Eur Respir J
1989, 2, 721–726

Bronchoscintigraphic visualization of the acute effect of tobacco exposure and terbutaline on mucociliary clearance in smokers

J. Mortensen, S. Groth, P. Lange, N. Rossing

Bronchoscintigraphic visualization of the acute effect of tobacco exposure and terbutaline on mucociliary clearance in smokers. J. Mortensen, S. Groth, P. Lange, N. Rossing.

ABSTRACT: The aim of this study was to examine the acute effect of tobacco smoke exposure and inhaled terbutaline on mucociliary clearance in 9 healthy smokers. It was based on a recently described method for scintigraphic visualization of the bronchi (bronchoscintigraphy). After an initial bronchoscintigram had been made by having the subjects inhale aerosolized ^{99m}Tc-albumin, they inhaled either terbutaline or placebo from a metered-dose inhaler. Subsequently, data acquisition for production of bronchoscintigrams was repeated at 10 min intervals for 120 min, and the mucociliary clearance was estimated from the movement of radioactivity in the series of bronchoscintigrams thus obtained. On two study days the subjects remained tobacco abstinent, while on two occasions they chain-smoked during the examination. Acute tobacco exposure resulted in an increased clearance rate in the lobar bronchi in 8 of the 9 smokers ($p<0.03$), while in the main bronchi and the trachea the effect was inconsistent. In all subjects terbutaline systematically increased the clearance rate in all visible bronchial structures compared to placebo ($p<0.04$). The combination of smoking and terbutaline caused a faster clearance rate in the lobar bronchi in most subjects than tobacco smoke or terbutaline alone. It is concluded that both acute tobacco exposure and terbutaline increase mucociliary clearance in healthy smokers.

Eur Respir J., 1989, 2, 721–726

Depts of Clinical Physiology and Nuclear Medicine, Rigshospitalet, and Medicine P, Bispebjerg Hospital, Copenhagen, Denmark.

Correspondence: J. Mortensen, Dept of Clinical Physiology and Nuclear Medicine, Righospitalet, 49 Strandboulevard, DK-2100, Copenhagen , Denmark.

Keywords: Bronchoscintigraphy; drug effects; mucus; physiology; smoking; terbutaline.

Received: June, 1988; accepted after revision March 7, 1989.

This study was supported by grants from the National Union for the Fight Against Lung Diseases, and the Danish Medical Research Council.

Cigarette smoke may cause profound changes in the composition and function of the airway epithelium in both a time and dose-dependent manner [1, 2]. If mucociliary defence is affected it may imply disease [3]. There have been reports indicating a significantly slower mucociliary clearance in the central airways of asymptomatic smokers than of nonsmokers, while clearance in the peripheral airways seems to be the same [4, 5]. However, as to the acute effect of tobacco exposure on mucociliary clearance results are conflicting [6–11]. Different cilioactive compounds of cigarette smoke have been shown to settle on and affect the bronchial epithelium of different generations of the bronchial tree in a non-uniform manner [12]. In previous studies of the acute effect of tobacco smoke on mucociliary clearance the evaluation has often been confined to arbitrarily defined regions of interest rather than to selected airway generations. Thus, regional effects of tobacco smoke on mucociliary clearance may have been overlooked, and may explain the conflicting results.

Recently a method has become available that allows regional mucociliary clearance to be visualized by the movement of ^{99m}Tc-albumin in the airways by means of bronchoscintigraphy [13]. The aim of this study was to employ bronchoscintigraphy to examine whether the effect of cigarette smoke on mucociliary clearance in the large airways is so pronounced that it can be demonstrated during acute tobacco exposure of otherwise healthy smokers. The technique was also used to determine whether inhaled terbutaline has an acute effect on mucociliary clearance in healthy smokers.

We chose healthy, smoking subjects because we wanted to study airways in which the ciliated epithelium was not yet expected to be grossly disturbed, and because we wanted to perform the examinations without too much interference from coughing due to hypersecretion.

Methods

Subjects

Nine healthy smokers participated. Their age, sex, percentage predicted forced expiratory volume in one second (FEV_1), percentage predicted forced vital capacity (FVC), and smoking history are listed in table 1. All subjects had normal FEV_1 and FVC. The study was approved by the local Ethical Committee.

