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Mucociliary clearance during and after isocapnic hyperventilation with dry air in the presence of frusemide

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Mucociliary clearance during and after isocapnic hyperventilation with dry air in the presence of frusemide. E. Daviskas, S.D. Anderson, I. Gonda, D. Bailey, G. Bautovich, J.P. Seale. ©ERS Journals Ltd 1996.

ABSTRACT: We have previously shown that mucociliary clearance (MCC) decreased during and increased after isocapnic hyperventilation (ISH) with dry air, both in asthmatic and healthy subjects. Inhaled frusemide, an inhibitor of the $Na^+/K^+/2Cl^-$ and NaCl co-transporters on the basolateral membrane of the epithelial cell, prevents the airway narrowing provoked by ISH with dry air. The co-transport system controls epithelial cell volume and chloride secretion and, thus, frusemide has the potential to modify the rate of recovery of periciliary fluid volume during and after ISH with dry air, and hence affect MCC. Frusemide also blocks mediator release from mast cells, which may also modify the increase in MCC after ISH.

Eleven asthmatic and 11 healthy subjects inhaled frusemide (35.7±0.44 mg) or its vehicle, from a Fisoneb ™ ultrasonic nebulizer 30 min before ISH with dry air, on two separate occasions. MCC was measured using ^{99m}Tc-sulphur colloid and a gamma camera. Frusemide, compared to its vehicle, did not affect MCC during or 45 min after ISH. However, in the presence of frusemide, the onset of the increase of MCC after ISH was significantly delayed for approximately 10 min in the whole right lung ($p<0.002$) and central region ($p<0.01$) in the asthmatic but not in the healthy subjects.

These findings could be explained by frusemide delaying the recovery of the periciliary fluid volume after ISH with dry air and/or interfering with the stimulus that causes the increase in MCC in the asthmatic subjects after ISH.
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Isocapnic hyperventilation (ISH) with dry air results in significant water loss from the airways whilst bringing the inspired air to alveolar conditions. The water loss from the airways is the stimulus whereby hyperpnoea provokes airway narrowing [1, 2]. Mucociliary clearance (MCC) decreases during and increases after ISH with dry air, compared to ISH with warm humid air and nasal breathing at rest [3]. The effect of ISH with dry air on MCC is most likely to be due to a reduction of the depth of the periciliary fluid layer, with the subsequent hyperosmolarity leading to release of mediators [4]. Frusemide, a loop diuretic known to inhibit the $Na^+/K^+/2Cl^-$ and NaCl co-transport systems at the basolateral membrane [5], inhibits the airway narrowing induced by exercise [6], ISH [7, 8], and hypertonic saline [9]. Inhibition of the co-transport systems affects the regulatory volume increase (RVI) of the hypertonically shrunken epithelial cells [10], and chloride secretion [5]. Therefore, frusemide has the potential to interfere with the water availability to the periciliary fluid layer.

The mechanism by which frusemide protects against dry air and hyperosmolar challenges in asthma is not clearly understood. It is possible that it interferes with the transport of the chloride ion or the chloride channel under stimulatory conditions and, thereby, blocks mediator release from mast cells [11], which are located in the airway lumen or epithelium.

We decided to investigate the effect of frusemide on MCC during and after ISH with dry air because of its potential to affect the water transport to the airways, when the water demand is high. We also wished to further our knowledge of the mechanism whereby frusemide inhibits airway narrowing.

Material and methods

The study was approved by the Ethics Review Committee of Central Sydney Area Health Service and informed consent was obtained in writing from all subjects before participating in the studies.

Subjects

Eleven asthmatic and 11 healthy volunteers took part in the study. Their characteristics are given in table 1. The asthmatic subjects had stable asthma and a resting forced expiratory volume in one second (FEV_1) >85% of predicted with the exception of subject No. 5 (table 1).

Table 1. – Anthropometric data, baseline lung function, ventilation rate (% maximum voluntary ventilation (MVV)) duration of isocapnic hyperventilation (ISH), responses to ISH with dry air of asthmatic and healthy subjects and medications taken by the asthmatic subjects

