Research article

Effects of intravenous furosemide on mucociliary transport and rheological properties of patients under mechanical ventilation

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Abstract

The use of intravenous (IV) furosemide is common practice in patients under mechanical ventilation (MV), but its effects on respiratory mucus are largely unknown. Furosemide can affect respiratory mucus either directly through inhibition of the $NaK(Cl)_2$ co-transporter on the basolateral surface of airway epithelium or indirectly through increased diuresis and dehydration. We investigated the physical properties and transportability of respiratory mucus obtained from 26 patients under MV distributed in two groups, furosemide ($n = 12$) and control ($n = 14$). Mucus collection was done at 0, 1, 2, 3 and 4 hours. The rheological properties of mucus were studied with a microrheometer, and *in vitro* mucociliary transport (MCT) (frog palate), contact angle (CA) and cough clearance (CC) (simulated cough machine) were measured. After the administration of furosemide, MCT decreased by $17 \pm 19\%$, $24 \pm 11\%$, $18 \pm 16\%$ and $18 \pm 13\%$ at 1, 2, 3 and 4 hours respectively, $P < 0.001$ compared with control. In contrast, no significant changes were observed in the control group. The remaining parameters did not change significantly in either group. Our results support the hypothesis that IV furosemide might acutely impair MCT in patients under MV.

Keywords furosemide, mechanical ventilation, mucociliary transport, mucus rheology

Introduction

Although mechanical ventilation (MV) is necessary to improve ventilatory support in respiratory failure, it is generally known that this procedure markedly increases the incidence of pulmonary infection and consequently the morbidity and mortality of patients. Mucociliary clearance has been reported to be impaired in patients under MV and this is probably an important underlying mechanism in the pathogenesis of pulmonary infection in these patients [1]. Mucociliary clearance has a pivotal role in the protection of the respiratory tract against inhaled noxious agents that are trapped in the blanket of mucus and transported towards the pharynx by ciliary beating or coughing. The efficiency of the mucociliary system depends not only on the integrity of the epithelium and on ciliary activity but also on the amount of mucus, the depth of the periciliary layer and the viscoelastic properties of mucus [2].

Airway epithelium is an absorptive and secretory type of epithelium [3]; the transepithelial movement of electrolytes generates osmotic gradients that are responsible for the

CA = contact angle; CC = cough clearance; HME = heat and moisture exchanger; IV = intravenous; MCT = mucociliary transport; MV = mechanical ventilation.

secretion or absorption of water. Pulmonary epithelial ion transport systems are important in the modulation of the ionic content and volume of periciliary fluid, which in turn modulates the physical properties and transportability of mucus. Small changes in the depth of periciliary fluid could greatly alter the efficiency of interaction between mucus and cilia [4]. Diuretics with an action on ionic channels present in the airway epithelium can alter ionic movement and change the physical properties and transportability of mucus. For instance, inhaling amiloride, a diuretic with action on the apical Na^+ channel, has been reported to increase mucociliary clearance and alter the physical properties of mucus in patients with cystic fibrosis [5–8].

Intravenous (IV) furosemide is frequently used in patients under MV with the aim of equilibrating a cumulative positive fluid balance. However, the possible effects of IV furosemide on respiratory mucus are largely ignored. Furosemide is a potent diuretic that acts by inhibiting the $NaK(Cl)_2$ co-transporter in the ascending limb of the loop of Henle. Besides its renal action, furosemide can also affect epithelial ion transport in the airway. Earlier studies demonstrated that furosemide inhibits the $NaK(Cl)_2$ co-transporter in canine airway epithelium [9] and also decreases intracellular Cl^- activity in cultured human airway epithelium [10]. The effects of inhaled furosemide have also been investigated. Inhaled furosemide prevents exercise-induced bronchoconstriction in asthmatic patients [11]. Hasani *et al*. [12] reported that inhaled furosemide had no effects on mucociliary clearance in humans. However, the primary site of furosemide action is the basolateral membrane of the airway, where it inhibits the $NaK(Cl)_2$ co-transporter. Therefore the effects of the drug on the respiratory epithelium might depend on the route of administration. The aim of the present study was to investigate the effects of IV furosemide on the transportability and rheological properties of mucus in patients under MV.