The radioaerosol procedure

The radioaerosol generation and inhalation procedure have been described in detail previously [13]. A technetium-bound human albumin aerosol was generated ultrasonically. The mass median aerosol diameter (MMAD) was 3.5 μm, geometric standard deviation (GSD) 1.9. Subjects inspired slowly (inspiratory flow rate ≤15 $l \cdot min^{-1}$) starting at residual volume, until 500 ml had been inspired. Exhalation was by maximal forced expiration. Inhalations were made until a count rate of 2,000 $counts \cdot s^{-1}$ was achieved. On average this was reached after 20 inhalations. Subsequently, the subjects gargled 3 times and finally swallowed some water. In between the following gamma camera acquisitions, they cleared the oesophagus with water.

Table 1. – Anthropometric data, lung function and smoking history

	Sex	Age	FEV_1 %pred	FVC %pred	$Cig \cdot day^{-1}$	yrs
1	F	42	103	107	15	22
2	M	39	106	112	20–25	18
3	F	29	102	101	15–20	9
4	F	26	97	100	10–15	8
5	F	24	93	114	15	6
6	M	37	96	110	15	20
7	F	26	94	95	15	6
8	F	24	85	81	20	10
9	F	46	112	115	10–15	24
Median		29	97	107	10 pack-yrs	
Range		(24–46)	(85–112)	(81–115)	(4.5–20)	

FEV_1: forced expiratory volume in one second; FVC: forced vital capacity.

Immediately after inhalation of the aerosol, the subjects were placed in the supine position, with their back against a gamma camera. A static acquisition was repeated every 10 min for 120 min to follow mucociliary clearance of ^{99m}Tc-albumin. The acquisition time remained unchanged apart from being corrected for the physical decay of ^{99m}Tc. In this way it was intended that changes in scintigrams contained biological information only.

The acute effect of terbutaline and tobacco exposure on mucociliary clearance

The subjects were studied by two series of examinations, each consisting of two investigations at least 48 h apart. Subjects abstained from smoking at least 2 h before the examination. In the first series (days A and B), the subjects inhaled, in a randomized double-blind crossover fashion, either 5 puffs of placebo or terbutaline from a metered-dose inhaler, immediately after the first gamma camera acquisition. The days of placebo treatment are called days A, and terbutaline treatment days B. The second series (called days C and D) was a repeat of the first series but after the terbutaline or placebo administrations, the subjects started smoking. They were instructed to smoke at random in a relaxed state as normal and on average they smoked 7 cigarettes (range 5–9 cigarettes) during the next 120 min. Smoking was interrupted only during data acquisition. The number of smoked cigarettes was held constant in each subject on days C and D. The subjects used their own brand of cigarettes (all filter-tipped). Only one of the subjects coughed a few times (non-productive) while chain-smoking. Therefore, it was not necessary to correct for cough as an additive clearance mechanism.

Data analysis

The number of different bronchi that could be identified on the bronchoscintigrams was counted and the time after inhalation when they could no longer be visualized was noted. The scintigrams were evaluated independently, in blind fashion, by two readers and the mean of their values was used. The comparison of mucociliary clearance between the four different days was performed by a Friedman two-way analysis of variance [14]. The Wilcoxon matched pairs signed rank test (two-tailed test) was used to evaluate the significance of the effect of either terbutaline or tobacco smoke exposure to placebo (or tobacco abstinence).

