cough clearance, it was necessary in our experiment to maintain a constant peak flow, whatever the cough frequency.

To our knowledge, the present work is the first which reports, in relation to mucus structure, the existence of changes involved in cough clearance. The gel viscoelastic and elasto-thixotropic structure of mucus depends on the concentration and molecular weight of the macromolecular components and on the conformation of the macromolecules. These macromolecules form a three-dimensional network with intermolecular entanglements and cross-links. The typical time-dependent behaviour of mucus is attributed to a reduction in the concentration of entanglement coupling during flow. If flow is interrupted, a denser state of entanglements is re-established [17]. This breaking down and building up of structures has been well-described for other biological fluids by QUEMADA and DROZ [15], who described a unified model for characterizing transient rheological properties of biofluids. Preliminary studies for modelling respiratory mucus with such a model are under consideration and might be useful for a better understanding of mucus transport mechanisms [18].

The present results are consistent with those of KIM *et al.* [14] and CHANG *et al.* [19] who found that a mouthward bias of air velocity in asymmetrical oscillatory flow would lead to a net mouthward movement of the mucus layer. All these results are fundamentally linked to the shear stress at the air-mucus interface.

The use of chest physiotherapy is widespread in hospital practice: postural drainage, percussion, vibration, clapping, breathing exercises. The aim of all these components is to promote mucus clearance and to finally expectorate the mucus by a cough manoeuvre. With regard to the results reported in the present work, the physiotherapists should take into account the cough conditions in order to improve its effectiveness.

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Rôle de la toux simulée et reférée dans la clearance du mucus.
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RÉSUMÉ: Le rôle de la toux répétée simulée sur le transport d'un simuli- mucus gel a été étudié. La répétition de la toux entraîne une augmentation significative ($p<0.01$) du transport du mucus (139.3 ± 78.7 mm) par comparaison avec une toux simple (24.9 ± 27.5 mm). De plus, l'augmentation de la fréquence de la toux répétée se traduit par une augmentation significative du transport du mucus (75.4 ± 51.1 mm et 139.3 ± 78.7 mm à 0.1 Hz et 1.6 Hz, respectivement). Une corrélation significative a été obsérvée entre l'augmentation du transport du mucus par la toux et les index de fluidification ($r=0.62$) et de thixotropie ($r=0.63$). Ces résultats suggèrent que les propriétés physiques du mucus (dépendantes du cisaillement), associées à une toux répétée, améliorent le transport du mucus par la toux.
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