Materials and methods

Patients

We studied 26 patients under MV in the Respiratory Intensive Care Unit of the Pulmonary Division, Hospital das Clínicas, University of São Paulo. The study was approved by the Ethics Committee of the University of São Paulo. All patients were clinically and haemodynamically stable for at least 24 hours before the study. In each of these patients we registered their clinical data, including arterial pressure, heart rate, fluid balance, urine output and temperature, during the 24 hours before and during the study. We also registered the mode of MV, tidal volume, respiratory rate, minute volume, fraction of inspired oxygen and system of humidification. The time interval between the initiation of MV and the study was also recorded.

The patients were distributed in two groups: the furosemide group consisted of 12 patients (8 female and 4 male) who received IV furosemide; the control group consisted of 14 patients (3 female and 11 male) who did not receive any diuretic during the study. Their ages (means ± SD) in the furosemide and control groups were, respectively, 66 ± 15 years with a range of 30–82 years, and 49 ± 20 years with a range of 20–76 years. The indication and dose of furosemide were determined by each patient's clinical conditions and in all cases were because of a positive fluid balance. The aim of including a control group was to make sure that there were no time-dependent changes in the variables analysed. When recruiting patients for the control group, our main goal was to match them in terms of the MV parameters.

Collection of mucus

Respiratory mucus was collected from the endotracheal tube by sterile technique with a suction catheter. The samples were extracted from the catheter with a sterile needle and were immediately immersed in mineral oil to prevent mucus dehydration. The suction conditions were kept to a minimum to decrease the degree of shear thinning and the incorporation of air bubbles [13]. Mucus samples were stored at −70°C in sealed plastic containers for later analysis.

We collected mucus at 0, 1, 2, 3 and 4 hours. The first sample (0 hours) in the furosemide group was just before the administration of the diuretic.

Mucus analysis

Mucus transportability by cilia

Mucociliary transport (MCT) was determined *in vitro* in the frog palate preparation, which possesses an epithelium that is similar to that in the upper airways in humans [14]. All animals were cared for in compliance with the Guide for Care and Use of Laboratory Animals published by the National Institutes of Health (NIH publication 85-23, revised 1985). To deplete the palate mucus, the palate was stored for 2 days at 4°C in a humidified chamber covered with plastic wrap [15]. Ciliary activity is maintained under these experimental conditions. The frog mucus was collected and used as a control for measurements of transport rate. Measurements of transport rate were determined with a stereomicroscope (Zeiss) equipped with a reticulated eyepiece. We timed the displacement of the mucus samples across a segment between the anterior and posterior parts of the palate. During the experiments the palate was kept at ambient temperature (20–25°C) and 100% humidity, provided by ultrasonic nebulization [13,16]. The results were expressed as relative transport velocity and corresponded to the ratio of velocity of the test mucus sample to that of the control frog mucus.

Contact angle (CA)

Respiratory mucus is a complex material that possesses both rheological properties, which are directly involved in the transportability of mucus, and physical properties such as wettability, which is an important property in the interaction between the mucus and the respiratory epithelial surface. Wettability is the tendency of a biological fluid to spread when deposited

on a solid plane surface owing to the interaction between the surface and the molecules of the mucus. The degree of wettability is determined by the contact angle between the tangent to the liquid–air interface and the horizontal at the triple point where the three phases meet [17].

CA was determined by an eyepiece that had a goniometer with a scale of 0° to 180°. Mucus samples were placed on a plate pretreated with sulphochromic acid to remove electrical charges, which interfered with measurements. During the experiments a water bath kept at 37°C allowed humidification to prevent the dehydration of mucus [13,16].

Mucus transportability by cough

Cough clearance (CC) experiments were performed *in vitro* in a simulated cough machine adapted from King *et al*. [18]. This machine consisted of a cylinder of compressed air serving as gas supply, a solenoid valve that controlled the release of gas, and a cylindrical acrylic tube 4 mm in internal diameter and 133 mm in length as a model trachea. Mucus was introduced into the tube and connected to the simulated cough machine. The solenoid valve released the air for 0.5 s under a pressure of 280 kPa. Clearance was quantified by determining the displacement of mucus in millimetres [13,16].

Rheological properties

The rheological properties of mucus samples were determined in the present study with a magnetic microrheometer as described by King and Macklem [19] and modified by Silveira *et al.* [20]. The microrheometer measured the displacement, resulting from a sinusoidal oscillating magnetic field, of a small steel ball inserted in the mucus sample. The motion of the ball was opposed by viscous and elastic forces.