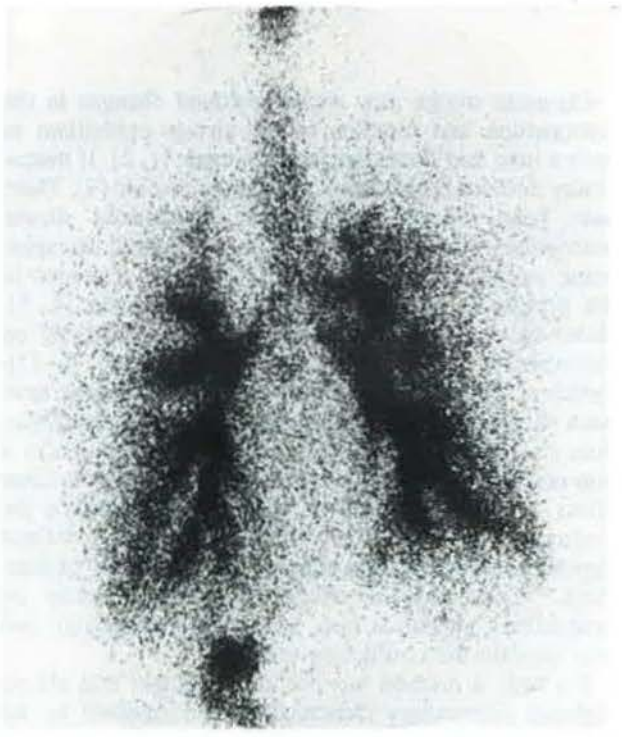

Fig. 1. – An example of a bronchoscintigram. The large airways from the trachea to the segmental bronchi are visualized.

Results

Figure 1 is an example of the quality of the morphological information that may be obtained by the technique for bronchoscintigraphic imaging. Individual bronchial structures are easily identified, but peripheral to the segmental bronchi, the structures merge. The radioactivity in the stomach below the left lung comes from the aerosol, which was originally deposited in the mouth. A small bolus in the oesophagus is visualized.

A typical example of the series of bronchoscintigrams that was used to evaluate mucociliary clearance is seen in fig. 2. The deposition of the radioactivity in the lung immediately after inhalation is seen on the uppermost scintigram (left panel). The initial images appeared similar on all 4 days, indicating a high degree of reproducibility of the deposition pattern. The major part of the retained activity was deposited on the large airways.