No.	Age	Sex	Height	Baseline FEV_1	Ventilation			Fall FEV_1 Dry ISH	Atopy	Medication		
					$L \cdot min^{-1}$	% MVV	Duration			Drug	Steroid dose μg	Time on
	yrs		cm	% pred			min	%				months
Asthmatic subjects												
1	23	M	176	94	90	59	8	20	Y	S,BEC	1000	18
2	21	M	189	95	104	57	8	16	Y	NIL		
3	20	M	182	102	94	56	6	18	Y	S,BUD	400	1
4	21	M	168	98	87	61	8	9	Y	S,BUD	400	4
5	24	M	171	74	86	60	6	4	Y	S,BUD	1000	1.5
6	28	M	182	96	97	60	8	23	Y	NIL		
7	31	M	174	111	87	60	8	13	Y	NIL		
8	28	M	168	91	74	54	6	18	Y	S,BUD	1600	4
9	19	M	174	86	95	61	8	12	N	S		
10	20	M	163	99	85	63	6	22	Y	S,BUD,SCG	800	2
11	24	F	163	157	64	59	8	10	Y	T,BUD	1200	52
Mean	24		174	100	90			15				
±SD	4		8	21	8			6				
Healthy subjects												
12	39	M	173	100	90	52	8	3	Y	-	-	-
13	20	M	184	107	103	60	8	5	Y	-	-	-
14	19	M	184	96	100	58	8	3	Y	-	-	-
15	23	M	178	115	96	61	8	0	N	-	-	-
16	37	M	180	102	85	56	8	2	N	-	-	-
17	23	M	176	103	93	60	8	2	Y	-	-	-
18	33	M	192	146	127	72	8	3	N	-	-	-
19	21	M	172	107	94	63	8	5	N	-	-	-
20	41	F	168	118	80	59	8	2	N	-	-	-
21	23	F	162	112	60	55	8	0	N	-	-	-
22	25	F	166	133	67	59	8	0	N	-	-	-
Mean	28		176	113	90			2				
±SD	8		9	15	18			2				

M: male; F: female; FEV_1: forced expiratory volume in one second; Y: yes; N: no; BEC: beclamethasone; BUD: budesonide; S: salbutamol; SCG: sodium cromoglycate; T: terbutaline.

Most of the asthmatics were on inhaled beta-agonists daily or as needed, and on inhaled corticosteroid medication (either beclomethasone or budesonide). All asthmatics withheld their medications for at least 8 h. No asthmatic subject was taking theophylline. All subjects were asked not to take any nonsteroidal anti-inflammatory drugs for at least 48 h prior to each study day. The subjects did not have a history of smoking. They did not have a lower respiratory tract infection in the 6 weeks prior to each study.

Study design

The study involved three visits. All subjects had their lung function measured before and after ISH with dry air, prior to mucociliary clearance study days (Visit 1). MCC was measured on visits 2 and 3, and the procedure on each day was as follows: 1) spirometry; 2) inhalation of frusemide or its vehicle for about 7 min; 3) radioaerosol inhalation; 4) emission anterior/posterior gamma camera images (static) to obtain initial dose in the lung; 5) ISH for 6–8 min; and 6) emission anterior/posterior gamma camera images (dynamic) for 45 min. The order of MCC studies involving frusemide or its vehicle was random.

Measurement of lung function

Spirometry, using a hot wire anemometer (Minato, AS-500, Osaka, Japan), was measured before and after ISH challenge with dry air, on the first visit. All subjects had normal and reproducible spirometry at rest. Subjects were included in the study if they had a spontaneous recovery within 30 min after the challenge and they were not distressed by the induction of asthma. To avoid possible changes in mucociliary clearance with the forced manoeuvres, lung function after ISH was not measured on the mucociliary clearance study days. The airway responses after ISH, in the presence of frusemide, were not measured. In all studies, predicted values for lung volumes were taken from GOLDMAN and BECKLAKE [12] for adults.

Delivery of frusemide and its vehicle

Ampoules of frusemide (Lasix; Hoechst, Germany) each containing 20 mg frusemide in 2 mL (pH 9, osmolarity

303 mosm) were used in this study. The vehicle was 0.9% saline adjusted to pH 9 by adding 0.1 M sodium hydroxide, prior to delivery. A pH meter (Radiometer PHM 62; Copenhagen, Denmark) was used to measure the pH of frusemide and its vehicle. Frusemide or its vehicle was delivered as an aerosol *via* a Fisoneb ™ ultrasonic nebulizer (Fisons, Rochester, New York, USA) which produces a dense aerosol with a mass median aerodynamic diameter of 4.7 μm. Six millilitres of frusemide were placed in the Fisoneb ™ and inhaled for about 7 min. The subject inhaled the aerosol through a mouthpiece, at a resting rate of breathing, while wearing a noseclip. The nebulizer was weighed (Sartorius Analytic, Gottingen, Germany) before and after nebulization with a stopper placed in the output hole to reduce loss of volume by evaporation. The difference in weight was recorded as the amount that was nebulized. Approximately 35.7±0.44 mg of frusemide was nebulized.

Isocapnic hyperventilation

The technique used was similar to the one described by PHILLIPS *et al*. [13]. Dry compressed air containing 21% oxygen, 4.9% carbon dioxide, and nitrogen to balance was passed *via* a demand resuscitator to a rotameter and then to a metereological balloon (approximately 30 L capacity) that served as a target. The subject, wearing a noseclip, breathed through a low resistance two-way valve (Hans-Rudolf No. 2700; Kansas City Mo, USA). The subject was asked to ventilate for 6–8 min at a rate equivalent to 60% of predicted maximum voluntary ventilation (MVV), (taken as $FEV_1 \times 37.5$) [14]. The ventilation and duration of ISH was kept the same for the three study days for the same subject.