The plexiglass container with the drop of mucus sample and the steel ball was placed into the gap of a magnetic toroid that was mounted on the stage of a projecting microscope and driven by a sine-wave generator. The shadow of the ball was projected onto two photocells that captured its oscillatory movement and provided an electrical output in proportion to the displacement of the moving ball. The toroid current and the output of the photocells were transmitted to a digital oscilloscope connected to an IBM-compatible personal computer for storage and off-line processing [13,16].

Measurements were made at two different frequencies: 1 radian/s (ciliary movement) and 100 radians/s (cough) [21]. Two parameters were obtained: first, the relation between stress and strain, representing the overall impedance of the mucus (G^*), and second, the phase lag between stress and strain, representing the ratio between viscosity and elasticity ($\tan \delta$).

Statistical analysis

Statistical analysis was performed by profile analysis [13], which takes into account time correlation between different sampling times (0, 1, 2, 3 and 4 hours). This is a multivariate method in which only one statistical model is applied. This method considers the group along the time and basic hypotheses can be tested enabling post hoc corrections to be performed through contrasts so as to identify, or discriminate, significant differences. Basic hypotheses are the following: H_{01}, in which there is no interaction between the factors group and time (parallelism); H_{02}, in which there is no difference between the use of either control or furosemide group (coincidence); and H_{03}, in which there is no time effect.

When H_{01} was accepted, hypotheses H_{02} and H_{03} were tested. When H_{01} was rejected, hypotheses H_{02} and H_{03} were not tested and post hoc corrections for multiple comparisons were performed through contrasts.

$P < 0.05$ was considered statistically significant.

Results

Demographic and MV parameters are described in Tables 1 and 2. The time lag between the initiation of MV and the study was 9 ± 6 and 9 ± 6 days for the furosemide and control groups, respectively ($P = 0.9$). In the furosemide group, two patients were using the heat and moisture exchanger (HME), and 10 were using the heated humidifier. In the control group, six patients were using the HME and eight were using the heated humidifier.

The results of mucus transportability in the frog palate (MCT) and cough (CC) are presented in Figs 1 and 2, respectively. MCT decreased significantly after furosemide administration and did not recover to baseline values by 4 hours ($P = 0.0001$). In contrast, MCT remained constant in the control group (Fig. 1). There was a trend that did not reach statistical significance for a decrease in CC in the furosemide group (Fig. 2).

The results of the remaining parameters, contact angle, log G^* and $\tan \delta$ measured at 1 and 100 radians/s, are presented in Table 3. There were no significant differences between groups.

Discussion

To our knowledge this is the first study to investigate the effects of IV furosemide on mucus transportability *in vitro* and the physical properties of mucus from patients under MV. Our results suggest that IV furosemide might acutely impair MCT for up to 4 hours after administration.

The mucociliary escalator of the lungs is an important protective transport system by means of which inhaled particles and microorganisms are removed from the tracheobronchial system. Lung mucociliary clearance is influenced by several factors, including the integrity of the ciliated epithelium and the thickness and physical properties of the periciliary or mucous layer [12]. Under normal circumstances, active ion transport in the respiratory epithelium is important in the pro-

Table 1

Demographic characteristics and mechanical ventilation parameters of the control group

Sex	Age	Diagnosis	Mode	F_{iO2} (%)	V_E (L/min)	Vasoactive drugs	Tracheal secretion	Fluid balance (ml)		Diuresis (ml)	
								24 hours	Study	24 hours	Study
F	70	Mediastinal tumor resection	AMV	40	11.7	–	*S. viridans*	+31	+233	1150	75
M	20	Head trauma	VAPS	45	9.6	–	*P. aeruginosa*	+78	−10	2350	400
M	20	Head trauma	VAPS	40	9.2	–	*P. aeruginosa* *S. aureus*	+341	+83	2450	400
M	63	Cerebrovascular accident, heart failure, osteomyelitis	VAPS	46	10.5	Dobutamine	*A. calcoaceticus* *P. aeruginosa* *S. aureus*	−1310	−154	3180	480
M	47	Lung neoplasm	SIMV	40	9.7	Dobutamine	–	+564	+321	1920	120
M	76	Lung neoplasm, pneumonia	AMV	36	8.7	Dobutamine Dopamine	*P. aeruginosa* *X. maltophilia*	+1408	+132	780	100
M	66	Heart failure, pneumonia, pulmonar lobectomy	PC	50	8.7	Dobutamine	*A. calcoaceticus*	+534	+373	2300	120
M	22	Craniotomy, pneumonia	AMV	40	9.4	–	*A. calcoaceticus* *E. cloacae*	+1411	+171	2400	140
M	41	Hyperosmolar coma, cerebrovascular accident	PC/SIMV	40	10.7	–	*A. baumanii* *S. viridans* *S. coagulase* neg	−518	+86	550	150
M	61	Lung neoplasm, COPD, acute renal insufficiency	PS	45	11.7	Dobutamine	*S. marcescens* *A. baumanii* *X. maltophilia*	+1871	+183	60	20
F	58	Drug intoxication, pneumonia	PC/SIMV	40	7.7	–	*S. aureus*	+286	−98	2000	171
M	74	Pulmonary tumor resection	AMV	30	8.3	–	–	−462	+133	1650	125
M	51	Respiratory failure	PC/SIMV	40	10.5	Noradrenaline	–	+1452	−59	2000	720
F	23	Thyroidectomy	SIMV	40	5.0	–	–	+3551	+290	2570	200
Mean ±SD	50 ±20			40 ±4	9.4 ±1.7			671 ±1169	82 ±212	1824 ±847	258 ±218