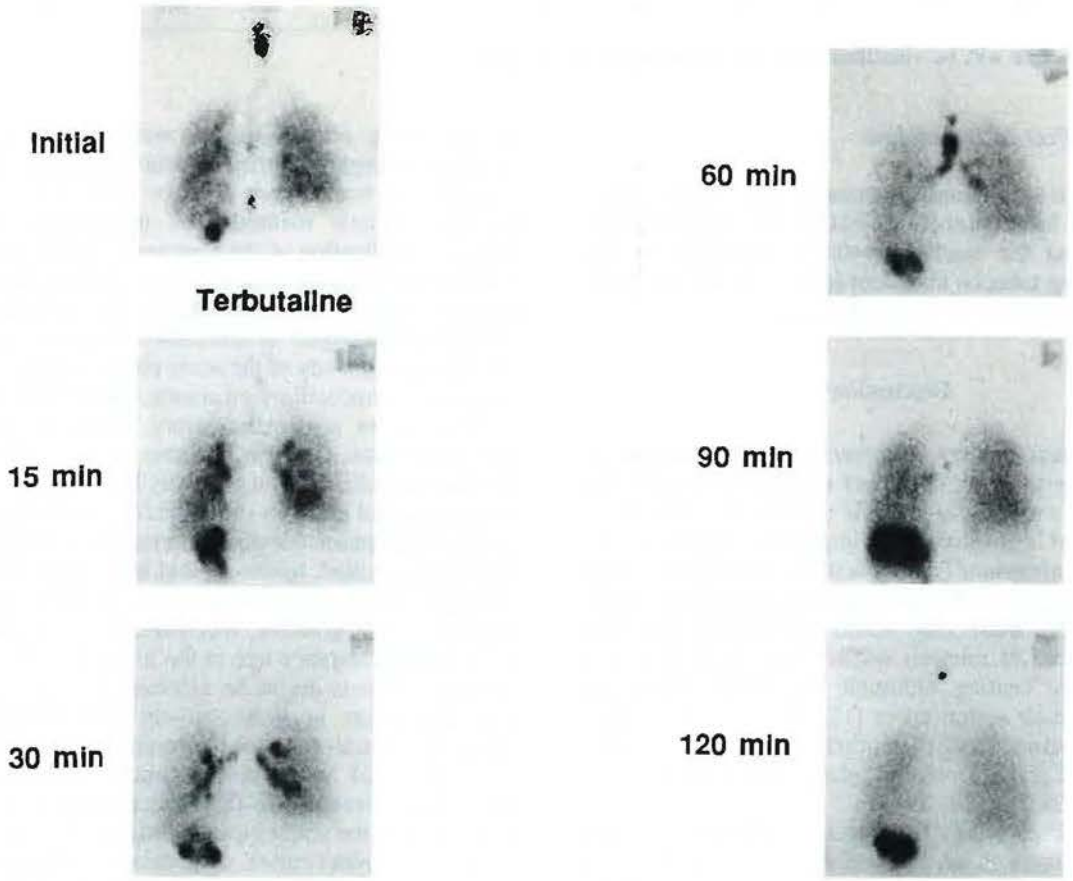

Fig. 2. – A series of bronchoscintigrams.

Table 2 shows the individual results for the longest time at which the lobar bronchi, the main bronchi, and the trachea were seen on the series of bronchoscintigrams on the four study days. In some cases the trachea was not seen at the end of examination (120 min) while the main bronchi still contained so much radioactivity that they could be visualized. The "disappearance" of the trachea could, therefore, only be temporary since the radioactivity would reach the trachea at a later point. These cases are indicated in table 2 by the value >120 min. The Friedman test revealed a significant difference in the mucociliary clearance rate between all visualized structures on the four study days. The differences were most pronounced in the lobar bronchi ($p<0.001$), which always became scintigraphically indiscrete faster than both the main bronchi ($p<0.01$) and the trachea ($p<0.001$).

The acute effect of cigarette smoke

The acute effect of cigarette smoke was assessed by comparing the regional mucociliary clearance on the days of placebo treatment (day A) and placebo+tobacco smoking (day C) (table 2). The lobar bronchi were cleared significantly faster in 8 of 9 subjects while chain-smoking ($p<0.03$). There was no significant acute effect of tobacco smoke on the mucociliary clearance of the main bronchi or the trachea.

The effect of chain-smoking was also assessed by comparing differences in clearance on days B (terbutaline) and D (terbutaline and tobacco). Again, the lobar bronchi were cleared faster on the day of tobacco smoke exposure in all but one subject ($p<0.02$), and again there was no significant effect on mucociliary clearance of the main bronchi or the trachea. The acute effect of tobacco smoke exposure on mucociliary clearance thus seems to be most pronounced in the lobar bronchi.

Table 2. – Longest time (min) at which the different anatomical structures were seen on the bronchoscintigrams, expressed as the mean values for the trachea, the main bronchi and the lobar bronchi, respectively, on study days A, B, C and D

	Trachea				Main bronchi				Lobar bronchi			
Subject No.	A	B	C	D	A	B	C	D	A	B	C	D
1	>120	>120	120	120	120	120	60	95	114	104	45	42
2	>120	100	>120	100	120	80	120	80	120	106	116	62
3	80	40	50	30	70	35	20	20	72	22	13	13
4	120	120	>120	120	120	70	120	75	120	65	110	10
5	100	40	120	60	80	26	100	30	44	32	70	19
6	>120	50	100	90	40	35	50	20	116	45	66	10
7	120	90	110	50	115	85	50	40	98	72	18	30
8	>120	120	>120	120	120	75	120	70	120	80	114	42
9	>120	80	120	120	110	40	110	75	112	30	82	38
Median	>120	90	120	100	115	70	100	70	114	65	70	30
Range	80–>120	40–>120	50–>120	30–120	40–120	26–120	20–120	20–95	44–120	22–106	13–116	10–62

>120: the structure will be visualized after the examination has stopped.

The acute effect of terbutaline

Terbutaline significantly increased mucociliary clearance in the lobar bronchi ($p<0.02$), the main bronchi ($p<0.04$), and the trachea ($p<0.02$) regardless of the subjects being tobacco abstinent (day A *versus* day B) or chain-smoking (day C *versus* day D).