Measurement of mucociliary clearance

Inhalation of radioaerosol. Mucociliary clearance was assessed using a radioaerosol technique. ^{99m}Tc-sulphur colloid (Australian Radioisotopes; ANSTO, Sydney, Australia) approximately 1 GBq, was diluted in 5 mL of isotonic saline. The radioaerosol was generated by an Acorn nebulizer (Medic-Aid, Peckham, Sussex, UK), using oxygen from a cylinder set to 6 $L \cdot min^{-1}$. The droplets had a mass median aerodynamic diameter (MMAD) of 6 μm and a geometric standard deviation (GSD) of 1.7 measured by a seven stage cascade impactor (DCI6; Delron, Columbus, Ohio, USA). The dilution air supplementing the flow to the mouthpiece was humidified to maintain the characteristics of the droplets [15]. The radioaerosol was delivered with a controlled breathing pattern in order to maximize deposition in the conducting ciliated airways. A closed breathing circuit was linked to an IBM computer, that allowed monitoring and control of the breathing pattern using a target volume and target inspiratory and expiratory times [16]. The target volume, set to 450 mL, was displayed on the screen together with the target line oscillating at set rates, aiming at a peak inspiratory flow rate of 60 $L \cdot min^{-1}$. The inspiratory time was set higher than the expiratory time, and the subject tried to breath according to the set tidal volume and flow rates. The patient inhaled the radioaerosol for approximately 2 min. This delivery time was chosen so that the gamma camera count rate was about 2,000 $counts \cdot s^{-1}$ over the posterior thorax. Upon termination of the delivery of radioaerosol, the subjects removed the radioactivity from their oropharynx and the oesophagus by rinsing and gargling with water and expectorating, and by swallowing some bread and water.

Imaging. Lung images were obtained using a gamma camera (Phillips Diagnost Tomo; Hanburg, Germany) fitted with a low energy, all purpose, collimator and linked to an on-line computer (DEC PDP11; Maynard, MA, USA). The images were collected in a 64 × 64 matrix.

In order to delineate the lung fields, for regions of interest definition in the analysis, the subjects had an anterior and posterior transmission image [17], taken using a flood source containing approximately 1.5 GBq of ^{153}Gd in water. An anterior and a posterior emission image (static), 1 min each, were collected for each subject approximately 10 min after the inhalation of the radioaerosol. The purpose of this image was to define the initial deposition of the radioaerosol in terms of distribution and intensity. As soon as the intervention had finished, the subject was placed in the supine position and successive anterior and posterior emission images (dynamic) of 20 s each were collected dynamically for 45 min [18]. Care was taken that the collection of the dynamic emission images started within a maximum of three min post intervention. In order to align the lung fields between the transmission, static and the dynamic images, images of markers placed on premarked positions on the subject's body were also collected at the end of each study. Care was taken that all emission images were taken at the same time after the mid-inhalation time of the radioaerosol for the 3 study days.

Image and data analysis

All the images were decay corrected to the mid-inhalation time of the radioaerosol. Geometric mean (GM) images were obtained from the anterior and posterior images for the transmission, static and dynamic emission images [18]. The left lung was not analysed as there could be possible interference from the activity in the stomach. The right lung was divided into three regions of interest: central, intermediate, peripheral [19], in order to estimate the initial deposition of the radioaerosol and subsequent clearance. An estimate of the initial homogeneity of deposition was obtained from the static emission GM image by calculating the penetration index (PI) as follows:

$$PI = \text{activity in peripheral region} / \text{activity in central region}$$

A bi-exponential function was fitted to the curve obtained from the dynamic GM images, using a nonlinear least squares method (PCNONLIN, SCI, Software, Lexington, KY, USA). The fitted data were extrapolated to 1 min post intervention, if the start of the dynamic emission images was delayed up to 3 min. The activity of the whole right lung and the defined regions in the static emission image, measured before the intervention, was taken as the initial activity and expressed as 100%

retention. The activity of the whole right lung and the defined regions in the dynamic emission images, measured after the intervention, was converted to counts per minute (cpm) and expressed as a percentage of the initial activity. Mucociliary clearance was the difference between the initial activity and the percentage retained at the specified time. Data from the best fit were used to calculate the % clearance during and post intervention as follows:

$$\%\ \text{clearance during intervention} = \frac{\text{initial activity - activity at 1 min post intervention}}{\text{initial activity}} \times 100$$

$$\%\ \text{clearance post intervention} = \frac{\text{activity at 1 min post intervention - activity at 45 min post intervention}}{\text{activity at 1 min post intervention}} \times 100$$

Statistical analysis

Analysis of variance (ANOVA) with repeated measures (two-factor ANOVA) was performed to compare the two groups in terms of the effect of frusemide on clearance during and post ISH. The same analysis was also carried out for the clearance rate for every 10 min interval post intervention. If the two groups were different, then ANOVA with repeated measures (one-factor ANOVA) was performed for each group separately.

Comparison of the deposition of radioaerosol on the two study days of both groups was made with ANOVA with repeated measures (two-factor ANOVA).

Spearman's correlation analysis was performed between the % fall in FEV_1 and the difference in the clearance rate between the frusemide and the vehicle in the first 10 min post-ISH in the asthmatic subjects.