Abbreviations: AMV, assisted mechanical ventilation; COPD, chronic obstructive pulmonary disease; F_{iO2}, fraction of inspired oxygen; PC, pressure-controlled ventilation; PS, pressure-support ventilation; SIMV, synchronized intermittent mandatory ventilation; VAPS, volume-assured pressure support, V_E, minute volume. *$P < 0.05$.

duction and regulation of the volume and composition of the respiratory tract secretion, which in turn is important for adequate mucociliary interaction [22]. Pharmacological interference in ionic transport is caused by a new class of drugs that can change MCT. For instance, inhalation of amiloride increases MCT in patients with cystic fibrosis by inhibiting the active absorption of salt and water from airway surfaces [23,24].

The effects of furosemide on the respiratory epithelium have attracted interest in the decade since Bianco *et al.* [11] reported that inhaled furosemide prevents exercise-induced bronchoconstriction in asthmatic patients. The mechanism of this protective effect remains to be established. The effects of inhaled furosemide on mucociliary clearance have been investigated and the results are controversial. Hasani *et al.* [12] reported that nebulized furosemide does not affect mucociliary clearance measured with a radioaerosol technique in healthy and asthmatic subjects. It must be stressed that the primary site of furosemide action is the basolateral membrane of the airway, where it inhibits the $NaK(Cl)_2$ co-transporter. Inhaled furosemide might therefore not reach the basolateral membrane of airway epithelial cells *in vivo* [11,25]. In fact, experimental studies have demonstrated that, in contrast with the serosal application of furosemide, mucosal application has no effect on co-transporter function [26]. Winters and Yeates [27] have reported an increase in lung mucociliary clearance *in vivo* after the inhalation of aerosolized furosemide and the IV administration of furosemide in dogs and baboons. However, in this study the

Table 2

Demographic characteristics and mechanical ventilation parameters of furosemide group

Sex	Age	Diagnosis	Mode	F_{iO2} (%)	V_E (L/min)	Vasoactive drugs	Tracheal secretion	Fluid balance (ml)		Diuresis (ml)	
								24 hours	Study	24 hours	Study
F	61	Pneumonia	VAPS	45	7.5	–	*X. maltophilia*	+380	−15	2600	500
F	64	Acute renal failure	CMV	45	6.9	Noradrenaline Dopamine	*S. aureus* *X. maltophilia*	+768	+178	482	275
M	66	Pulmonar lobectomy, respiratory failure	SIMV	40	8.6	Dobutamine	*P. aeruginosa* *A. calcoaceticus*	+1175	−200	1580	540
M	30	Wound of gunshot injury	SIMV	30	4.7	–	–	+960	−1475	1250	1800
F	86	Burn	PC	40	4.2	Dobutamine	–	−610	−366	3400	860
F	59	Penetrating thorax wound	CPAP	40	5.8	–	–	−740	−734	1750	890
F	82	Pneumonia	AMV	50	8.6	–	*A. calcoaceticus*	+1242	+264	1440	300
F	82	Pneumonia	AC	45	9.5	–	*A. calcoaceticus*	+418	+192	1120	540
F	67	Sjogren syndrome	CMV	40	8.7	–	*P. aeruginosa*	+502	+604	1850	200
M	74	Wegener's granulomatosis, pneumonia	VAPS	40	12	Dopamine Dobutamine	*P. maltophilia*	+1988	−1194	1670	1700
M	61	COPD, respiratory failure	PC	60	9.9	Dopamine	*S. marcescens* *A. baumanii* *X. maltophilia*	+2244	−1	290	100
F	63	Cerebrovascular accident, PE, pneumonia	AC	55	8.9	Dobutamine	*A. calcoaceticus*	+2190	−22	1460	440
Mean	66.2			44	8.3			813	−231	1575	679
±SD	±14.7			±8	±2.1			±1036	±616	±836	±554
P	0.03*			0.20	0.17			0.64	0.19	0.23	0.01*