Discussion

In vitro tests are not necessarily good predictors of events taking place in the intact respiratory system, but can provide a means for specific evaluation of the effect of individual constituents of cigarette smoke on the mucociliary apparatus (ciliary activity, viscoelastic properties and quantity of mucus and the periciliary layer). Several of the more than 2,000 compounds that have been identified in tobacco smoke, have been shown to affect ciliary beating although the effectiveness and duration of their action differ [12, 15–17]. For instance, small nicotine doses slightly stimulate ciliary co-ordinated activity, while higher concentrations seem to cause a decline [18, 19].

In man, observations have been conflicting. In young smokers CAMNER *et al.* [6] and ALBERT *et al.* [7] have found a stimulatory effect from chain-smoking on lung mucociliary clearance. GOODMAN *et al.* [8] and YEATES *et al.* [9], on the other hand, have found no acute effect on tracheal mucus velocity of the smoking of up to 12 cigarettes, while NAKHOSTEEN *et al.* [10] reported a decrease in tracheal mucus velocity in a two-case study after acute tobacco exposure. PAVIA *et al.* [11] found in 22 long-term smokers (19 pack-yrs) that smoking 2–5 cigarettes decreased clearance in 10 subjects, while in the others there were no significant effects.

There may be many explanations for the discrepancy between the different observations, the most important perhaps being related to the methods employed, in particular as regards correction for deposition of tracer material on nonciliated airways and for coughing. Recently, a new method was introduced [13] that allowed visualization of the clearance process on a semi-morphological basis, thus enabling studies of regional clearance defects that might easily be overlooked by a conventional analysis of arbitrarily defined regions.

In the present study of the acute effect of tobacco smoke exposure on mucociliary clearance, the method provided evidence of an acute stimulatory effect on an airway generation basis, but also that this effect is confined to the lobar bronchi. This might easily have been overlooked in conventional analysis of arbitrarily defined regions of interest. The reason for this observation is unclear. It has been demonstrated, however, that individual compounds of tobacco smoke settle on different locations in the airways [12]. It is possible, therefore, that our findings of an increased clearance rate in the lobar bronchi of chain-smoking subjects might be associated with a predominant deposition in these airway generations of the cilioactive substances of the cigarette smoke. The stimulatory effect of acute tobacco smoke exposure on the mucociliary clearance of the large airways is interesting in as much as the study group consisted of young healthy smokers whereas after long-term smoking, mucus hypersecretion often ensues. The results of the present study may, therefore, be an expression of a general stimulatory effect of cigarette smoke on the airway epithelium.

We found a significantly faster clearance in asymptomatic smokers after inhalation of terbutaline than after placebo administration, just as we have previously observed in healthy nonsmokers [13]. In comparison with the results of the nonsmokers in the previous study, the mucociliary clearance of the smokers was not significantly slower after placebo. After terbutaline, the increase in mucociliary clearance of the nonsmokers was larger

than the increase in the smokers. After a combination of terbutaline and cigarette smoking, however, the clearance rate in the smokers further increased to reach similar values as seen in the nonsmokers after terbutaline. In a previous study of FOSTER *et al.* [20], employing an open design, there was a similar effect of $beta_2$-agonists on mucociliary clearance in healthy smokers. To obtain blindness we compared the effect of terbutaline to placebo, and to our knowledge, therefore, the present study is the first controlled study to demonstrate a stimulatory effect of an inhaled $beta_2$-agonist on mucociliary clearance in healthy smokers. The possibility that the aerosolized freon propellants contained in the placebo (and terbutaline) canister *per se* might have an effect on the mucociliary clearance has been dealt with in a study by SACKNER *et al.* [21]. They were unable to demonstrate any effect.

The additive effect of terbutaline and tobacco smoke on the mucociliary clearance need not necessarily be mediated *via* the same mechanism. Thus, exposure to cigarette smoke stimulates mucus discharge [22, 23], inhibits epithelial ion movement *in vivo* and *in vitro* [24, 25], initiates biochemical changes in the composition of mucus [23] and alters ciliary beat frequency [12, 15–19]. $Beta_2$-agonists, on the other hand, increase ciliary beating in a dose-related manner [18, 36] and change the composition of the periciliary fluid by affecting ion transport [25, 27]. Bronchodilatation can facilitate mucus transport by altering the depth and the viscoelastic properties of the mucus layer. Furthermore, mucus discharge may be stimulated by $beta_2$-agonists [23, 28].