Results

The increase in mucociliary clearance following ISH with dry air was significantly delayed, in the presence of frusemide, in the whole right lung ($p<0.002$) and central region ($p<0.01$) of the asthmatic subjects (fig. 1) but not in the healthy subjects (fig. 2). This delay in the presence of frusemide was manifested by a significantly slower clearance rate in the first 10 min (figs 1 and 3), on average, after the ISH, although in two asthmatic subjects it persisted for 17 and 20 min (fig. 4). The mean % clearance·min^{-1} was 43 and 57% slower in the whole right lung and central region, respectively, in the first 10 min post-ISH, in the presence of frusemide compared to its vehicle, in the asthmatic subjects (fig. 1). In the asthmatic subjects, the mean difference (95% confidence intervals) in the % clearance·min^{-1} between frusemide and vehicle in the first 10 min in the whole right lung and central region was -0.865 (-1.323 to -0.407) and -1.214 (-2.074 to -0.354), respectively. This delay was observed in 9 out of 11 asthmatic subjects. For seven subjects, the delay occurred in both the whole right lung and central region, and in a further two asthmatic subjects in the central region only. By contrast, for the healthy subjects there was no significant effect of frusemide on MCC rates (figs. 2 and 5), although one subject had

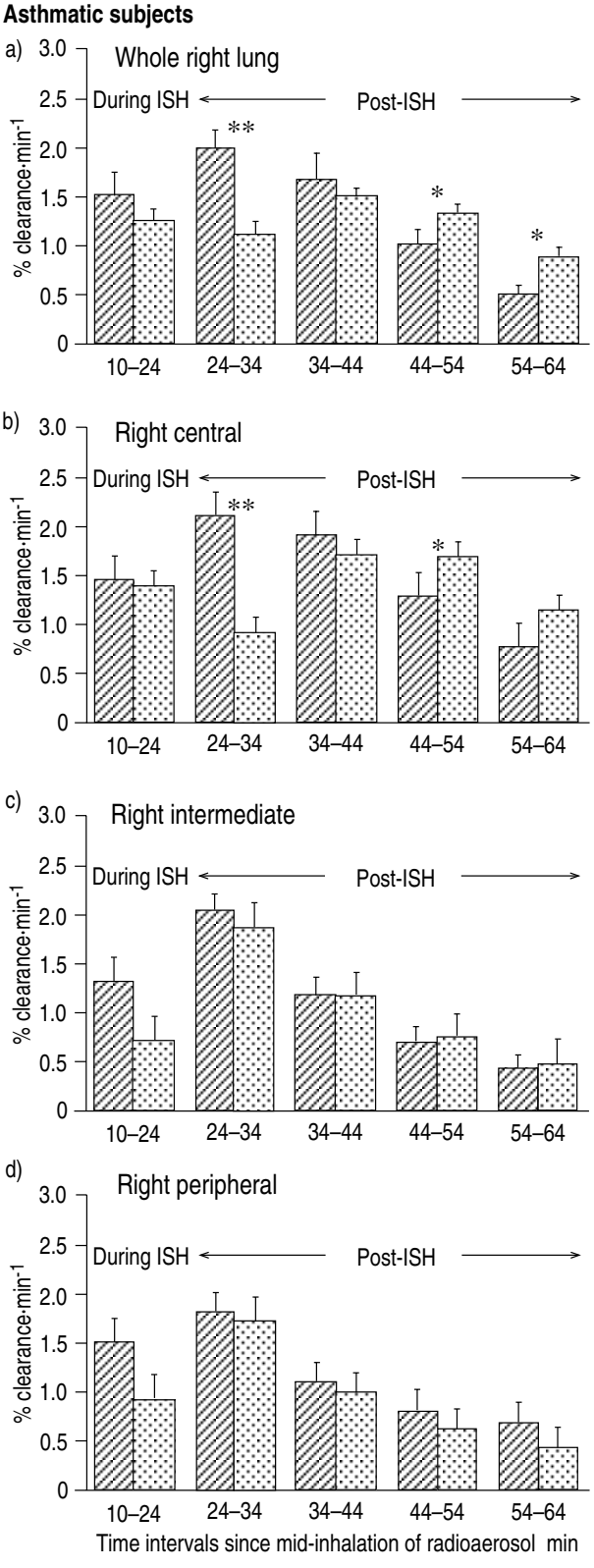

Fig. 1. – Mean % clearance rates of: a) the whole right lung; and b) the central region; c) the right intermediate region; and d) the right peripheral region, in the presence of frusemide ⁘ and its vehicle ▨ in the asthmatic subjects. Frusemide compared to its vehicle significantly delayed the increase in the mucociliary clearance rate for about 10 min after isocapnic hyperventialtion (ISH) with dry air in the whole right lung and central region of the asthmatic subjects. Values are presented as mean±SEM. *, **: $p<0.05$, $p<0.01$, respectively.