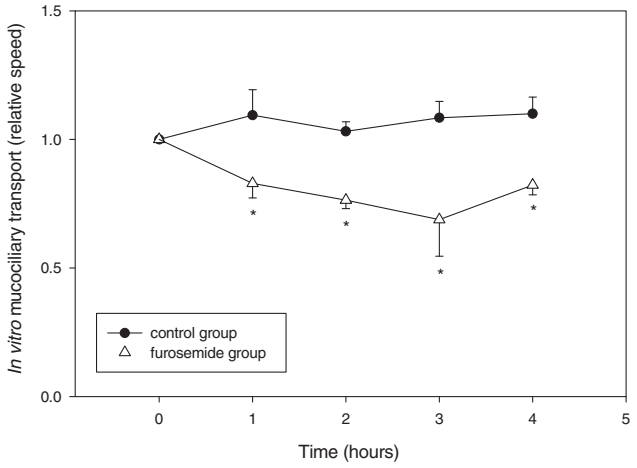
Abbreviations: AC, assist/control ventilation; AMV, assisted mechanical ventilation; CMV, controlled mechanical ventilation; COPD, chronic obstructive pulmonary disease; F_{iO2}, fraction of inspired oxygen; PC, pressure-controlled ventilation; PE, pulmonary embolism; SIMV, synchronized intermittent mandatory ventilation; CPAP, continuous positive airway pressure; VAPS, volume-assured pressure support, V_E, minute volume.
*$P < 0.05$.

properties and *in vitro* transportability of mucus were not determined.

In our study we observed a decrease in MCT after furosemide administration that did not recover to the baseline by 4 hours. Furosemide inhibits the $NaK(Cl)_2$ co-transporter, which is one of the physiological mechanisms involved in the respiratory hydration of mucus; its inhibition could therefore interfere in the rheological properties of mucus [4,28]. The ionic concentration of Na^+ and Cl^{2-} in mucus can also influence the rheology and transportability of mucus independently of its total water content [6,29]. In addition, diuresis might lead to systemic dehydration and impairment of mucociliary clearance [30,31]. In our study, furosemide administration was a clinical decision based on cumulative positive fluid balance and determined by the medical staff. Interestingly, the furosemide and control groups had similar fluid balance in the 24 hours before the onset of the study. As expected, furosemide promoted increased diuresis. It must be stressed that in our study the patients were not monitored invasively. Fluid balance, diureses and haemodynamic status can give only gross estimates of fluid balance. In summary, from this study it is not possible to determine the mechanism involved in the effects of furosemide on MCT.

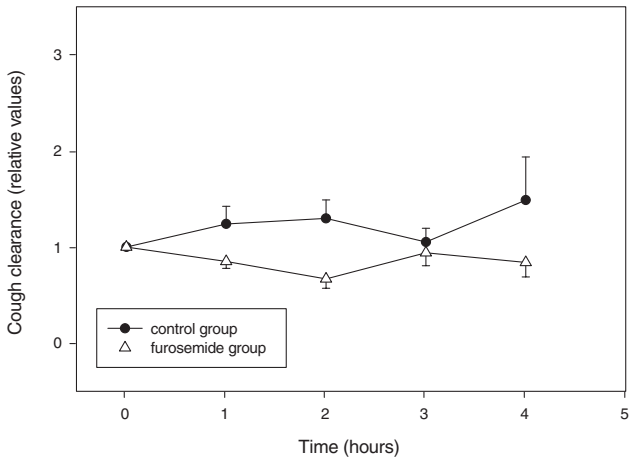
The mode of humidification was not uniform between the groups. Nakagawa *et al.* [13] have recently compared the effects of two systems of humidification (HME with a Pall BB 100 F, and a heated humidifier) on respiratory mucus and its transportability in patients under MV. The effects were evaluated for up to 72 hours of MV. They observed a decrease in CC in the HME group only after 72 hours of MV. Because the present study was limited to an intervention in a short period (4 hours), baseline clinical conditions, including age, MV parameters and the mode of humidification, probably did not influence the results. Indeed, our control group showed no time-dependent changes in all parameters studied. Infection also affects respiratory mucous and epithelium. However, the occurrence of pulmonary infection was similar in both groups (10 patients in the control group and 9 in the furosemide group), suggesting that this factor did not influence our results.