Another explanation of the additive effect may be that we have not reached the top of the dose-response by giving 1.25 mg terbutaline or having the subjects chain-smoking. Systematically performed dose-response studies analysing the effect of inhaled terbutaline and tobacco smoke on mucociliary clearance are still required.

To conclude, both acute tobacco exposure and inhaled terbutaline increase mucociliary clearance in healthy smokers.

Acknowledgement: We thank DRACO (Denmark and Sweden) for supplying the drugs.

References

1. Cosio MG, Hale KA, Niewoehner DE. – Morphologic and morphometric effects of prolonged cigarette smoking on the small airways. *Am Rev Respir Dis*, 1980, 122, 265–271.
2. Ebert RV, Hanks PB. – Mucus secretion by the epithelium of the bronchioles of cigarette smokers. *Br J Dis Chest*, 1981, 75, 277–282.
3. Wanner A. – State of the art: clinical aspects of mucociliary transport. *Am Rev Respir Dis*, 1977, 116, 73–125.
4. Agnew JE, Pavia D, Clarke SW. – Mucus clearance from peripheral and central airways of asymptomatic cigarette smokers. *Bull Eur Physiopathol Respir*, 1986, 22, 263–267.
5. Vastag E, Matthys H, Zsamboki G, Köhler D, Diakeler G. – Mucociliary clearance in smokers. *Eur J Respir Dis*, 1986, 68, 107–113.
6. Camner P, Philipson K, Arvidsson T. – Cigarette smoking in man. Short-term effect on mucociliary transport. *Arch Environ Health*, 1971, 23, 421–426.
7. Albert RE, Peterson HT, Bohning DE, Lippmann M. – Short-term effects of cigarette smoking on bronchial clearance in humans. *Arch Environ Health*, 1975, 30, 361–367.
8. Goodman RM, Yergin BM, Landa JF, Golinvaux MH, Sackner MA. – Relationship of smoking history and pulmonary function tests to tracheal mucous velocity in nonsmokers, young smokers, ex-smokers, and patients with chronic bronchitis. *Am Rev Respir Dis*, 1978, 117, 205–214.
9. Yeates DB, Aspin N, Levison H, Jones MT, Bryan AC. – Mucociliary tracheal transport rates in man. *J Appl Physiol*, 1975, 39, 487–495.
10. Nakhosteen JA, Lindemann L, Vieira J. – Mukoziliäre klärfunktion. Ergebnisse bei rauchern, nichtrauchern und patienten mit atemwegskrankheiten. *Dtsch Med Wschr*, 1982, 107, 1713–1716.
11. Pavia D, Thomson ML, Pocock SJ. – Evidence for temporary slowing of mucociliary clearance in the lung caused by tobacco smoking. *Nature*, 1971, 231, 325–326.
12. Hee J, Guillerm R. – Dicussion on smoke and mucociliary transport. *Eur J Respir Dis*, 1985, 66 (Suppl. 139), 86–88.
13. Groth S, Mortensen J, Lange P, Munch EP, Sørensen PG, Rossing N. – Imaging of the airways by bronchoscintigraphy for the study of mucociliary clearance. *Thorax*, 1988, 43, 360–365.
14. Siegel S. – *In:* Nonparametric statistics for the behavioral sciences. McGraw-Hill Kogakusha Ltd., Tokyo, 1956.
15. Kitamura S. – Effects of cigarette smoking on metabolic events in the lung. *Environ Health Perspect*, 1987, 72, 283–296.
16. Kaminski EJ, Fancher OE, Calandra JC. – *In vivo* studies of the ciliastatic effects of tobacco smoke. *Arch Environ Health*, 1968, 16, 188–193.
17. Bernfeld P, Nixon CW, Homburger F. – Studies on the effect of irritant vapors on ciliary mucus transport. 1. Phenol and cigarette smoke. *Toxicol Appl Pharmacol*, 1964, 6, 103–111.
18. Iravani J, Melville GN. – Wirkung von pharmaka und milieuänderungen auf die flimmertätigkeit der atemwege. *Respiration*, 1975, 32, 157–164.
19. Ballenger JJ, Dawson FW, DeRuyter MG, Harding HB. – Effects of nicotine on ciliary activity *in vitro*. *Ann Otol*, 1965, 74, 303–311.
20. Foster WM, Langenback EG, Bergofsky EH. – Disassociation in the mucociliary function of central and peripheral airways of asymptomatic smokers. *Am Rev Respir Dis*, 1985, 132, 633–639.
21. Sackner MA, Epstein S, Wanner A. – Effect of beta-adrenergic agonists aerosolized by freon propellant on tracheal mucous velocity and cardiac output. *Chest*, 1976, 69, 593–598.
22. Peatfield AC, Davies JR, Richardson PS. – The effect of tobacco smoke upon airway secretion in the cat. *Clin Sci*, 1986, 71, 179–187.
23. Reid L, Bhaskar K, Coles S. – Clinical aspects of respiratory mucus. *In:* Advances in experimental medicine and biology. E.N. Chantler, J.B. Elder, M. Elstein eds, Plenum Press, New York, 1982, pp. 369–391.
24. Welsh MJ. – Cigarette smoke inhibition of ion transport in canine tracheal epithelium. *J Clin Invest*, 1983, 71, 1614–1623.
25. Welsh MJ. – Electrolyte transport by airway epithelia. *Physiol Rev*, 1987, 67, 1143–1184.
26. Van As A. – The role of selective $beta_2$-adrenoceptor stimulants in the control of ciliary activity. *Respiration*, 1974, 31, 146–151.
27. Davis B, Marin MG, Yee JW, Nadel JA. – Effect of terbutaline on movement of Cl^- and Na^+ across the trachea of the dog *in vitro*. *Am Rev Respir Dis*, 1979, 120, 547–552.
28. Phipps RJ, Williams IP, Richardson PS, Pell J, Pack RJ, Wright N. – Sympathomimetic drugs stimulate the output of secretory glycoproteins from human bronchi *in vitro*. *Clin Sci*, 1982, 63, 23–28.