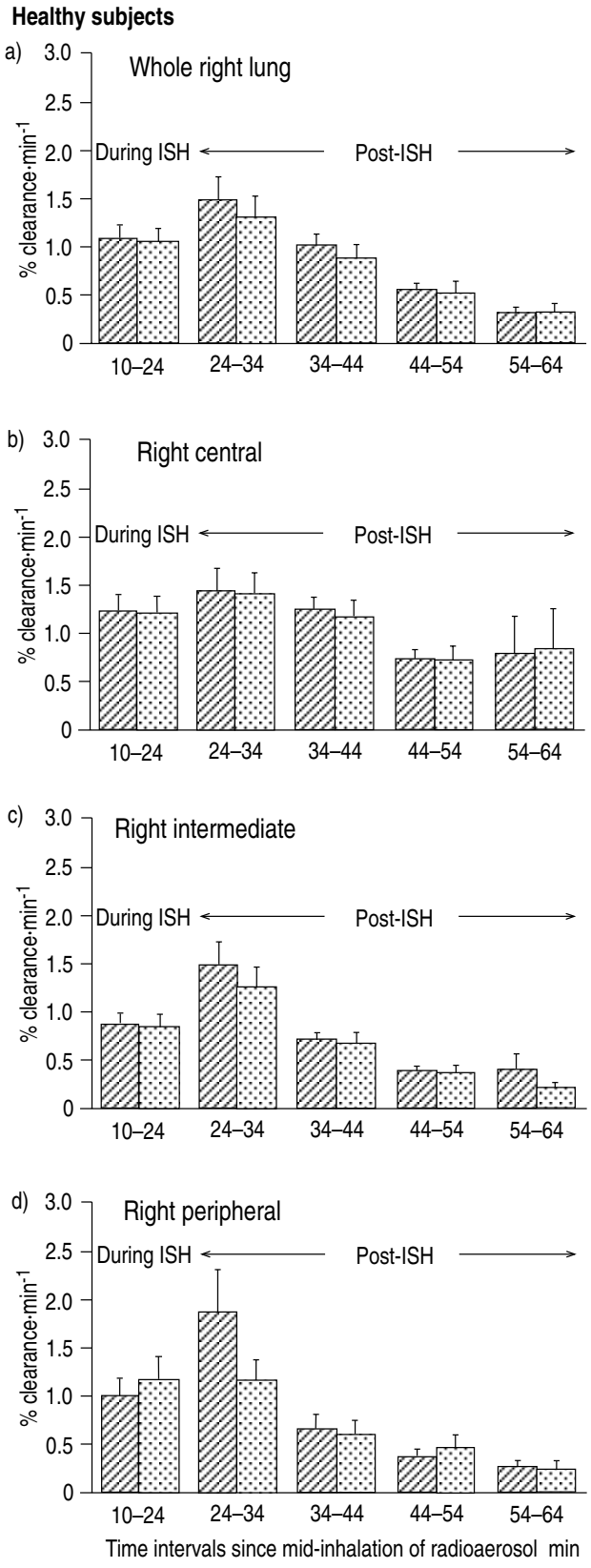

Fig. 2. – Mean % clearance rates of: a) the whole right lung; b) the right central region; c) the right intermediate region; and d) the right peripheral region, in the presence of frusemide ⊡ and its vehicle ▨ in the healthy subjects. Frusemide in comparison with its vehicle had no effect on the mucociliary clearance rate. Values are presented as mean±SEM. ISH: isocapnic hyperventilation.

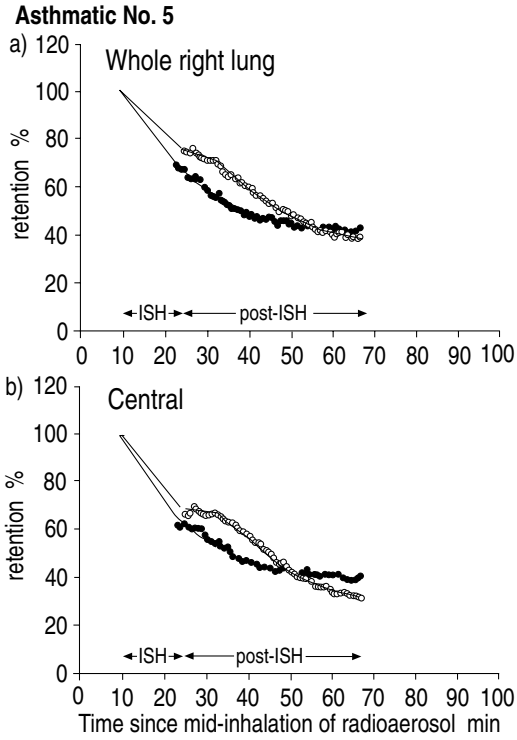

Fig. 3. – Example of mucociliary clearance (MCC) of: a) the whole right lung; and b) the central region, in the presence of frusemide and vehicle in an asthmatic subject (No. 5). This figure demonstrates the average delay in the increase of MCC rate after isocapnic hyperventilation (ISH) with dry air. ●: vehicle; ❍: frusemide.