Figure 1



Results of mucociliary transport *in vitro* in frog palate. There was a significant decrease in MCT after furosemide administration that did not recover to the baseline by 4 hours. * $P < 0.05$.

Figure 2



Results of mucus transportability by cough measured with a simulated cough machine. The results are shown in terms of relative change in CC (CC at 1, 2, 3 and 4 hours divided by CC at time 0, i.e. before drug administration).

Table 3

Mucus analysis (means ± SD)

	MCT (relative speed)		CA (degrees)		CC (mm)		logG^*, 1 radian/s	
Time (hours)	C	F	C	F	C	F	C	F
0	0.83 ± 0.22	1.01* ± 0.21	44.14 ± 8.78	37.75 ± 8.13	58.21 ± 30	74.25 ± 29.46	1.66 ± 0.38	1.45 ± 0.43
1	0.88 ± 0.24	0.81 ± 0.16	45.43 ± 8.53	41.25 ± 10.9	60.43 ± 29	62.5 ± 29.44	1.49 ± 0.44	1.57 ± 0.49
2	0.85 ± 0.21	0.77 ± 0.2	44.93 ± 8.11	40.92 ± 7.8	63.6 ± 37.44	46.92 ± 28.6	1.62 ± 0.35	1.55 ± 0.42
3	0.88 ± 0.2	0.82 ± 0.22	45 ± 10.2	41.75 ± 9	57.6 ± 34.25	63.1 ± 28.93	1.37 ± 0.57	1.61 ± 0.38
4	0.88 ± 0.18	0.82 ± 0.2	44.29 ± 6.29	39.92 ± 11.4	60.57 ± 26.57	57.75 ± 31.22	1.46 ± 0.4	1.48 ± 0.27
P	0.88	0.0001*	0.25		0.54		0.60	

	logG^*, 100 radians/s		tanδ, 1 radian/s		tanδ, 100 radians/s	
Time (hours)	C	F	C	F	C	F
0	1.65 ± 0.24	1.61 ± 0.42	0.51 ± 0.12	0.51 ± 0.19	0.73 ± 0.22	0.84 ± 0.41
1	1.68 ± 0.3	1.71 ± 0.38	0.57 ± 0.14	0.54 ± 0.25	0.75 ± 0.23	0.79 ± 0.34
2	1.67 ± 0.35	1.69 ± 0.26	0.49 ± 0.13	0.61 ± 0.26	0.86 ± 0.27	0.78 ± 0.26
3	1.40 ± 0.36	1.71 ± 0.32	0.47 ± 0.15	0.63 ± 0.36	0.63 ± 0.17	0.72 ± 0.16
4	1.51 ± 0.23	1.62 ± 0.25	0.6 ± 0.15	0.57 ± 0.14	0.66 ± 0.22	0.82 ± 0.39
P	0.16		0.16		0.14	

Abbreviations: C, control group; CA, contact angle; CC, cough clearance; F, furosemide group; MCT, mucociliary transport. *$P < 0.05$.

In our study, impairment in MCT was not matched with significant changes in other physical properties of mucus. It is possible that MCT is a more sensitive method for detecting mucociliary impairment. Because our study involved a relatively small number of patients, we cannot discard a type 2 error to explain the absence of furosemide effect on other mucus parameters. An alternative explanation is that furosemide has direct effects on the ciliary beating frequency of the frog palate.

In conclusion, our preliminary results support the hypothesis that IV furosemide might acutely impair mucociliary clearance. In patients with respiratory failure and MV, many factors can potentially impair MCT, such as ventilation with a high concentration of oxygen, the activation of inflammatory mediator systems, colonization by bacteria, suction-induced lesions of the mucous membrane, infections and drugs [1]. The mechanisms and the clinical relevance of our findings remain to be established.

Competing interests

None declared.

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