Visualisation broncho-scintigraphique des effets aigus de l 'exposition au tabac et à la terbutaline sur la clearance muco-ciliaire des fumeurs. J. Mortensen, S. Groth, P. Lange, N. Rossing.

RÉSUMÉ: L'objectif de cette étude est d'examiner les effets aigus de l'exposition à la fumée de tabac et à l'inhalation de terbutaline sur la clearance muco-ciliaire chez 9 fumeurs bien portants. Elle s'est fondée sur une méthode récemment décrite pour la visualisation scintigraphique des bronches (broncho-scintigraphie). Après un broncho-scintigramme initial, réalisé par l'inhalation de ^{99m}Tc-albumine en aérosol, les sujets ont inhalé, soit de la terbutaline soit un placebo par un aérosol doseur. Ultérieurement, les données acquises pour la production du broncho-scintigramme ont été répétées à des intervalles de 10 minutes pendant 120 minutes, et la clearance muco-ciliaire a été estimée par le déplacement de la radioactivité dans les séries de broncho-scintigrammes obtenus de la sorte. Les sujets sont restés sans fumer pendant deux jours d'étude, alors que, pendant deux examens, ils ont fumé à la chaîne pendant toute la durée de l'examen. L'exposition aiguë à la fumée de tabac a entraîné un taux de clearance accru dans les bronches lobaires de 8 des 9 fumeurs ($p<0.03$), alors que l'effet sur les bronches principales et la trachée était inconsistant. Chez tous les sujets, la terbutaline augmentait systématiquement le taux de clearance dans toutes les structures bronchiques visibles, par comparaison avec le placebo ($p<0.04$). La combinaison de la fumée et de la terbutaline a entraîné une accélération du taux de clearance dans les bronches lobaires chez la plupart des sujets, par rapport à l'effet isolé du tabac ou de la terbutaline. L'on conclut que, aussi bien l'exposition aiguë au tabac que la terbutaline en aérosol, augmentent la clearance muco-ciliaire chez les fumeurs bien portants.

Eur Respir J., 1989, 2, 721–726