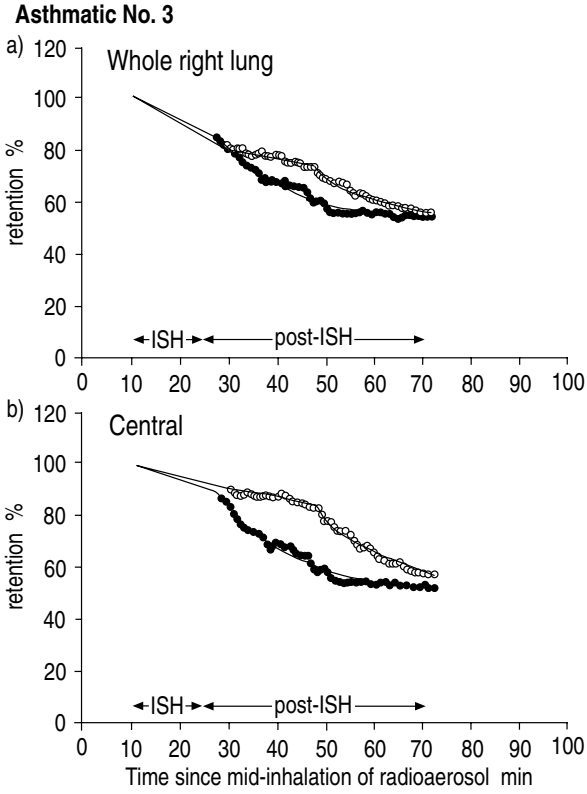

Fig. 4. – Example of mucociliary clearance (MCC) of: a) the whole right lung; and b) the central region, in the presence of frusemide and vehicle in an asthmatic subject (No. 3). This figure demonstrates a prolonged delay (about 20 min) in the increase of MCC rate after ISH with dry air. ISH: isocapnic hyperventilation. ●: vehicle; ❍: frusemide.

a reduction in both the whole right lung and central region, and a further two subjects displayed similar findings to the asthmatic subjects in the central region.

Frusemide had no significant effect (p>0.3) on the mucociliary clearance rate during ISH in either group, although in the intermediate region the difference approached statistical significance (p=0.08) (figs. 1 and 2). After ISH, in the asthmatic subjects, there appeared to be a "catch up" period in MCC with frusemide, after the initial delay. The rate of MCC in the presence of frusemide was significantly higher compared to its vehicle 30 min (p<0.02) after ISH (interval 44–54 min since mid-inhalation of the radioaerosol), in the whole right lung and central region of the asthmatic subjects (fig. 1). Thus, 45 min after ISH, the total clearance in the presence of frusemide was the same (p>0.2) as for the vehicle in the whole right lung and central region of the asthmatic subjects (table 2). Frusemide, compared to its vehicle, had no significant effect in the % clearance·min^{-1} for each 10 min interval after ISH in the intermediate and peripheral region (p>0.2) in either group (figs. 1 and 2). However, frusemide compared to its vehicle caused a 5 and a 7% reduction in the total clearance 45 min post-ISH of the peripheral region in asthmatic and healthy subjects, respectively. This small reduction of the clearance in the peripheral region reached statistical significance (p<0.03) (table 2), despite the lack of effect of frusemide in the % clearance·min^{-1} either during ISH or for each 10 min interval after ISH.

The distribution of deposited radioaerosol was well-matched (p>0.8) on both study days, as shown by the penetration indices (table 3), and not significantly different (p>0.8) between the two groups.

The airway response to ISH (%fall in FEV1), the ventilation (L·min^{-1}) and the duration of ISH are shown in table 1 both for asthmatic and healthy subjects. Two of

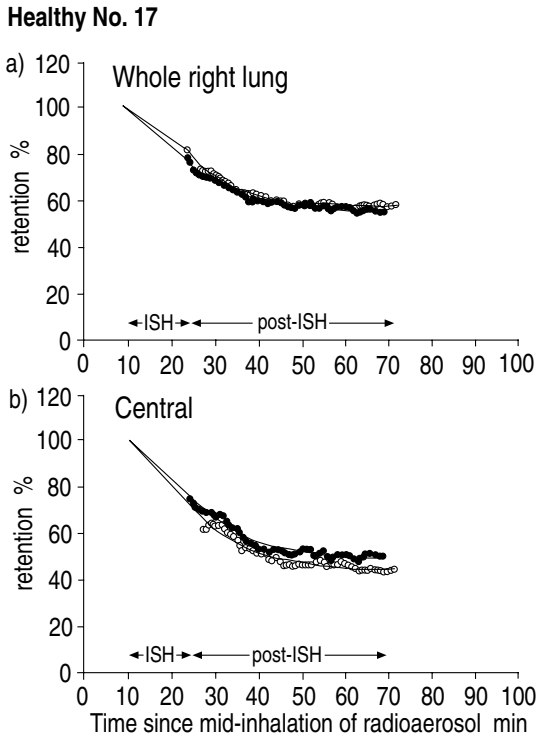

Fig. 5. – Example of mucociliary (MCC) of: a) the whole right lung; and b) the central region, in the presence of frusemide and vehicle in a healthy subject (No. 17). This figure demonstrates the lack of any effect on MCC in the presence of frusemide in the healthy subjects. ISH: isocapnic hyperventilation. ●: vehicle; ○: frusemide.

Table 2. – Total % clearance during and 45 min post-ISH in asthmatic and healthy subjects with frusemide and vehicle

Region	Asthmatic		Healthy	
	Frusemide	Vehicle	Frusemide	Vehicle
During ISH				
Whole right lung	18±3	21±3	16±2	17±2
Central region	19±4	20±4	18±3	18±3
Intermediate region	10±3*	18±3	12±2*	13±2
Peripheral region	14±4	21±2	17±4	15±3
45 min-post ISH				
Whole right lung	42±4	44±4	29±4	31±3
Central region	47±4	49±5	33±5	34±3
Intermediate region	38±4	39±4	24±3	27±3
Peripheral region	33±4**	38±5	22±4**	29±4

Values are presented as mean±SEM. ISH: isocapnic hyperventilation. No statistical significant differences (p>0.3) between frusemide and vehicle were observed except in the peripheral region. *: p=0.08; **: p<0.03.

Table 3. – Penetration index (PI) of the frusemide and vehicle study for asthmatic and healthy subjects

No.	Vehicle	Frusemide	% change PI
Asthmatic subjects			
1	0.208	0.213	-2.4
2	0.365	0.369	-1.1
3	0.26	0.321	-23.5
4	0.26	0.254	2.3
5	0.139	0.122	12.2
6	0.412	0.397	3.6
7	0.273	0.267	2.2
8	0.119	0.188	-58.0
9	0.408	0.422	-3.4
10	0.294	0.229	22.1
11	0.276	0.27	2.2
Mean	0.274	0.277	-4.0
±SD	0.096	0.092	21.0
		p=>0.8	
Healthy subjects			
12	0.226	0.215	4.9
13	0.372	0.353	5.1
14	0.237	0.206	13.1
15	0.365	0.358	1.9
16	0.147	0.197	-34.0
17	0.507	0.488	3.7
18	0.352	0.345	2.0
19	0.36	0.361	-0.3
20	0.154	0.177	-14.9
21	0.294	0.278	5.4
22	0.18	0.246	-36.7
Mean	0.290	0.293	-4.5
±SD	0.112	0.096	16.6
		p>0.8†	

†: there was no significant difference between the penetration indices of frusemide and vehicle study days (p>0.8).

the asthmatic subjects had a fall in FEV1 less than 10% (attributable to treatment with inhaled corticosteroids). It is of interest that the MCC of one of these two asthmatic subjects (No. 4) was not affected at all by the frusemide. However, no correlation ($p>0.2$; $r_s=0.019$) was found between the % fall in FEV1 and the difference in clearance rate between frusemide and vehicle in the first 10 min in the asthmatic subjects.

Frusemide had a diuretic effect immediately after hyperventilation in all subjects.

Discussion

Inhaled frusemide, an inhibitor of the Na^+ and Cl^- co-transport systems, significantly delayed the onset of the increase in mucociliary clearance (MCC) for about 10 min after ISH with dry air in the asthmatic subjects. However, despite causing the delay in the increase in MCC, frusemide had no significant effect on the total mucociliary clearance 45 min after ISH when compared to its vehicle.

The delay in the onset of the increase of MCC in the presence of frusemide was observed in the whole right lung and central region in the asthmatic but not the healthy subjects. The clearance rate in the intermediate and peripheral regions was unaffected by the presence of frusemide, either during or after ISH. Therefore, the delay in the increase of clearance immediately after the ISH, in the central region and the whole right lung, did not appear to be a consequence of an increase in the clearance rate from the small airways. The delay in the onset of the increase of MCC was also of short duration, and it would have been missed if the measurements of MCC were not continuous for at least 30 min after ISH.

Under conditions of resting ventilation with ambient air, frusemide has no effect on MCC either in asthmatic or healthy subjects [20]. However, there is recent evidence that, under resting conditions, frusemide decreases the clearance of ^{99m}Tc-diethylenetriomine penta-acetic acid (DTPA) in asthmatic subjects [21], suggesting that frusemide may alter the mucosal permeability in asthmatic but not in healthy subjects. In the present study, under the conditions of hyperpnoea with dry air, while frusemide did not affect the magnitude of the reduction in MCC in response to dry air, it did have an effect in delaying the increase in MCC after ISH with dry air in the asthmatic subjects. It is unlikely, however, that this effect of frusemide in the asthmatic subjects could be due to a slower clearance of frusemide from the asthmatic airways, because of the immediate diuretic effect of frusemide in the asthmatic as well as the healthy subjects. It is possible that the sensitivity to the drug may be different in asthmatic compared to healthy subjects, as has been suggested [21].

The rate of MCC can be affected if the depth of the periciliary fluid layer is not at the optimum level. Hyperventilation with dry air results in evaporative water loss from the airway surface and hyperosmolarity of the periciliary fluid layer, in agreement with experimental evidence from animal studies [22–24]. The immediate source of water of the periciliary fluid layer is the epithelial cells and the submucosa below the basement membrane. The epithelial cells initially behave like sensitive osmometers [25, 26]. The cells shrink and the tight junctions get tighter and remain so while hyperosmolarity persists. The mechanism by which the water is restored to normality in the airway surface after hyperpnoea is not clearly understood. The results of the present study suggest that frusemide may transiently interfere with the availability of water to the airway surface of the asthmatic subjects. Water can move onto the airway surface in response to an osmotic gradient, either passively or following the transport of chloride ion. If the mechanism of water transport in response to an osmotic gradient depended largely on the chloride secretion, then its inhibition by frusemide would have affected the MCC of the healthy subjects as well. The absence of an effect of frusemide on the MCC of the healthy subjects suggests that passive diffusion of water in response to an osmotic gradient is the primary mechanism for restoring the water of the airway surface [27, 28] and this diffusion may be slower in asthmatic subjects [3].

We have previously found that MCC decreases during ISH with dry air [3], and we were expecting frusemide to cause an additional reduction in MCC but it did not. This suggests that inhibition of chloride secretion by frusemide had no additional effect on MCC over the effect of the dry air alone [3].

The 10 min delay in the increase in the MCC after ISH, in the presence of frusemide, in the asthmatic subjects, could relate to the time required for the epithelial cells to recover their volume and possibly to a slower rate of water diffusion. The swelling of the hypertonically shrunken cells, termed regulatory volume increase (RVI), involves an influx of NaCl into the cell predominantly *via* the activation of $Na^+/K^+/2Cl^-$ co-transport system, which is activated by the cell shrinkage and inhibited by loop diuretics, such as frusemide and bumetanide [10, 25, 26]. The RVI can take up to 20 min to be complete [25, 26]. There is evidence that the apical membrane of the airway epithelium is more permeable to water than the basolateral membrane [29]. There is also evidence that asthmatic subjects have thickening of the basement membrane [30, 31], which could limit the rate of water diffusion when there is a high demand. It is possible that the delaying effect of frusemide in the increase of the mucociliary clearance, immediately after ISH with dry air in the asthmatic subjects, could be explained by the time needed for the epithelial cell to recover its volume after dehydration and the possible slower water diffusion due to thickness of the basement membrane.

The increase in MCC after the ISH with dry air is most likely to be due to mediators released in response to transient hyperosmolarity from epithelial cells (*e.g.* prostaglandin E_2), mast cells (*e.g.* histamine), and sensory nerves (*e.g.* substance P) [32], as these mediators are known to stimulate ciliary activity and mucus transport mechanisms [33–35]. It is possible that inhibition or blocking of mediator release from mast cells [11], and neuropeptides from sensory nerves [36], by frusemide could cause a transient delay in the increase in MCC after ISH with dry air in asthmatic subjects.

In the present study, we did not measure MCC at baseline or during ISH as we have previously determined, using the same methodology as this study, that MCC decreased during ISH [3]. The values of MCC

with vehicle or frusemide are similar to those obtained with ISH previously [3].

It is of interest that frusemide also delays rather than prevents the bronchoconstricting response to 4.5% saline aerosol [9], and ISH with dry air [7, 8]. Frusemide when inhaled prior to 4.5% saline caused a 2.6 doubling dose increase in the provocative dose causing a 20% fall in FEV_1 (PD_{20}) [9]. In addition, frusemide, inhaled prior to ISH with dry air, increased the provocative volume causing a 20% fall in FEV_1 (PVE_{20}) by 21 L [8], and in the study by GRUBBE *et al*. [7] a significant inhibition of the bronchoconstricting effect occurred only during the first 10 min post challenge. It is possible that the effect of frusemide on bronchoconstriction and on mucociliary clearance in asthmatic subjects may be due to a common mechanism.

Although frusemide delayed the onset of the increase in mucociliary clearance in the asthmatic subjects, it had no effect on the total clearance measured 45 min post-ISH either in asthmatic or healthy subjects. The fast clearance of frusemide from the airways, as evidenced by its systemic diuretic effect, may explain why its effect on the MCC in the asthmatic subjects was transient, and the absence of any effect 45 min post-ISH in either group. Rapid clearance could also explain the transient effect on bronchoconstriction [7–9].

In summary, frusemide in comparison with its vehicle caused a significant delay in the onset of the increase of mucociliary clearance after ISH with dry air in asthmatic but not healthy subjects. The present data suggest that after hyperpnoea with dry air: 1) frusemide may interfere with the availability of the water required to restore the depth of the periciliary fluid layer in asthmatic subjects; 2) frusemide may interfere with the mechanism, probably involving local chemical mediators, that stimulates MCC in asthmatic subjects. The short duration of the delaying effect is probably explained by the rapid elimination of frusemide from the airways.

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